Graff's Textbook of Urinalysis and Body Fluids

THIRD EDITION

Lillian A. Mundt   Kristy Shanahan

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Design Coordinator: Teresa Mallon
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Prepress Vendor: Aptara, Inc.

Third edition
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9 8 7 6 5 4 3 2 1

Printed in China

ISBN: 978-1-4963-2016-2
Library of Congress Cataloging-in-Publication Data available upon request from the Publisher.

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We dedicate this book to our families who were patient and encouraged us while we spent valuable time away from them working on this project.

We also dedicate this book to medical laboratory students, instructors, and practitioners to whom this text and its ancillaries will prove to be a valuable resource.

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The authors would like to thank the following for reviewing current and previous editions.

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Preface

Sister Graff's *A Handbook of Routine Urinalysis* had served the clinical laboratory profession as a classic reference text of urine testing and atlas of urinary sediment from 1983 to 2011, when it was revised for the second edition to add chapters on body fluid analysis. Our goal for the third edition of this text is continually to preserve the integrity and importance of Sister Graff's work while expanding its use as a valuable resource for students, instructors, and laboratory professionals.

The second edition had substantially revised and enhanced the content to bring it up-to-date and expanded the text by including other body fluids, Chapter Objectives, Key Terms, Terms, Study Questions, Case Studies, and a Glossary to make the text more user friendly in the classroom. These updates and additions were reflected in the new title, *Graff's Textbook of Urinalysis and Body Fluids.*

This, the third edition continues to provide students, instructors, and laboratory professionals with updated information concerning urine and body fluid analyses. The expanded urinalysis and body fluids coverage in this text is comprehensive, clearly presented, and explained in easy-to-understand language. Continuing the standard set by Sister Graff, the book uses numerous color photomicrographs to familiarize readers with both the normal and abnormal structures found in the urinary sediment and body fluids while tables and illustrations help clarify concepts. The third edition is divided into three sections for better grouping of content.

Section I includes foundational information about renal anatomy (Chapter1) and disorders that may be detected by analyzing urine (Chapters 2 and 3), as well as introductory information regarding clinical laboratory operations including safety (Chapter 4) and microscopy (Chapter 5).

Section II focusses on the urinalysis, beginning with urine specimen collection and preservation (Chapter 6), then progressing through physical examination (Chapter 7), chemical analysis (Chapter 8), and microscopic examination including 17 new images (Chapter 9), ending with the central chapter and jewel of *Graff's Textbook of Urinalysis and Body Fluids,* the atlas of urinary sediment containing (Chapter 10). Instructors, students, and clinicians will find that no other text includes a comparable atlas.

Section III contains revised information as well as new information about various body fluid analyses beginning with an introduction (Chapter 11) and goes into further depth about: cerebrospinal fluid (Chapter 12), serous body fluids (Chapter 13), synovial fluid (Chapter 14), gastric fluid and fecal analysis (Chapter 15), semen analysis (Chapter 16), amniotic fluid (Chapter 17), vaginal secretions (Chapter 18), and miscellaneous body fluids (Chapter 19). Several new images have been added to this section to provide for better comprehension of the information presented.

The book wraps up with a chapter on automated methods and equipment (Chapter 20); Appendices provide answers to the study and case questions, historically relevant urinalysis information, reagent strip color chart, and a detailed explanation of manual hemocytometer cell count. A glossary of terms that is ideal for study and review summaries key terms highlighted in chapter; and for those who rely on the text as a reference, a complete index.

We note that the information concerning reactions of the various reagent strip methods is up-to-date at the time of publication, but because manufacturers continually improve their products, the reagents, sensitivities, detection ranges, and timings may change. Therefore, following manufacturers' most recent directions is of utmost importance.

We are grateful for the thoughtful suggestions of our reviewers and readers, to which we have given consideration when revising and updating the current text to meet the needs of our audience.

Graff's Textbook of Urinalysis Body Fluids will continue to serve the medical laboratory profession

Content Highlights

SECTION I FOUNDATIONAL INFORMATION

- **Chapter 1, *Urinary System Anatomy and Physiology and Urine Formation,*** contains the basic information on anatomy and physiology that is key to understanding the urinary system and urinalysis. While retaining the previous anatomy sections and the 17 related illustrations, the revised chapter has more information on urinary tract functions and on assessing renal function than the previous edition. The chapter has added information on creatinine clearance and calculating and estimating the glomerular filtration rate and using that information to assess chronic kidney disease stages. It also has included information on additional testing to assess renal function and basic information on assessing tubular reabsorption and secretion.
- **Chapter 2, *Renal and Urinary Tract Diseases and Related Urinalysis Findings***, has been split from the metabolic diseases that were together in Chapter 7 of the previous edition. This chapter includes expanded and updated information on acute kidney injury (AKI), which is a common problem in hospitalized patients, and recognizing the AKI stages. AKI has poor prognosis with a high associated mortality in hospitalized patients, particularly in the intensive care unit. This chapter also has expanded information and images on urolithiasis, which is a relative common and frequently recurring malady that occurs in the urinary tract. This is often a painful condition that has been found to have comorbidities with other conditions that affect the urinary tract.
- **Chapter 3, *Metabolic Diseases and Related Urinalysis Findings*** has been split from the renal diseases that were together in Chapter 7 of the previous edition. This chapter provides an overview of the most common metabolic diseases that are detectable with urine testing such as aminoacidurias, and carbohydrate metabolism disorders. A table explaining urine findings in porphyria, metabolic pathways, and five new images enhance the reader's understanding.
- **Chapter 4, *Urinalysis Clinical Laboratory Operations*** (Chapter 1 in the second edition) provides an overview of various regulatory agencies that have an influence laboratory operations and explains levels of testing as categorized in CLIA'88. The fundamentals of quality assessment are also covered as are principles for safe practices in the medical laboratory including an explanation of the Globally Harmonized System for labeling chemicals. Five new images have been added to this chapter, for a total of seven.
- **Chapter 5, *Microscopy*** is a new chapter for this edition drawing from the work of Landy McBride. This chapter includes the basics of microscope handling along with principles of illumination and how to achieve the best contrast for proper viewing. Several types of microscopy are presented. This chapter contains 25 images new to this text.

SECTION II THE URINALYSIS

- **Chapter 6** pulls out the **Collection and Preservation of Urine** information that was contained in Chapter 3 of the second edition, Collection and Physical Examination of Urine, into its own chapter. The previous edition only included one image for the collection and preservation information. The third edition contains seven additional images, including illustration of suprapubic puncture for the collection on urine directly from the bladder, collection containers, methods of transfer from collection container to urine testing tubes, and an assortment of urine preservative tubes. This chapter now contains eight images (seven new plus one reused as a chapter review question).
- **Chapter 7** pulls out the **Physical Examination of Urine** information that was contained in Chapter 3 of the second edition, Collection and Physical Examination of Urine, into its own chapter. The third edition contains images of abnormal urines, and an example of a "flow-through" set up for refractometry. This chapter now contains eight images (four new plus one reused as a chapter review question).
- **Chapter 8, Chemical Examination of Urine,** the third edition contains tables, chemical reactions, and images from the previous edition and clarifies procedures by enclosing this information in "boxes." In addition, urine reagent strip tests for ascorbate, calcium, creatinine, and microalbumin are included along with new images. This chapter now contains 23 images (12 new).
- **Chapter 9, *Microscopic Examination of Urinary Sediment*** continues to detail the observance of urinary sediment and includes 17 new images, some of rarely seen sediment. This chapter now contains 97 images.
- **Chapter 10, *Atlas of Urinary Sediment*** continues to be one of the most complete resources for urinary sediment

images. Most of the 190 images have been resized, while three have been replaced.

SECTION III BODY FLUIDS ANALYSIS

- **Chapter 11,** ***Introduction to Body Fluids***, along with its six images, is a basic overview of the types of fluids in the body, the formation of effusions and the methods for enumerating cells in these fluids.
- **Chapter 12,** ***Cerebrospinal Fluid*** contains a brief overview of the anatomy and physiology of CSF. Laboratory testing is detailed, and conditions affecting CSF test results are outlined. Eight new images have been added to this chapter for a total of 29.
- **Chapter 13,** ***Serous Body Fluids*** contains information about laboratory analysis of pleural, pericardial, and peritoneal fluids. Five new images have been added to this chapter for a total of 22.
- **Chapter 14,** ***Synovial Fluid*** contains a brief overview of the composition and function of synovial fluid. Laboratory analysis of synovial fluid is explained along with interpretation. This chapter contains 22 images.
- **Chapter 15,** ***Gastric Fluid and Fecal Analysis*** has been expanded to include gastric fluid information, which was not included in the second edition. An image of gastric fluid collection is included as well as an image for the testing of gastric fluid. An image has been added to the physiology section at the beginning of the chapter to provide students with an overview or review of the digestive system; and an additional image is added to the fecal analysis section. This chapter contains nine images (three new).
- **Chapter 16,** ***Semen Analysis*** provides information about the composition of semen and sperm formation, classification of semen by using various test parameters, and macroscopic and microscopic evaluation of semen. This chapter contains 27 images.
- **Chapter 17,** ***Amniotic Fluid*** contains information moved from the chapter on miscellaneous body fluids in the second edition into its own chapter. Three of this chapter's six images are new to this edition.
- **Chapter 18,** ***Vaginal Secretions*** contains information moved from the chapter on miscellaneous body fluids in the second edition into its own chapter and now covers the detection of fetal substances in vaginal secretions at the time of fetal membrane rupture prior to delivery. Six of this chapter's ten mages are new to this edition.
- **Chapter 19,** ***Miscellaneous Body Fluids***, is the most revised of all the chapters in the third edition. Sections from the second edition Chapter 14, Miscellaneous Body Fluids, that were removed and made into their own chapters include Amniotic Fluid and Vaginal Secretions. The information remaining from the previous Chapter 14, Urine Pregnancy Testing, and Bronchoalveolar Lavage and Bronchial Washings, is found in this chapter and includes six new images including an illustration to help students visualize the collection of bronchial fluid. New information not contained in the second edition, includes sections on Urine Eosinophils, Ear Fluidand Vitreous Fluid, along with three new images. The chapter now contains 18 images (10 new).
- **Chapter 20,** ***Automation of Urinalysis and Body Fluid Examination***, presents up-to-date information and images from several manufacturers of instrumentation for the analysis of urine and body fluids, including semen. In addition, 11 new images, screen shots from automated microscopic analysis of urine, are included for common urinary sediments. This chapter includes 32 images.

APPENDICES

- **Appendix A,** ***Answers to Study Guide Questions and Case Studies***, allows students to verify their comprehension of the material presented in the end of chapter questions.
- **Appendix B,** ***Reagent Strip Color Chart***, gives students to opportunity to view the color chart for manual interpretation of urine chemistry analysis prior to hands on laboratory sessions.
- **Appendix C,** ***Hemocytometer Cell Counts***, details manual cells counting principles. Seven new images are included to enhance understanding.
- **Appendix D,** ***Historically Relevant Urinalysis Information***, preserves methods used in the past as it provides for an understanding of the evolution of current methods. In addition, this appendix includes procedures that may still be performed by some laboratories.
- **Glossary,** now contains over 180 additional words. Watch for crossword puzzles to be made available on ThePoint.

ANCILLARY MATERIALS

Ancillary materials for users of *Graff's Textbook of Urinalysis and Body Fluid 3e* can be found online at thePoint.lww.com

- PowerPoint Presentations
- Test Generator
- Image Bank
- Quiz Show PowerPoints
- Flashcards
- Crossword Puzzles
- Additional Case Studies

About the Authors

Lillian A. Mundt, EdD, MLS(ASCP)SH, LMT (NCBMT) is a medical laboratory scientist, massage therapist, curriculum designer, and author. Her background includes a bachelor's degree in medical technology, a master's degree in health professions education, and a doctorate in educational leadership. For over 30 years, she has developed and taught phlebotomy programs, clinical laboratory science programs, and graduate programs at both hospital-based and university-based institutions. She has authored and developed course materials used in online education programs for colleges and universities, as well as online continuing education companies. In addition, Dr. Mundt has authored several articles for professional journals, and has co-authored books on urinalysis, phlebotomy, a book chapter in clinical chemistry, and has published her dissertation. Dr. Mundt has presented posters and lectures at local, state, and national conventions. Her current focus is on developing educational materials for medical laboratory science and health professions education. She remains professionally current maintaining licensure as a massage therapist, employment as a medical laboratory scientist at a hospital laboratory, and developing and teaching courses on a contractual basis.

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Acknowledgments

Thank you to Landy James McBride PhD, MT(ASCP), Professor Emeritus of Biology (1983–2002) California State University, Bakersfield, CA for content and images that comprise most of Chapter 5: Microscopy.

We thank our reviewers for their insightful suggestions for improving the third edition of this text. We greatly appreciate the expertise, cooperation, and patience of the publisher and editing managers who assisted us.

In addition, we thank the various manufacturers for images of their instruments.

Finally, we thank the Pathology Laboratory at Adventist Hinsdale Hospital and Adventist LaGrange Memorial Hospital, IL, for supplying specimens from which new images were created; and co-worker for her modeling of laboratory procedures.

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Urinary System Anatomy and Physiology and Urine Formation

Chapter 1

KEY TERMS

Adrenal Gland
Aldosterone
Angiotensin-Converting Enzyme
Angiotensin I and II
Antidiuretic Hormone (ADH) (Vasopressin)
Anuria
Azotemia
Blood Urea Nitrogen (BUN)
Bowman Capsule (Glomerular Capsule)
Carbonic Anhydrase
Collecting Ducts
Cortex
Countercurrent Multiplication
Creatinine Clearance
Cystitis
Cystatin C
Diabetes Insipidus
Distal Convoluted Tubule
Endogenous Substance
Estimated Glomerular Filtration Rate (EGFR)
Exogenous Substance
Glomerular Filtrate
Glomerular Filtration Rate (GFR)
Glomerulonephritis
Glomerulus (Renal Corpuscle)
Hilus
Juxtaglomerular Apparatus
Loop of Henle
Macula Densa Cells
Major Calyx
Medulla
Microalbumin
β_2-Microglobulin
Minor Calyx
Nephritis
Nephron
Nephrosis (Nephrotic Syndrome)
Oliguria
Osmolarity
Osmolar and Free Water Tests
Oval Fat Bodies
Peritubular Capillaries
Podocytes
Polyuria
Proximal Convoluted Tubule
Pyelonephritis
Reabsorption
Renal Columns
Renal Pelvis
Renal Pyramids
Renal Sinus
Renin
Secretion
Shield of Negativity
Syndrome of Inappropriate Antidiuretic Hormone (SIADH)
Threshold Substances
Ultrafiltrate
Uremia
Ureter
Urethra
Vasa Recta

LEARNING OBJECTIVES

1. List six major functions of the kidney and urinary system.
2. Sketch the urinary tract, labeling each of the four basic anatomical components.
3. Diagram the kidney and the structures it contains.
4. Identify the main functional unit of the kidney.
5. Identify the structures and components of the nephron.
6. Describe the functions of the glomerulus, the tubule, and the loop of Henle.
7. Sketch the structures of Bowman capsule and the glomerulus.
8. Summarize the blood flow through the kidney from the renal artery through the renal vein, including the glomerulus.
9. Describe the process of glomerular filtration and list what is filtered and what is not filtered from blood.
10. Discuss the glomerular filtration rate and how filtration is affected by blood flow and by the dilation and contraction of the afferent arteriole.
11. Describe what happens to the glomerular ultrafiltrate as it becomes the urine that is excreted.
12. Define renal threshold and countercurrent mechanism. State the renal threshold range for glucose.
13. Discuss the reabsorption process and what is reabsorbed.
14. Summarize the process of tubular secretion in the nephron.
15. Explain the role of the kidney in ion secretion and acid–base balance and identify the roles of (a) hydrogen ions, (b) bicarbonate ions, and (c) ammonium ions in accomplishing this balance.
16. Describe the process of formation of urine.
17. Describe the effect of each of the following and their effect on urine production: (a) aldosterone, (b) renin, and (c) vasopressin (antidiuretic hormone [ADH]).
18. List the major organic and inorganic constituents of urine.
19. List and sketch the three types of epithelial cells that can be found in a routine urinalysis, name their source, and explain their clinical significance.
20. List three areas of patient's renal function assessment.
21. Write the formula for the classic creatinine clearance and discuss its value.
22. List three factors that can affect classic creatinine clearance testing.
23. Name three methods used for calculating an estimated glomerular filtration rate (eGFR) and discuss their uses.
24. Evaluate the use of the cystatin C test.
25. Name two tests for assessing tubular secretion function.
26. Write the formula for the renal blood flow calculation and state the use of this calculation.

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The urinary system is composed of four main components: the kidney, where urine is formed from the filtration of blood; the ureters that carry the urine to the bladder; the bladder that stores the urine produced; and the urethra that delivers the urine for excretion outside the body (Figs. 1-1 to 1-4). The kidneys are paired organs that are located inside the small of the back. They are essential for maintaining homeostasis including the regulation of body fluids, acid–base balance, electrolyte balance, and the excretion of waste products. They are also concerned with the maintenance of blood pressure and erythropoiesis. Renal function is influenced by the blood volume, pressure, and composition, as well as by hormones from the adrenal and pituitary glands.

The importance of blood flow to the kidneys in the process of urine formation cannot be underestimated. One major function of the kidney and the urinary system is removal of waste products of metabolism, and toxins and drugs, from the circulatory system into the urine for excretion from the body. Without the proper blood volume and pressure, urine cannot be formed. The circulatory system is crucial for the retention of water and key organic molecules from the initial renal filtrate to prevent dehydration and loss of essential nutrients. The kidney and urinary system have additional critical functions; maintaining the proper water level (total water volume) for the body, regulating blood pressure, regulation of ion concentrations, the regulation of **osmolarity** (the amount of solute per unit of volume) of bodily fluids, and the release of hormones including erythropoietin, renin, and the active form of vitamin D.

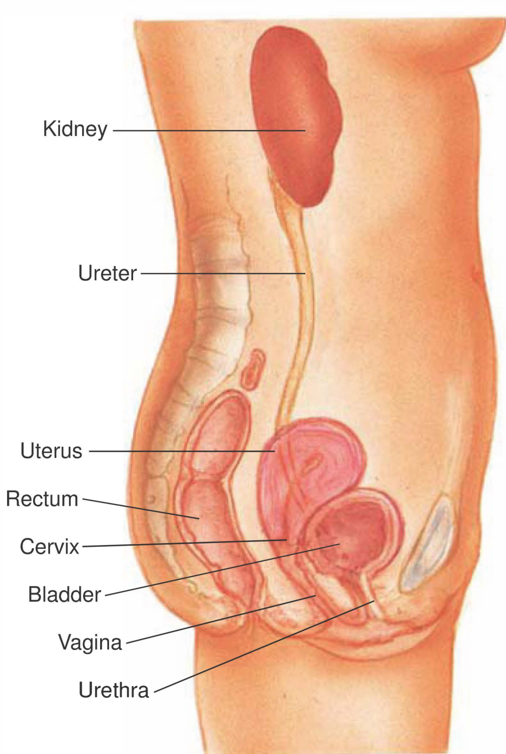


Figure 1-2. Female urinary tract—lateral view. (Adapted by permission from Anatomical Chart Company, Inc., Skokie, IL, USA.)

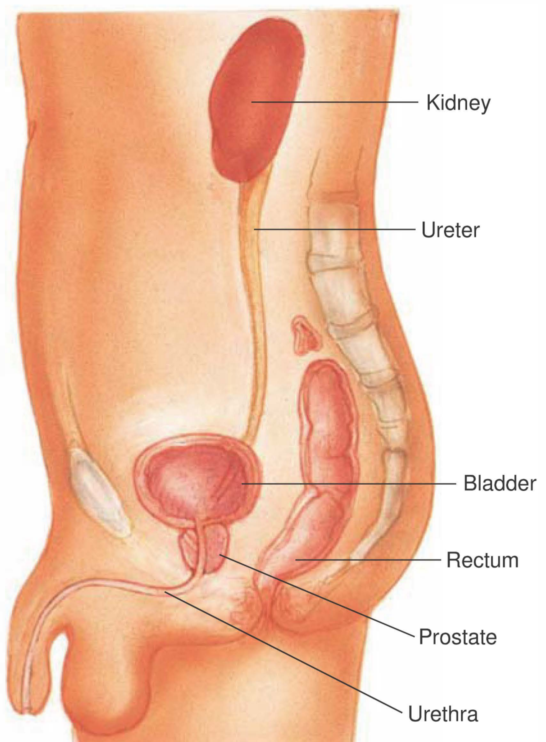


Figure 1-1. Male urinary tract—lateral view. (Adapted with permission from Anatomical Chart Company, Inc., Skokie, IL, USA.)

The formation of urine involves the complex processes of blood filtration, the **reabsorption** of essential substances including water, and the tubular **secretion** of certain substances. After formation in the kidney, the urine passes down the ureter into the bladder, where it is temporarily stored before being excreted through the **urethra**.

Renal Anatomy

The two kidneys are fist-sized organs situated near the middle of the posterior wall of the abdominal cavity, just below the rib cage, with one on each side of the vertebral column. Because of the anatomical location of the liver, the right kidney is slightly lower than the left kidney. The kidney is a bean-shaped organ and its medial border contains an indentation, the renal **hilus**, through which the renal artery enters the kidney and the renal vein and the **ureter** leave the kidney. Each kidney is covered by a capsule and capped with an **adrenal gland**, which is an endocrine gland (Figs. 1-5 and 1-6).

The kidney contains three internal regions: the **cortex**, the **medulla**, and the **renal pelvis**. The cortex is the outer layer of the kidney, located just below the renal capsule. Regions of the cortex, called **renal columns**, extend into the renal medulla or middle areas of the kidney. Blood vessels that supply the cortex

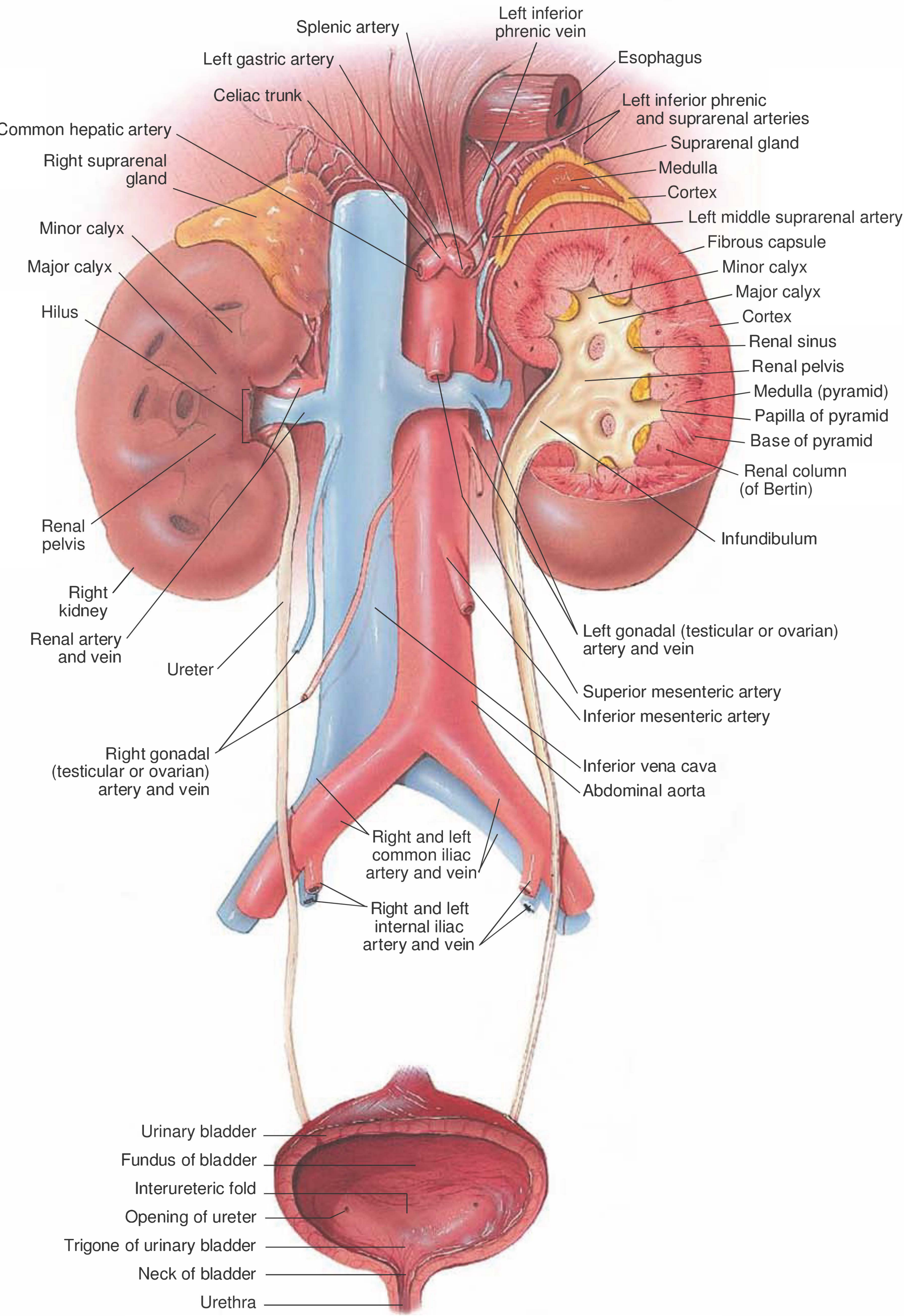


Figure 1-3. The urinary system. (Asset provided by Anatomical Chart Co.)

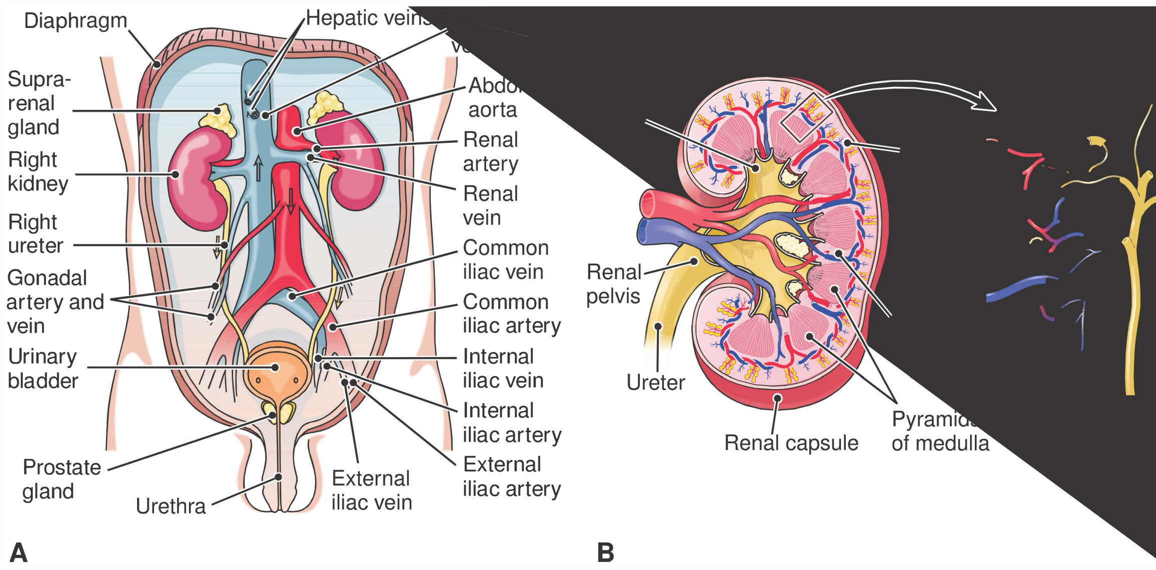


Figure 1-4. Structures of the urinary tract **(A)**, and cross-section of the kidney **(B)**.

and the medulla pass through the renal columns. Also within the medulla are the triangular **renal pyramids**, located between the renal columns. The tips of the renal pyramids, the papillae, project into a funnel-shaped space, a **minor calyx**, and several minor calyces join together to form a **major calyx**. The major calyces join with one another to form the renal pelvis, which is an expansion of the upper ureter. The hilus opens into this space, the **renal sinus**, in which the renal pelvis and the renal blood vessels are located.

The renal cortex and medulla contain the renal tubules, which include the nephrons tubules and the **collecting ducts**. There are approximately 1 million or slightly more nephrons in each kidney. The nephron is the main functional unit of the kidney.

Within the cortex of the kidney, the cells of the afferent arteriole make contact with the **macula densa cells** of the distal tubule to form the **juxtaglomerular apparatus**. The juxtaglomerular apparatus and the macula densa cells of the distal convoluted

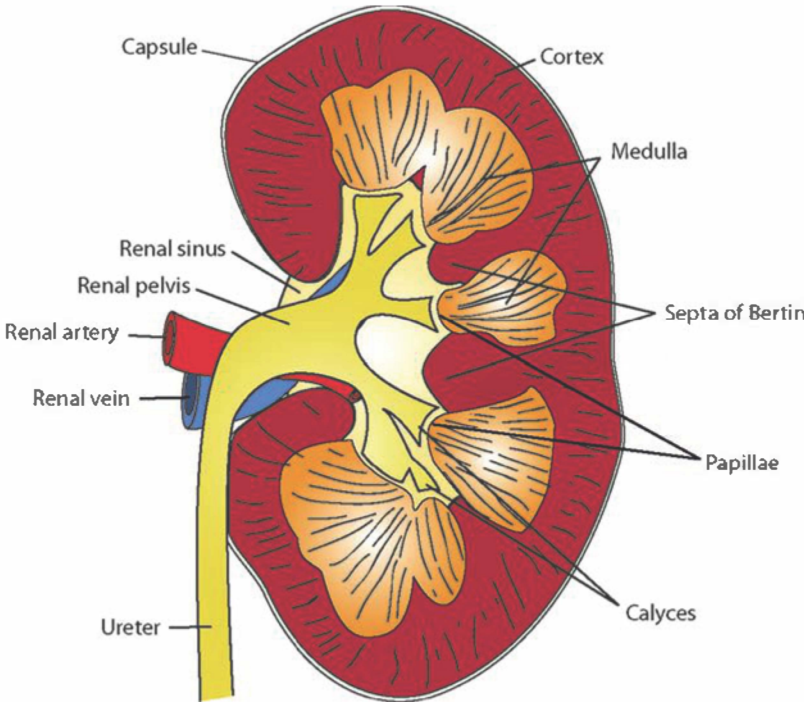


Figure 1-5. The kidney.

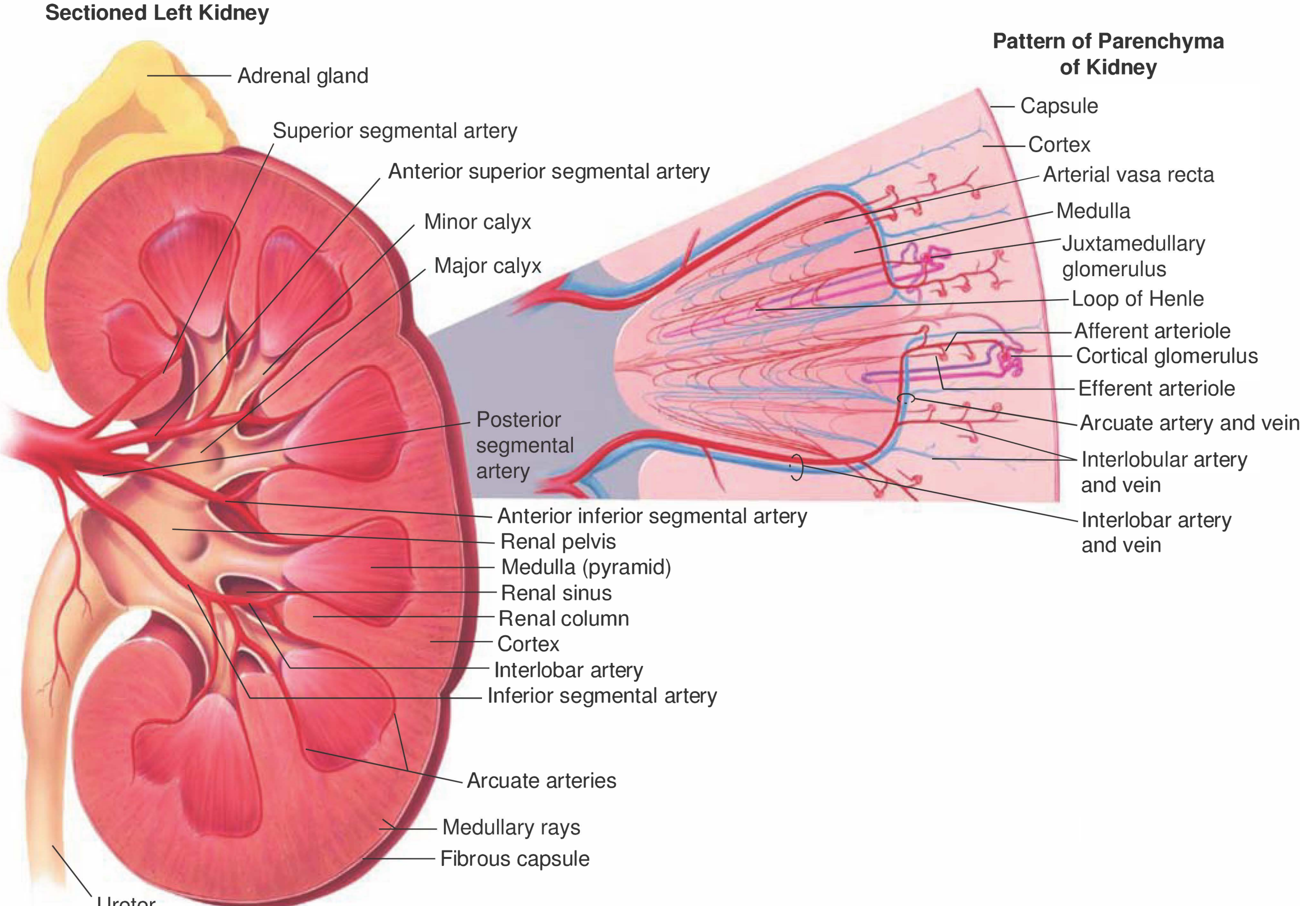


Figure 1-6. The sectioned left kidney.

tubule maintain the blood pressure at a relatively constant rate regardless of fluctuations in the systemic blood pressure through regulation of the dilation and constriction of the afferent arteriole. **Renin**, an enzyme produced by the juxtaglomerular cells, is secreted and reacts with the precursor angiotensinogen in the blood to produce **angiotensin I**. Angiotensin I passes through the lungs where the enzyme **angiotensin-converting enzyme** changes it to the active **angiotensin II**. Angiotensin II corrects renal blood flow (RBF) by dilating the afferent arteriole and constricting the efferent arteriole, by stimulating sodium reabsorption in the proximal convoluted tubule, and by triggering the release of the hormone **aldosterone** from the adrenal gland and **antidiuretic hormone** (**ADH**, and also known as **vasopressin**) from the pituitary gland.

ANATOMY AND PHYSIOLOGY OF THE NEPHRON

The **nephron** (Fig. 1-7) is the functional unit of the kidney and there are approximately 1 million or slightly more nephrons in each kidney. The nephron consists of a capillary network, called the **glomerulus** (also known as the **renal corpuscle**), and a long tubule which is divided into three parts: the **proximal convoluted tubule**, the **loop of Henle**, and the **distal convoluted tubule**. Each nephron empties into a larger collecting tubule to which other nephrons are connected. Nephrons that are located mostly within the cortex alone are referred to as cortical nephrons. Nephrons that extend deep into the medulla are called juxtamedullary nephrons. Each nephron consists of two major parts: a glomerulus and a tubule. Various regions of the nephron differ from one another anatomically and consist of differing types of epithelium corresponding to differing functions.

The urine then collects in the renal pelvis and empties into the ureter. The glomerulus and the convoluted tubules are located in the cortex of the kidney, while the loop of Henle extends down into the medulla (Fig. 1-8).

RENAL BLOOD FLOW AND THE GLOMERULUS

The kidneys receive a continuous large blood flow. Approximately 20% to 25% of the blood that leaves the left ventricle of the heart enters the kidneys by way of the renal arteries (Fig. 1-9). This means that in a normal adult the blood passes through the kidneys at a rate of about 1,200 mL/min, or

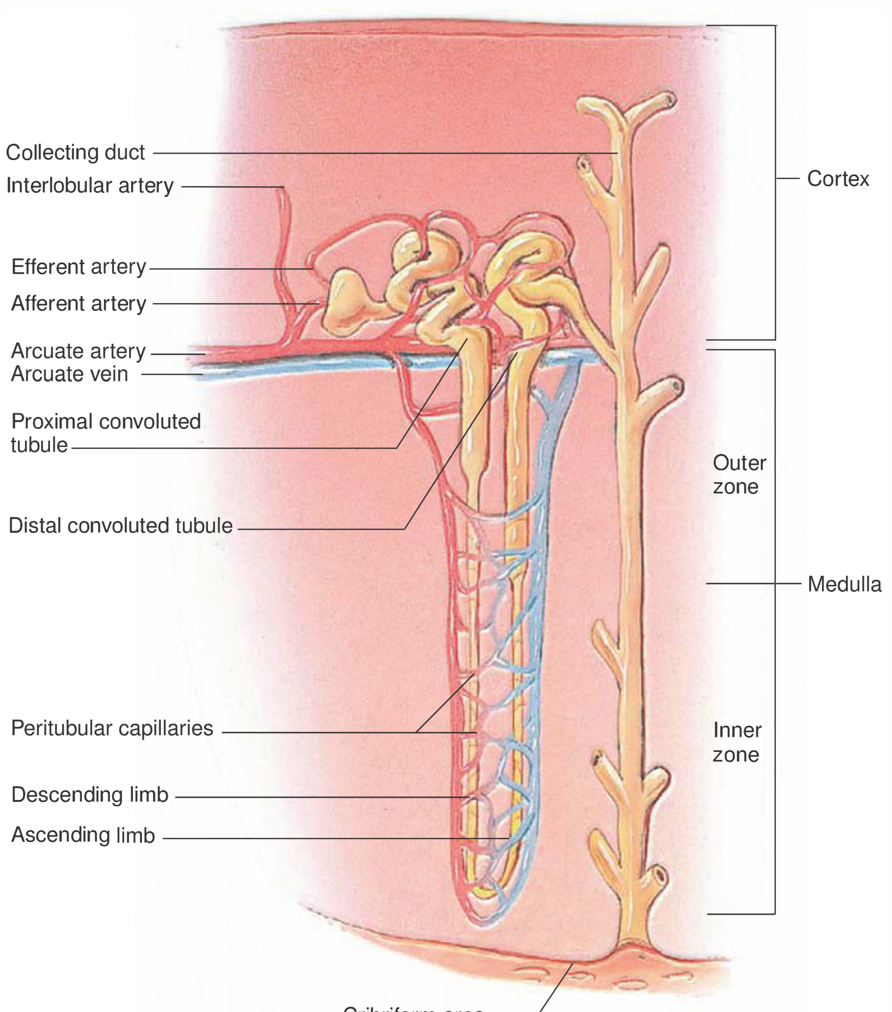


Figure 1-7. The nephron. (Asset provided by Anatomical Chart Co.)

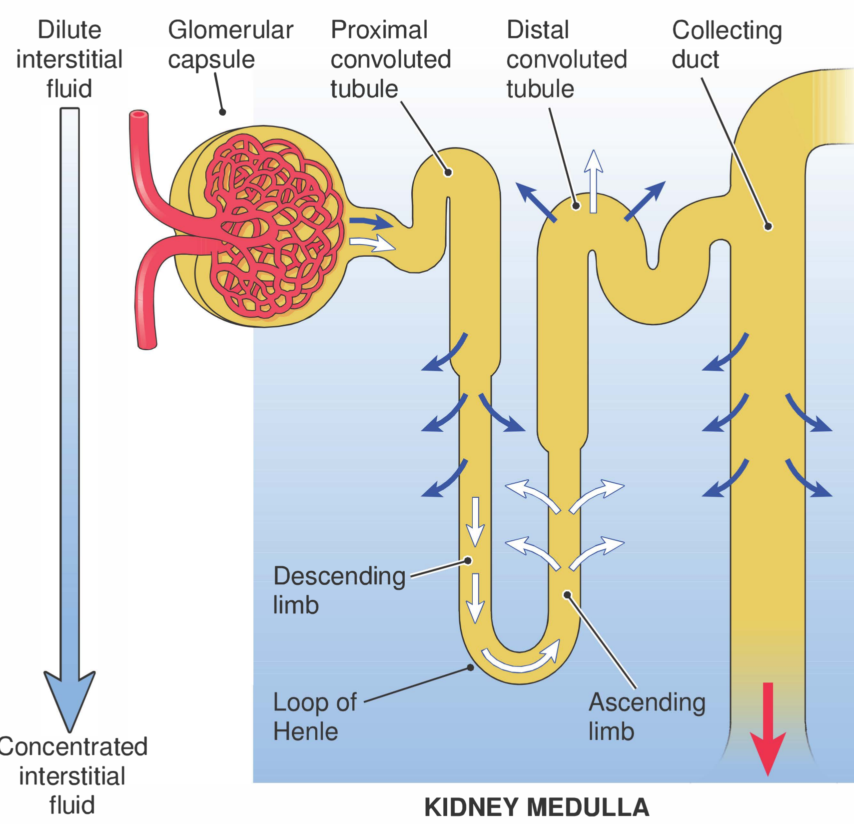
600 mL/min/kidney. After the renal artery enters the kidney it breaks up into smaller branches until thousands of tiny arterioles are formed (Fig. 1-10). These arterioles are called afferent arterioles because they carry the blood to the nephrons. Each afferent arteriole then forms the capillary network of a glomerulus. The glomerulus is unique in being a capillary tuft located between the two arterioles rather than between an arteriole and a venule. The glomerulus is surrounded by a structure called the **Bowman capsule (glomerular capsule)**, and the space that is formed between the capsule and the glomerulus is the Bowman space (Figs. 1-11 and 1-12).

The outer (parietal) layer of Bowman capsule is composed of squamous epithelium. This epithelial layer rests on a thin basal lamina. The inside (or visceral) layer of Bowman capsule is composed of specialized cells known as **podocytes**. The podocytes have several extending processes that adhere to a basement membrane covering the fenestrated, squamous endothelium of the glomerular capillaries (Fig. 1-12). In addition, the endothelial cells have a negative charge, referred to as the **shield of negativity**, which serves to repel most plasma proteins to prevent their loss from the blood. The extending podocyte processes form an elaborate network of small slits between them, called filtration slits. Together, these layers form a filtration barrier for filtering the blood and creating the ultrafiltrate.

As a result of its special structure, the glomerular wall acts as an ultrafilter which is very permeable to water. The pressure of the blood within the glomerulus forces water and dissolved solutes with a molecular weight of less than 50,000 through the semipermeable capillary membrane and into the Bowman space. The remainder of the blood including blood cells, plasma proteins, and large molecules, leaves the glomerulus via the efferent arteriole and enters a second capillary network, called the **peritubular capillaries**, which surrounds the tubules.

The Formation of Urine

Approximately 120 mL/min, or one-fifth, of the renal plasma is filtered through the glomeruli forming what is known as the **ultrafiltrate**, which is further processed as it travels



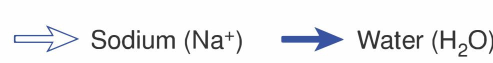


Figure 1-8. In this view the nephron has been stretched out and the surrounding blood vessels removed to illustrate the different sections of the tubule. The reabsorption of sodium and water is indicated. (From Cohen BJ, Taylor JJ. (2005). *Memmler's The Human Body in Health and Disease*, 10th ed. Baltimore, MA: Lippincott Williams & Wilkins.)

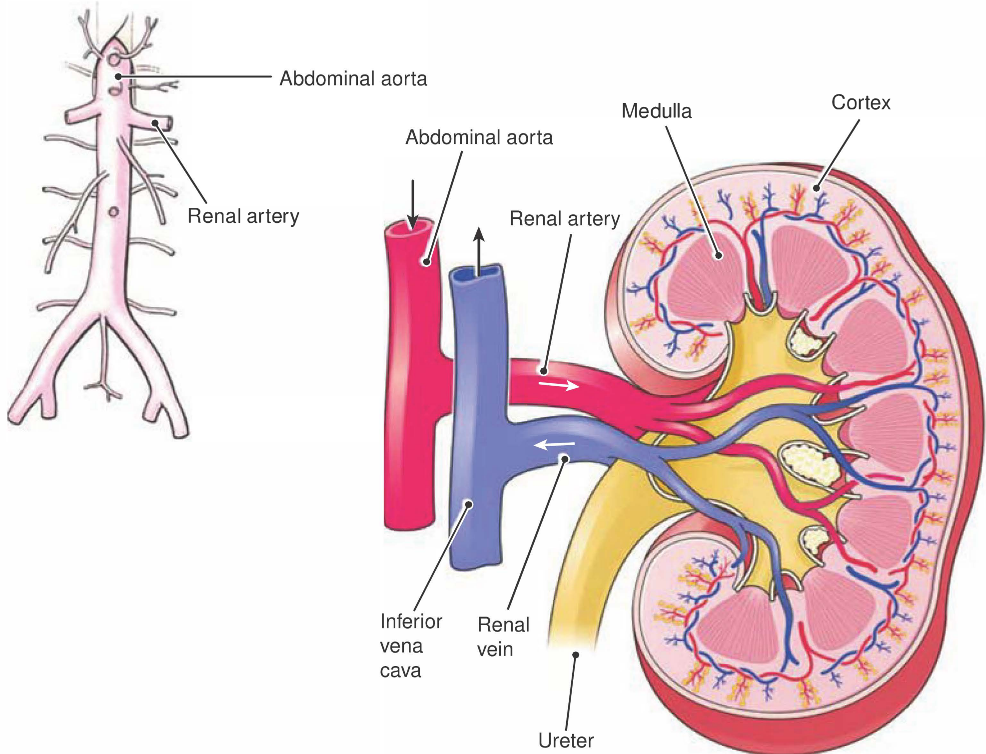


Figure 1-9. Renal arterial and venous blood flow through the kidney.

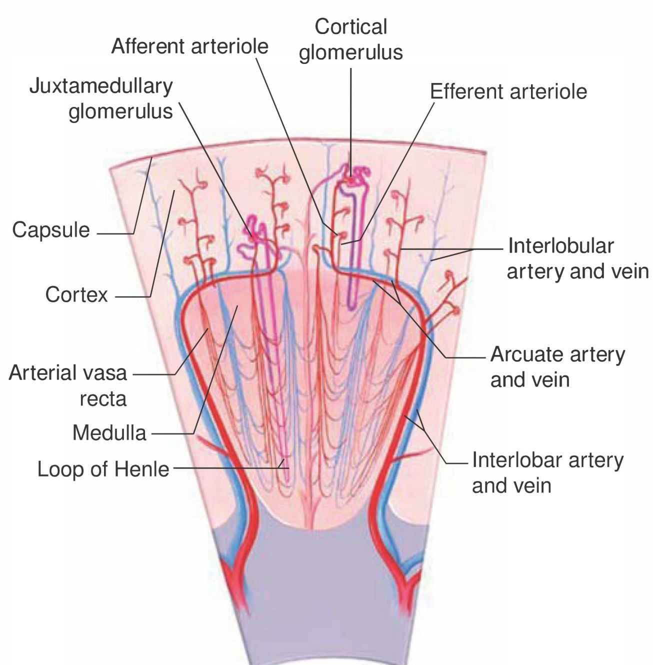


Figure 1-10. The renal pyramid with corresponding blood vessels. (Asset provided by Anatomical Chart Co.)

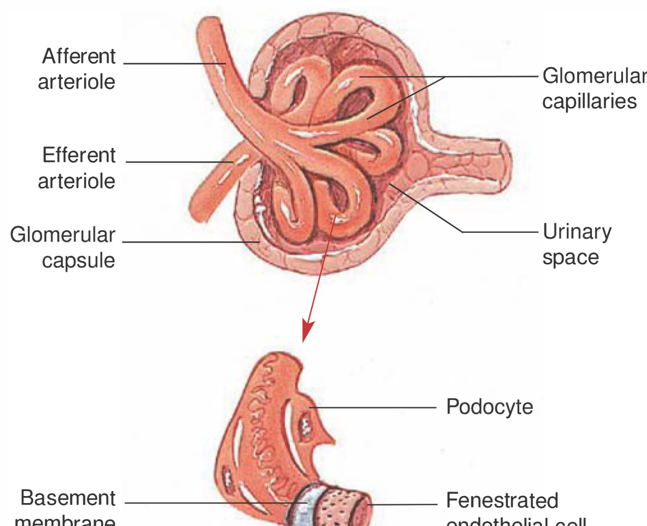


Figure 1-12. The glomerulus (renal corpuscle) and the filtration barrier of the glomerulus. (Asset provided by Anatomical Chart Co.)

through the nephron (Figs. 1-13 and 1-14). The ultrafiltrate has the same composition as blood plasma but it is normally free of protein except for about 10 mg/dL of low–molecular-weight protein. Some of the filtered substances from the glomerular filtration process include: water, glucose, electrolytes, amino acids and the nitrogen containing metabolic waste products, urea, uric acid, creatinine, and ammonia. Impaired glomerular filtration results in increased blood levels of these nitrogenous waste products. Nitrogenous waste products are frequently measured to assess kidney function. For example, the **blood urea nitrogen level, (BUN)**, is used to assess kidney function. Elevation of urea in the blood is referred to as **azotemia** and blood urea elevation with accompanying pathological manifestations is referred to as **uremia**. Additional glomerular function tests are also used to assess renal function.

The rate of filtration, the **glomerular filtration rate (GFR)**, is proportional to the body size and thus varies with age and sex. The GFR is an important indicator of renal function and is used to monitor kidney disease progression. It can be calculated with classic clearance tests or more frequently through a calculated **estimated glomerular filtration rate (eGFR)**. Classic clearance tests require collection of a 24-hour urine sample along with a blood sample. For earlier recognition of chronic kidney disease (CKD), it is strongly recommended that clinical laboratories automatically report an **eGFR**, along with values for serum creatinine, whenever serum creatinine is measured. The clinical laboratory

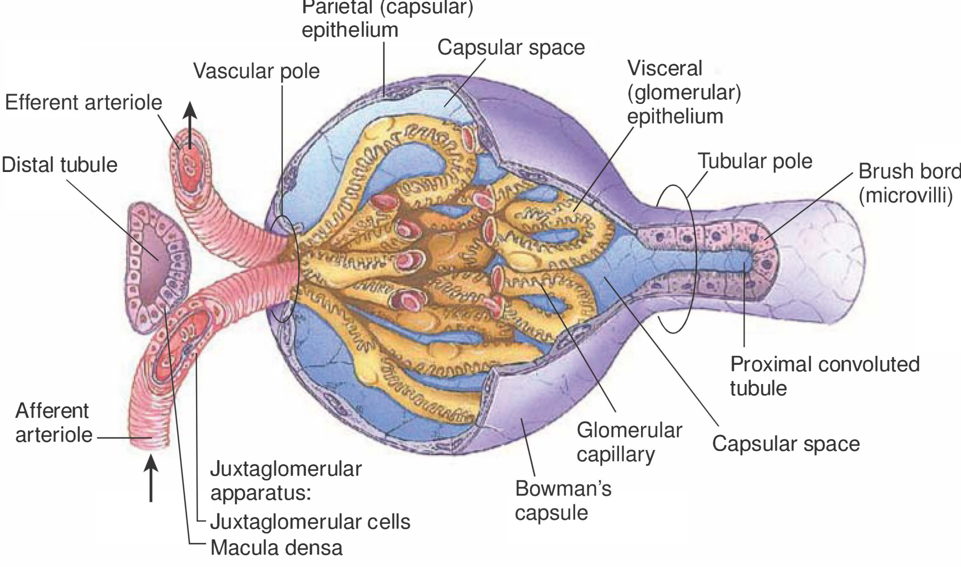


Figure 1-11. Bowman capsule, the glomerular tuft, and the juxtaglomerular apparatus. (From Gartner H. *Color Atlas of Histology*, 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2001.)

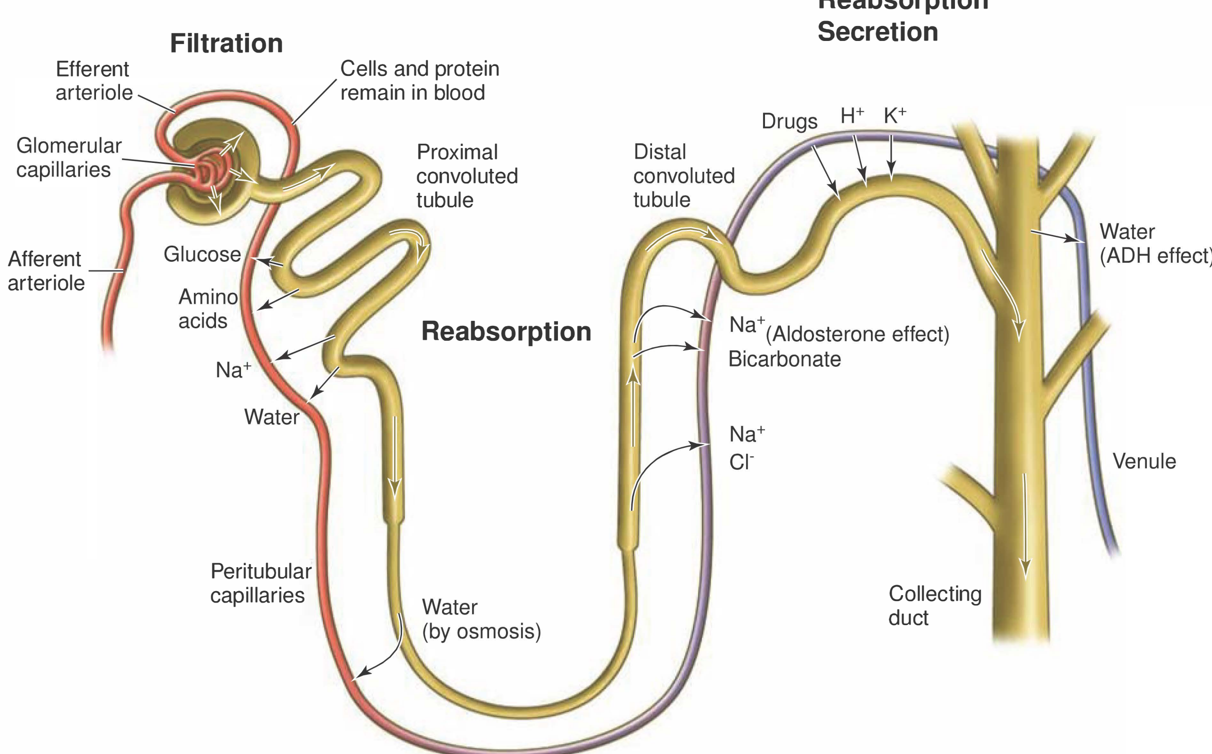


Figure 1-13 Formation of urine via filtration, reabsorption, secretion, and hormonal effects. (From Premkumar K. *The Massage Connection Anatomy and Physiology*. Baltimore, MA: Lippincott Williams & Wilkins, 2004.)

reported eGFR should be based on the abbreviated Modification of Diet in Renal Disease study equation that adjusts for body surface area without requiring measurement of height, weight, or the need for a 24-hour urine collection. The recommended formula is:

Modification of Diet in Renal Disease Formula

$$\text{GFR (mL/min/1.73 m}^2) = 175 \times (S_{cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$$

TUBULAR REABSORPTION

As the ultrafiltrate, also known as the **glomerular filtrate**, passes through the proximal tubules, a large portion of the water, sodium chloride, bicarbonate, potassium, calcium, amino acids, phosphate, protein, glucose, and other threshold substances needed by the body are reabsorbed and pass back into the bloodstream. These substances are reabsorbed in varying proportions so that while proteins and glucose, for example, appear to be almost completely reabsorbed, sodium chloride

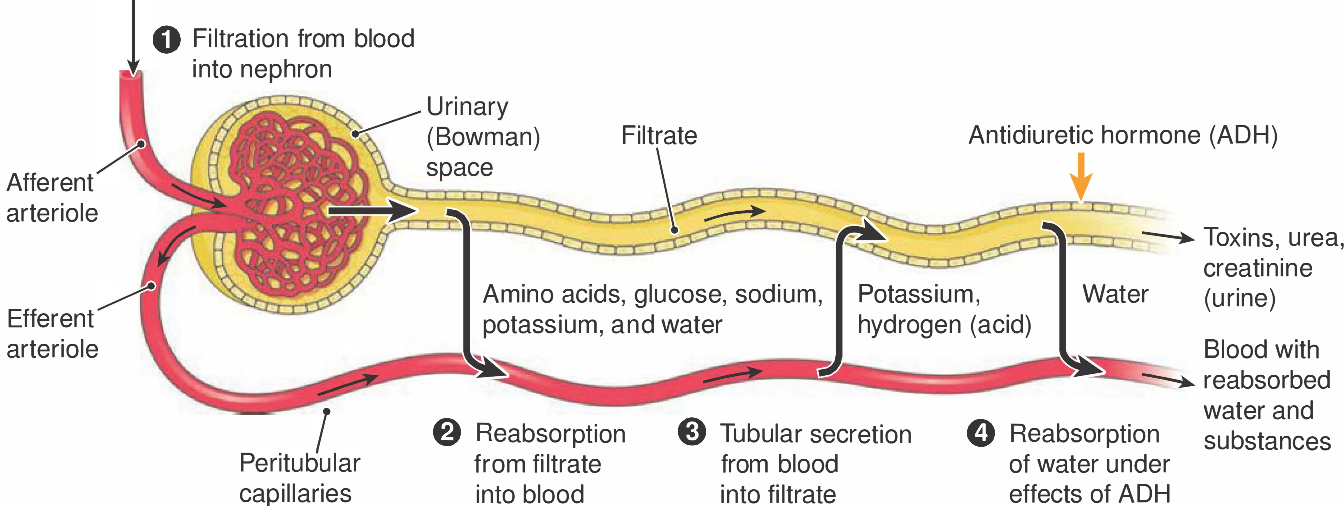


Figure 1-14. Filtration and tubular processing of the glomerular ultrafiltrate.

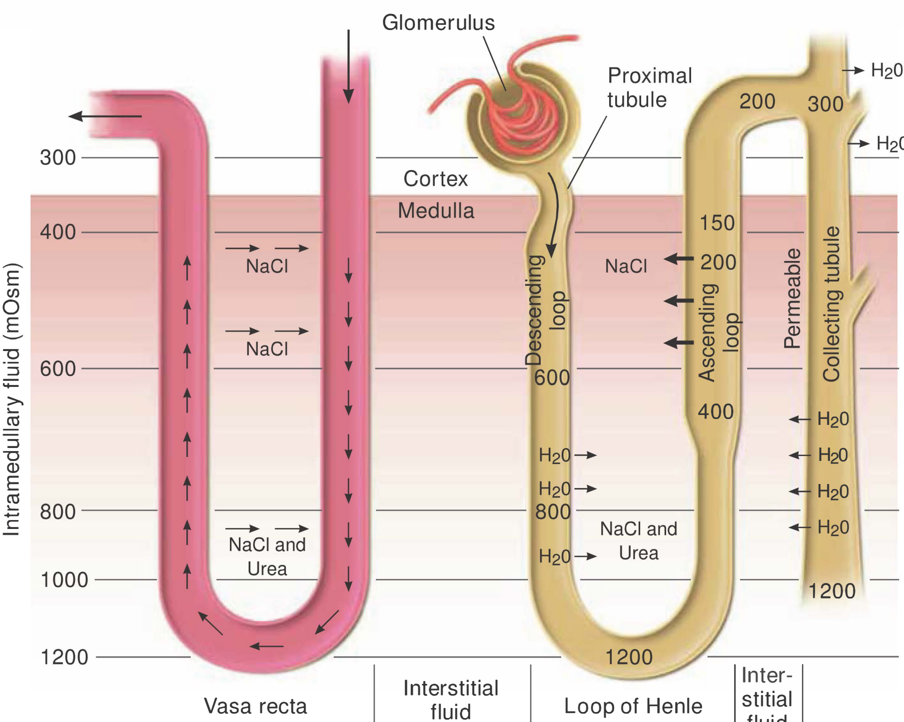


Figure 1-15. The countercurrent mechanism and antidiuretic hormone in urine concentration. (From Premkumar K. *The Massage Connection Anatomy and Physiology*. Baltimore, MA: Lippincott Williams & Wilkins, 2004.)

is only partly reabsorbed, and there is no reabsorption of creatinine. More than 80% of the filtrate is reabsorbed in the proximal tubule. The unique structure of the proximal tubule makes this reabsorption possible. The epithelial cells that line this portion of the tubule have a brush border of microvilli which provides a large surface area for reabsorption and secretion. These microvilli contain various enzymes such as carbonic anhydrase which help in this process.

Threshold substances are those substances which are almost completely reabsorbed by the renal tubules when their concentration in the plasma is within normal limits. When the normal plasma level is exceeded, the substance is no longer totally reabsorbed and therefore appears in the urine. Glucose is a high threshold substance because it usually does not appear in the urine until the plasma concentration exceeds about 160 to 180 mg/dL. Some of the other threshold substances include sodium chloride, amino acids, potassium, creatine, and ascorbic acid. As the filtrate moves through the tubules, various substances are added to it by the process of tubular secretion. In the proximal tubule, sulfates, glucuronides, hippurates, hydrogen ions, and drugs such as penicillin are some of the substances which are secreted. In the proximal as well as the distal tubule, the hydrogen ions are exchanged for the sodium ions of sodium bicarbonate. The hydrogen ions then combine with the bicarbonate in the filtrate to form carbonic acid which in the presence of **carbonic anhydrase** breaks down to water and carbon dioxide. The carbon dioxide then diffuses back out of the tubule, and thus, both the sodium and bicarbonate are reabsorbed.

Like the proximal tubule, the descending limb of the loop of Henle is very permeable to water, but the resorption of solutes does not occur in this part of the loop. The ascending limb, however, is nearly impermeable to water, but there is active resorption of sodium, chloride, calcium, and magnesium. Because of the loss of sodium chloride, the fluid that leaves the loop of Henle has a lower osmolality than plasma. In this section of the tubule and in the remaining tubule, hydrogen ion and ammonia are secreted. The mechanism that provides for the absorption of water from the descending loop, and the resorption of solute without water in the ascending limb, is called **countercurrent multiplication** (Fig. 1-15). There is a set of blood vessels called the **vasa recta** that is parallel to and shaped the same as loop of Henle. In the vasa recta, solutes diffuse out of the interstitium of the medulla and into the ascending limb and then out of the ascending limb back into the interstitium. Water, however, moves in the opposite direction or out of the descending limb and back into the ascending one. The net effect is to retain only solute, and not water, in the interstitium of the medulla. This process coupled with the resorption of solute from the ascending loop of Henle results in an interstitium which is hypertonic, thus, causing water to be absorbed from the descending loop and the collecting tubule. About 90% of the glomerular filtrate is reabsorbed by the time it reaches the distal tubule. Urea is also reabsorbed in the collecting duct. Some reabsorption is passive and some requires energy for active transport across cells.

TUBULAR SECRETION

In contrast to reabsorption, which removes substances from the tubules for retention by the body, tubular secretion involves

sending molecules from the blood in the peritubular capillaries into the tubular filtrate for excretion. The tubular secretion process (a) removes unneeded foreign waste substances that are not filtered by the glomerulus including various medications and toxins and (b) promotes secretion of hydrogen ions and other ions to help regulate acid–base and electrolyte balance. Medications and foreign substances are often bound to carrier proteins and thus cannot be removed from the circulation during glomerular filtration. To be removed from the circulation by the body, these foreign substances develop a higher affinity for cells of the proximal convoluted tubule than for their carrier molecules and are then transported across the tubular cells into the tubular filtrate. Various ions are also secreted including hydrogen ions, ammonium ions, sodium ions, potassium ions, bicarbonate ions, uric acid, and some weak acids and bases. Much of this activity requires active transport by cells and expenditure of energy.

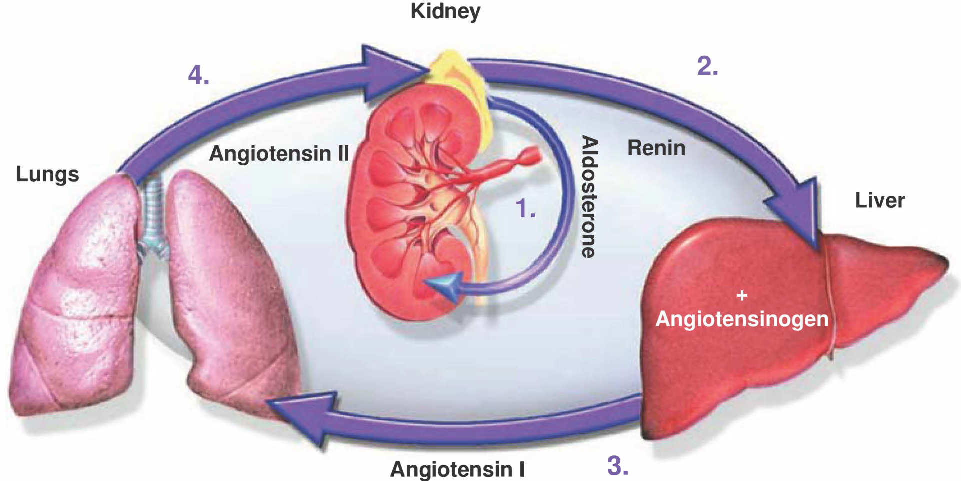
The Kidney's Role in Ion Secretion and Acid–Base Balance

An important kidney function is acid regulation. As cells metabolize, they continually produce acids. Foods that we eat can increase the acid in our body. The kidneys and the lungs have the crucial role of elimination of acid and regulation of acid–base balance. In the kidney, three secretory mechanisms play a key role in maintaining blood pH homeostasis. These three mechanisms each rely directly or indirectly on tubular secretion of acid as hydrogen ions (H^+ ions) and some on the secretion or reabsorption of alkali as bicarbonate ion (HCO_3^-). The mechanisms are (a) in acidotic blood conditions, H^+ ions are secreted in exchange for sodium and bicarbonate ions, (b) also in acidotic conditions, ammonia diffuses into the tubular lumen and subsequently sodium ions are reabsorbed while ammonium ions are excreted, and (c) in alkalotic blood conditions, tubular secretion of H^+ is minimized and additional bicarbonate is secreted from the body. The ammonia that is secreted combines with hydrogen ions to form ammonium ions ($NH_3^+ + H^+NH_4^+$) in the tubular lumen and this helps regulate the hydrogen ion (H^+) concentration of the urine. Hydrogen ions are produced as waste from metabolism and are generally secreted. Bicarbonate can also be secreted but is more often reabsorbed (usually up to 100%) to help maintain the proper blood pH.

The main function of the distal and collecting tubules is the adjustment of the pH, osmolality, and electrolyte content of the urine as well as the regulation of those substances still present in the filtrate. Potassium, ammonia, and hydrogen ions are secreted by this portion of the nephron, while sodium and bicarbonate are reabsorbed by the same mechanism as in the proximal tubule.

HORMONAL EFFECTS ON THE KIDNEY AND ON URINE PRODUCTION

Potassium ions are also exchanged for sodium ions, and this exchange is enhanced by aldosterone which is secreted by the adrenal cortex. Aldosterone increases blood sodium, which in turn increases body water as water follows salt, raising blood pressure. Aldosterone release is also triggered by angiotensin II as described above. The release of aldosterone via the angiotensin route contributes to hypertension and this process is the target of hypertensive therapy (Fig. 1-16). The absorption of water in the distal portion of the nephron is regulated by ADH that is secreted by the pituitary gland. When the body needs to conserve water, ADH is secreted, and the walls of the distal and collecting tubules are made



Mechanism of renovascular hypertension
1. Renal artery stenosis causes reduction of blood flow to kidneys.
2. Kidneys secrete renin in response.
3. Renin combines with angiotensinogen in the liver to form angiotensin I.
4. In the lungs, angiotensin I is converted to angiotensin II, a vasoconstrictor.

Figure 1-16. The renin–angiotensin–aldosterone cycle and hypertension. (Asset provided by Anatomical Chart Co.)

very permeable by ADH, thereby allowing water to be reabsorbed. If the body has excess water, less ADH is produced, the walls of the tubules become less permeable, and the volume of excreted urine increases. Insufficient ADH results in **diabetes insipidus**. The excretion of ADH when it is not needed is referred to as the **syndrome of inappropriate antidiuretic hormone (SIADH)**. SIADH can be a complication of brain injury, pneumonia, tumor growth, and certain medications. SIADH is a condition of continued ADH secretion in spite of plasma hypotonicity and a normal or expanded plasma volume that results in high plasma volume, low serum osmolarity, high urine osmolarity, low plasma sodium, and higher than normal urine sodium.

FINAL URINE VOLUME

Of the approximate 120 mL/min that was filtered at the glomerulus, only an average of 1 mL/min is finally excreted as urine. This quantity can range from 0.3 mL in dehydration to 15 mL in excessive hydration. For an adult the normal average daily volume of urine is about 1,200 to 1,500 mL, with more urine produced during the day than at night. However, the normal range may be from 600 to 2,000 mL/24 hr. **Polyuria** is an abnormal increase in the volume of urine (>2,500 mL), as in diabetes insipidus and diabetes mellitus. **Oliguria** is a decrease in urinary volume, as occurs in shock and acute nephritis. In an adult it is frequently defined as being <500 mL/24 hr or <300 mL/m^2/24 hr. The term **anuria** designates the complete suppression of urine formation, although in the wider sense of the term it is sometimes defined as being <100 mL/24 hr during 2 to 3 consecutive days, in spite of a high fluid intake.

FINAL URINE COMPOSITION

The main constituents of urine are water, urea, uric acid, creatinine, sodium, potassium, chloride, calcium, magnesium, phosphates, sulfates, and ammonia. In 24 hours the body excretes approximately 60 g of dissolved material, half of which is urea. In some pathologic conditions, certain substances, such as ketone bodies, protein, glucose, porphyrins, and bilirubin, appear in large quantities. For additional information on physical and chemical assessment of urine, see Chapters 7 and 8.

Urine can also contain structures such as casts, crystals, blood cells, and epithelial cells. Some of these are considered to be normal, while others are seen in various renal and metabolic disorders as described in the Chapter “9. Three categories of epithelial cells found in urine are squamous epithelial cells, urothelial (transitional) epithelial cells, and renal tubular epithelial cells. Squamous epithelial cells line the urethra and the vagina of the female and the distal portion of the urethra of males. Squamous epithelial cells are the most common and the largest number of the cells seen in urine. Squamous epithelial cells are generally found in urine due to vaginal contamination. Urothelial cells line the renal calyces, the renal pelvis, the ureters, the bladder, and in the male, much of the urethra. An occasional urothelial cell, also known as a transitional cell, can be seen in normal patients or after catheterization. Increased numbers of urothelial cells are seen in urinary tract infections and in transitional cell carcinoma. Urothelial cells vary greatly in size depending upon where in the urinary tract they arise. As described earlier, each part of the renal tubule is lined with a single layer of characteristically differing epithelial cells, called renal tubular epithelial cells. An occasional renal tubular epithelial cell might be seen in a healthy individual. Renal tubular epithelial cells may be seen in increased numbers or seen in fragments or casts of several cells in acute tubular ischemic, toxic renal tubular disease, or in tubular necrosis. In nephrotic syndrome, these cells absorb and become engorged with fat. These cells, filled with lipids, are also known as **oval fat bodies** (Fig. 1-17). These and other renal and metabolic disorders are discussed in the Chapters 2 and 3.

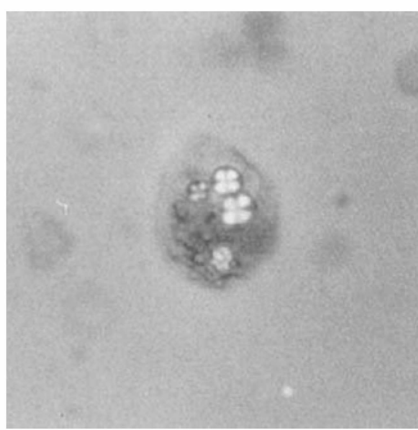


Figure 1-17. Renal cell becoming an oval fat body. Note the Maltese-cross formations inside the cell, indicating the lipid droplets contained in this cell. Eventually, the cell will become unrecognizable as a cell as it takes on more lipids, and it will become an oval fat body.

Some of the renal disorders that a urinalysis can help in diagnosing include **cystitis**, which is the inflammation of the bladder; **nephritis**, which is the inflammation of the kidney and can be present either with bacterial infection **(pyelonephritis)** or without infection **(glomerulonephritis)**; and **nephrosis (nephrotic syndrome)**, which is the degeneration of the kidney without inflammation. These and other renal and metabolic disorders are discussed in the Chapters 2 and 3.

Assessing Renal Function

Testing of a patient’s renal function can involve assessment in any of these areas: glomerular filtration, reabsorption or concentrating ability of the tubules, and secretion from the vasa recta into the tubule. These tests can be helpful in monitoring many renal diseases and in monitoring diabetics to assess the state of their kidney function.

ASSESSING GLOMERULAR FILTRATION RATE USING CLASSICAL CREATININE CLEARANCE TESTING

Assessment of glomerular filtration is critical to assessing renal damage in renal disease, diabetes, and various immunological conditions. Many substances have been used to assess filtration. Some substances used are endogenous (normally found in the body), such as creatinine and urea, and some substances used are exogenous (not normally found in the body) to the body, such as inulin. **Endogenous substances** are generally preferred for assessing the GFR. Creatinine is preferred over urea as about 40% of urea is reabsorbed rather than just being filtered and excreted as is creatinine. Various methods are used to assess glomerular filtration, including use of serum creatinine levels, classic creatinine clearance methods, estimated creatinine clearance methods, and **cystatin C** measurements.

The Classic Creatinine Clearance Test

Whatever substance is used (usually creatinine), the following classic clearance formula can be used to assess the GFR:

$$C\ (\text{mL/min}) = U\ (\text{mg/dL}) \times V\ (\text{mL/min}) / P\ (\text{mg/dL})$$

C = clearance of substance in milliliters per minute
U = urine concentration of substance being testing in milligrams per deciliter
P = plasma concentration of substance being testing in milligrams per deciliter
V = volume of urine excreted in a timed collection (usually 24 hours)

Note: The above formula is used for an adult with an average body surface area, 1.73 m^2. For a small adult, or for a child, or for a more accurate calculation, you would multiply the equation by 1.73 m^2/Patient body surface area, as shown below.

$$C\ (\text{mL/min}) = [\,U\ (\text{mg/dL}) \times V\ (\text{mL/min}) / P\ (\text{mg/dL})] \times [1.73\ \text{m}^2/\text{Patient body surface area}]$$

Obviously, since creatinine is so linked to muscle function, muscle mass is an important factor in **creatinine clearance**. This must be accounted for in children or patients with muscle wasting disease. Also, males and females have different normal ranges due to muscle mass and size differences. Both a blood sample and a timed (usually 24 hours) urine collection are needed for the traditional clearance test. The clearance tests are more prone to error due to the need for accurate timing in the collection of both blood and 24-hour urine collection specimens, but done properly they can be helpful in some situations.

CALCULATION METHODS TO ESTIMATE THE GLOMERULAR FILTRATION RATE

Review earlier in this chapter and in the Chapter 2. The National Kidney Disease Education Program and the National Kidney Foundation recommend that an eGFR be calculated routinely when a serum creatinine level is reported. This is a practical method to detect and monitor kidney disease. There are a variety of methods to perform this estimated rate calculation, depending upon the intended use for the information and upon individual patient factors. Some of the commonly used formulas are given in the following descriptions.

Cockcroft–Gault Formula

The older and most commonly used equation for estimating creatinine clearance is the Cockcroft–Gault formula. The Cockcroft–Gault calculation is preferred for calculating drug dosages.

$$\text{GFR (mL/min)} = [(140 - \text{age}) \times \text{Weight (kg)}/72 \times S_{cr}\ (\text{mg/mL})] \times (0.85\ \text{if female})$$

S_{cr} is serum creatinine in µmol/L.

GFR calculated by this equation correlates well with measured GFR when renal function is within the reference interval, but as renal function declines, it overestimates GFR because creatinine is removed not only by glomerular filtration but also by renal tubular secretion. When GFR is low, tubular secretion becomes an important factor.

Modification of Diet in Renal Disease Formula

Another formula, the MDRD (Modification of Diet in Renal Disease) equation, is the most widely used to estimate GFR and is used by clinical laboratories for eGFR calculations.

$$\text{GFR (mL/min/1.73 m}^2) = 175 \times (S_{cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742\ \text{if female}) \times (1.212\ \text{if African American})$$

S_{cr} is serum creatinine in µmol/L.

The MDRD study equation is the most thoroughly validated equation. It too has limitations, and is known to underestimate GFR values in healthy patients that have GFR values over 60 mL/min and underestimate the GFR in patients that are underweight. As with all clearance methods, the formula is only useful when renal function is stable. This formula is not recommended for those younger than 18 years, or those with extremes in muscle mass or diet (e.g., taking creatine dietary supplements).

The Chronic Kidney Disease Epidemiology Collaboration Formula

The CKD epidemiology collaboration formula was developed in 2009. It has been shown to perform better in patients with a higher GFR. It has not been as extensively validated as the MDRD formula has been and is not yet in wide use.

$$\text{GFR} = 141 \times \min(S_{cr}/k, 1)\ \alpha \times \max(S_{cr}/k, 1) - 1.209 \times 0.993\ \text{age} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}]$$

Where:

S_{cr} is serum creatinine in mg/dL,
κ is 0.7 for females and 0.9 for males,
α is −0.329 for females and −0.411 for males,
min indicates the minimum of S_{cr}/κ or 1, and
max indicates the maximum of S_{cr}/κ or 1.

CLASSIFYING THE STAGES OF CHRONIC KIDNEY DISEASE WITH THE GFR

A classification system of the stages of CKD has been developed for evaluating the progression of CKD using the GFR as follows.[22] Below are the five stages of CKD and GFR ranges for each stage:

- Stage 1 with normal or high GFR (GFR >90 mL/min)
- Stage 2 Mild CKD (GFR = 60–89 mL/min)
- Stage 3A Moderate CKD (GFR = 45–59 mL/min)
- Stage 3B Moderate CKD (GFR = 30–44 mL/min
- Stage 4 Severe CKD (GFR = 15–29 mL/min)
- Stage 5 End stage CKD (GFR <15 mL/min)

GFR values from the various calculation systems above can be used to assess CKD. The GFR becomes abnormal gradually with CKD. The additional tests that follow may also be of assistance in the assessment of kidney function.

ADDITIONAL TESTS TO ASSESS KIDNEY FUNCTION

The following tests are also used frequently to assess kidney function in various renal diseases and conditions.

Cystatin C

Cystatin C levels in the blood can be measured, because as the GFR decreases, the levels of cystatin C in the blood rises. It may be a more sensitive in detecting kidney function problems earlier than a decreased GFR.

GFRs and related tests like cystatin C are important in many aspects of medicine. They are essential in monitoring renal disease and renal failure. They give information in addition to evaluating uremia with the common BUN and creatinine level screening tests. They are necessary to calculate therapeutic drug dosages and to adjust medications to account for the patient's renal function.

Microalbumin

The urine **microalbumin** test or albumin/creatinine ratio (ACR) is used to screen people with chronic conditions, such as diabetes and hypertension, with an associated increased risk of developing kidney disease. Identifying microalbuminuria the very early stages of kidney disease helps doctors adjust treatment to achieve greater control of diabetes and hypertension and slow the progression of kidney disease. Microalbumin is not detected with most urine dipsticks. Microalbuminuria may also be correlated with creatinine levels to assess significance. A microalbumin to creatinine ratio of >30 mg/g is considered diagnostic of microalbuminuria.[23] The onset of diabetic renal complications is first predicted by the detection of microalbuminuria. Monitoring the GFR also helps track kidney disease progression. Detection of elevated urinary microalbumin is also associated with heart disease.

The National Kidney Foundation recommends that diabetics between 12 and 70 years of age have a urine test for albumin at least annually. According to the American Diabetes Association, everyone with type 1 diabetes should get tested annually, starting 5 years after onset of the disease, and all those with type 2 diabetes should start testing at the time of diagnosis. If microalbuminuria is detected, it should be confirmed by retesting and, if positive on two of three determinations over a 3- to 6-month period, it is considered to be present and appropriate treatment should be given.

If significant amounts of albumin are detected with screening tests performed on random, 4-hour, or overnight urine samples, they may be repeated and/or confirmed with a 24-hour urine albumin test. People with hypertension may be tested at regular intervals, with the frequency determined by their healthcare provider. Moderately increased albumin levels found in both initial and repeat urine tests indicate that a person is likely to be in an early phase of developing kidney disease. Very high levels are an indication that kidney disease is present in a more severe form. Undetectable levels are an indication that kidney function is normal. Blood in the urine, a urinary tract infection, vigorous exercise, or an acid–base imbalance can cause false-positive test results. Testing should be repeated after these conditions have resolved.

β_2-Microglobulin

Elevated serum and urine **β_2-microglobulin** indicate increased cellular turnover as seen in lymphoproliferative disorders, inflammatory conditions, viral infections, and in renal failure. β_2-Microglobulin measurements are used clinically to evaluate kidney disease and renal tubular disease. Increased levels of β_2-microglobulin in the blood and urine indicate that there is a problem that is likely of kidney origin but are not diagnostic of a specific disease or condition.

In someone with signs of kidney disease, increased levels of β_2-microglobulin in the blood along with low levels in the urine indicate that the disorder is associated with glomeruli dysfunction. If β_2-microglobulin is low in the blood and high in the urine, then it is likely that the person has renal tubule damage or disease.

In a patient who has been on long-term dialysis, an increase in β_2-microglobulin is associated with dialysis-related amyloidosis. Increases in urine β_2-microglobulin can occur in a person with a kidney transplant may indicate early kidney rejection. Increases of β_2-microglobulin in someone who is exposed to high amounts of mercury or cadmium may indicate early kidney dysfunction.

ASSESSING TUBULAR FUNCTION

Assessment of the tubular functions of reabsorption and the ability to concentrate urine require an understanding that the urine produced just after filtration has a set specific gravity of 1.010. Patients with loss of tubular function due

1. A frequent complication of diabetes is chronic kidney disease and kidney failure. Why does diabetes cause kidney failure with accompanying anemia?

2. Label the parts of the kidney.

3. Label the structures of the nephron that are involved in urine formation and excretion.

4. Compare and contrast the processes of reabsorption and tubular secretion.

5. Diagram the renin–angiotensin–aldosterone cycle and discuss its role in hypertension.

6. List the primary constituents of urine.

7. Patients with renal failure typically have no urine output volumes; this is referred to as:
   a. Oliguria
   b. Anuria
   c. Polyuria
   d. Pyuria

8. The glomerular filtration barrier is composed of:
   a. The capillary endothelium, basement membrane, and juxtaglomerular apparatus
   b. Capillary endothelium, podocytes, and basement membrane
   c. Podocytes, hilum, and basement membrane
   d. Capillary endothelium, podocytes, and juxtaglomerular apparatus

9. The glucose renal threshold is 160 to 180 mg/dL. This represents the:
   a. Concentration of glucose in the vasa recta
   b. Maximum rate of glucose reabsorption in the renal tubule
   c. Plasma concentration above which glucose is excreted in the urine
   d. Plasma level at the commencement of glucose reabsorption in the nephron

10. Which of these is not a mechanism to maintain blood pH through the kidney?
    a. Excretion of acetic acid
    b. Excretion of hydrogen ions
    c. Excretion of ammonium ions
    d. Reabsorption of bicarbonate

11. Which of these is the major functional unit of the kidney?
    a. The hilus
    b. The efferent arteriole
    c. The juxtaglomerular apparatus
    d. The nephron

12. Which of these urinary structures is involved in the countercurrent exchange mechanism?
    a. The afferent arteriole
    b. The efferent arteriole
    c. The vasa recta
    d. The juxtaglomerular apparatus

13. Which of the following is NOT true about antidiuretic hormone?
    a. It is the same as vasopressin
    b. It increases blood pressure
    c. It is made in the kidney
    d. Its release is inhibited by ethanol and caffeine

14. Aldosterone is involved in the reabsorption of:
    a. Potassium
    b. Sodium
    c. Bicarbonate
    d. Hydrogen ion

15. Which of the following is NOT a method for an estimated glomerular filtration rate?
    a. The Chronic Kidney Disease Epidemiology Collaboration Formula
    b. Cockcroft–Gault Formula
    c. Osmolar and Free Water Formula
    d. Modification of Diet in Renal Disease Formula

16. Which of these is a method to assess renal tubular function?
    a. Osmolar and Free Water Formula
    b. Cockcroft–Gault Formula
    c. The Chronic Kidney Disease Epidemiology Collaboration Formula
    d. Modification of Diet in Renal Disease Formula

CASE STUDIES

Case 1-1 A 61-year male banker, Tom Jones, was in good health until he lost control of his car, hit a light pole, and suffered head trauma. While hospitalized in the intensive care unit during recovery, he developed hospital-acquired pneumonia. During his long hospitalization, Tom began to show signs of depression and was treated with a selective serotonin reuptake inhibitor. His depression was not lessened in 2 weeks and the dosage of the antidepressant was increased. Six days after his dosage increase, his nurse noted the patient exhibited confusion and lethargy. Tom's blood pressure was slightly elevated. His basic blood work showed the following:

Serum sodium: 121 mEq/L, decreased
Blood urea nitrogen: 9 mg/dL, decreased
Serum chloride: 85 mEq/L, decreased
Serum uric acid: 2.2 mg/dL, decreased

The rest of his electrolytes and chemistry panel blood tests were normal, in addition to his thyroid and cortisol tests all being in normal range.

Tom's physician then ordered a serum and a urine osmolarity and a urine sodium level. He was started on a water restriction regimen and was given a saline IV and saline PEG tube flushes were begun. The serum osmolarity was low, while the urine osmolarity was elevated and the urine sodium was elevated.

1. What condition has hypotonic plasma, with lower serum osmolarity and higher urine osmolarity and an elevated urine sodium level with a lower plasma sodium level?
2. What other disorder is associated with ADH?
3. What conditions does the patient have that are associated with SIADH?
4. How does ADH affect the body?

Case 1-2 A 61-year-old Caucasian woman with no significant medical history has routine laboratory tests done as part of her annual physical examination. She weighs 135 lb (61.2 kg) and is 64 in (163 cm) tall. Her serum creatinine level is 1.1 mg/dL; her estimated GFR is 53 mL/min/1.73 m^2. A urinalysis dipstick is normal for protein and blood.

1. How does age affect GFR?
2. What is the difference between creatinine clearance and GFR?
3. Is this patient's GFR normal?

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Renal and Urinary Tract Diseases and Related Urinalysis Findings

Chapter 2

KEY TERMS

Acute Poststreptococcal Glomerulonephritis
Acute Kidney Injury (Acute Renal Failure)
Acute Tubular Necrosis
Alport Syndrome
Azotemia
Chronic Glomerulonephritis
Chronic Renal Failure
Cystitis
Diabetic Nephropathy (Kimmelstiel-Wilson Disease)
Edema
Estimated Glomerular Filtration Rate (eGFR)
Focal Segmental Glomerulosclerosis
Glomerular Disease
Goodpasture Syndrome
Immunoglobulin a Nephropathy (Berger Disease)
Microalbumin
Pyelonephritis (Acute and Chronic)
Rapidly Progressive (Crescentic) Glomerulonephritis
Tubulointerstitial Disease
Uremia
Urolithiasis or Renal Lithiasis

LEARNING OBJECTIVES

1. Define the following: Glomerulonephritis, nephrosis, glomerulosclerosis, cystitis, and pyelonephritis.
2. Describe and discuss the typical signs and symptoms of kidney stones, the types of stones, and laboratory tests that may be ordered for patients with stones.
3. Categorize the various renal diseases in this chapter as to whether they are glomerular, tubular, interstitial, or vascular in origin.
4. Describe how immunologic processes damage the kidney.
5. Match urinalysis findings with urinary tract and kidney diseases.
6. Differentiate between findings in cystitis and in pyelonephritis.
7. Differentiate between membranous and membranoproliferative glomerulonephritis.
8. Discuss the course of disease for IgA nephropathy and the associated laboratory findings.
9. Name a urinary finding that is diagnostic of renal tubular damage.
10. Discuss diabetic nephropathy and the development of end-stage renal disease in diabetic patients.
11. Differentiate between diabetic nephropathy, diabetes insipidus, and syndrome of inappropriate antidiuretic hormone.

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A wide variety of disorders can affect the upper and the lower urinary tract. Urinalysis findings can be a great help in the diagnosis and management of many of these disorders if the caregiver is familiar with changes that occur in these disorders. This chapter reviews many of these disorders of the kidney and the urinary system and their related changes in urinalysis findings.

Anatomical Conditions Affecting the Urinary Tract

Congenital abnormalities of the kidney and urinary tract arise during fetal development. Parts of the urinary tract can grow to an abnormal shape or size. Accidents and injuries can also damage the kidneys or urinary tract and also leave them more vulnerable to infections and disease.

Duplicate sets of ureters, horseshoe kidney (where the kidneys are fused in development), and vesicoureteral reflux are all anatomical conditions that arise prior to birth. Vesicoureteral reflux is an anatomical condition in which urine abnormally refluxes (or flows backward) from the bladder into the ureters. Urine backflow may even reach the kidneys. In all of these conditions, infection and subsequent scarring often occur over time.

Another common condition related to anatomic changes in the urinary tract is benign prostatic hyperplasia, the most common prostate problem for men older than 50 years. It is associated prostate enlargement around the urethra which causes urinary discomfort and frequency.

Infections of the Lower Urinary Tract

About one-half of all people develop a urinary tract infection (UTI) during their lifetimes. UTIs are a common cause for an outpatient visit to a physician and for nosocomial infections. Up to 10% of UTIs result in serious complications such as sepsis or chronic kidney infections with loss of renal function. Because of their short urethra offering less protection from invading organisms from the nearby vagina and rectum, females are also more prone to UTIs than men. About one in five women develop a UTI in their lives. UTIs may be symptomatic or asymptomatic. Pregnant women often have asymptomatic UTIs that can lead to pregnancy complications. Men not only have less UTIs than women do but also suffer from them in increased numbers with increasing age. It is relatively common for elderly persons to suffer UTIs. In children, UTIs occur in as many as 3% to 5% of female children and 1% of male children. With tissue damage and scarring from previous infections, it is also not unusual for patients to suffer reoccurring infections.

Infections can arise in the lower urinary tract via the urethra and ascend the urinary tract or can arise through infections in the bloodstream seeding the kidney with organisms deposited in the upper urinary tract and descend the urinary tract. Descending (or hematogenous) UTI implies that microorganisms, having gained access to the bloodstream from another source, then secondarily infect the kidneys and other parts of the urinary tract. Infections of the ascending type are more common. Upper UTIs **(pyelonephritis [Acute and Chronic])** are treated more aggressively as there is risk of permanent kidney damage. "Upper UTI" also encompasses intrarenal and perinephric abscesses. Obstruction, foreign bodies, or the presence of catheters can increase the risk of acquiring a UTI. While UTIs usually show evidence of increased neutrophils (in microscopic examination and positive leukocyte esterase) and presence of bacteria (in microscopic examination and often in the nitrate patch) and may show some blood or protein or increases in pH, there are some differences to keep in mind when distinguishing urinalysis results. If white blood cell (WBC) casts are present, this is an indication of kidney infection rather than a bladder infection, as casts are formed in the nephron, not below the kidney.

Urolithiasis

Kidney stones are the most common cause of upper urinary tract obstruction. Although stones can form anywhere in the urinary tract, the most common site is in the kidney. Men are affected more commonly than women. Kidney stones **(urolithiasis or renal lithiasis)** are small, hard deposits that form inside your kidneys. These stones can lodge in your ureters or urethra and can obstruct urine flow leading to hydronephrosis with fluid backup and further still to infections. The stones are made of mineral and acid salts. Kidney stones have many causes and can affect any part of your urinary tract—from your kidneys to your bladder or urethra. Different types of crystalline stones may form from the materials that are normally excreted in urine. Often, stones form when patient is dehydrated and the urine becomes concentrated, allowing minerals to crystallize and stick together. The basic types of stones include calcium oxalate or calcium phosphate, magnesium ammonium phosphate, uric acid, and cystine stones. A major manifestation of this condition is pain. Passing kidney stones can be quite painful, but the stones usually cause no permanent damage although they may cause a small amount of bleeding. Depending on your situation, you may need nothing more than to take pain medication and drink lots of water to pass a kidney stone. In other instances, lithotripsy or surgery may be needed. Your doctor may recommend preventive treatment of increased hydration, diet alterations, or even medications to reduce your risk of recurrent kidney stones if you are at increased risk of developing them again.

The urinalysis may or may not show blood and RBCs on the microscopic examination. Urine pH from the urinalysis

Figure 2-1. Comparison of different types of kidney stones: Clinical manifestations of kidney stones include hematuria and flank pain.

is related to crystal formation, and crystals that may be present in the urine microscopic examination that may contribute to stone formation. See Figure 2-1 for stone characteristics. Also, see the types of stones patients encounter in Figures 2-2 to 2-4. Specialized laboratory testing is available to determine the chemical composition of a stone that has been passed.

Diseases of the Kidney

Kidney functions are essential to our survival. If the kidneys fail, other means of replacing kidney functions, such as dialysis or transplant, are needed to cleansing the system of wastes and maintaining blood homeostasis in order to survive. If you decline dialysis or transplant treatment after kidney failure, you may live for only a few days to a few weeks, depending on your health status and your remaining kidney function.

Kidney disease and other diseases contributing to kidney disease are common. According to "Summary Health Statistics for U.S. Adults: National Health Interview Survey of 2006," 2% of US adults 18 years of age and older were told in the previous 12 months that they had kidney disease. Other chronic diseases often contribute to kidney disease. Chronic diseases that contribute to kidney disease are diabetes, hypertension, and immune and autoimmune disease. In the 2006 national health interview survey mentioned above, 8% of adults aged 18 and older reported learning that they had diabetes in the last 12 months; 23% reported that they were told that they had hypertension; and 21% reported some type of autoimmune disease, with some types of autoimmune disease more often linked to kidney disease and some less linked to kidney disease. The two most common diseases that affect the kidney are diabetes and hypertension. The high blood sugar of diabetes damages the nephrons. High blood pressure can prevent the small blood vessels in the kidneys from filtering and cleansing the blood well enough. Damage to the kidneys from infection, injury, or other disease also contributes to most kidney diseases. Other less common diseases, such as Wegener granulomatosis, which is also called granulomatosis with polyangiitis, often affects your kidneys, as well as lungs and upper respiratory tract, restricting blood flow to these organs causing damage. Less frequently, genetic abnormalities

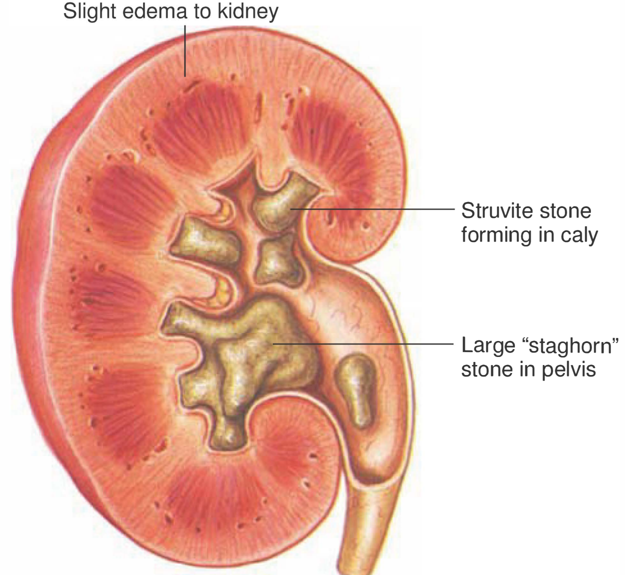

Figure 2-2. Magnesium ammonium Phosphate (Struvite) Stones in Kidney Labeled Asset provided by Anatomical Chart Co.

in the physical structures of the urinary tract or kidneys can also contribute to kidney disease. Most kidney diseases affect the nephrons, resulting in changes in the blood and urine that can be detected by the clinical laboratory.

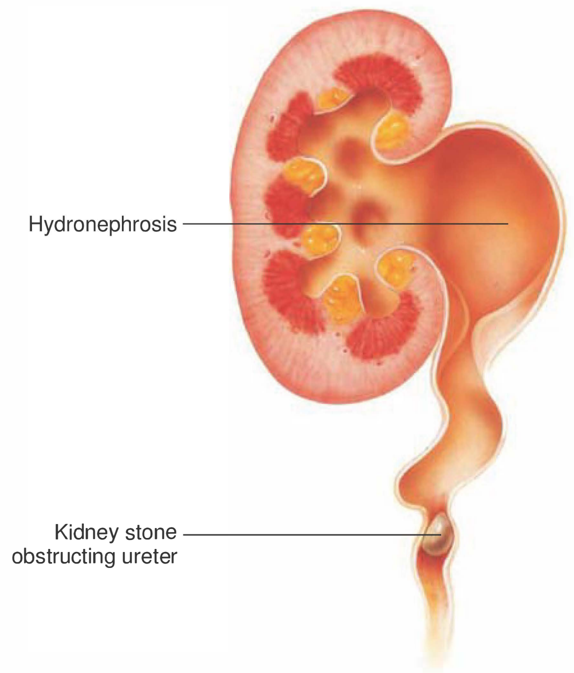

Figure 2-3. Hydronephrosis and Kidney Stone Labeled Asset provided by Anatomical Chart Co.

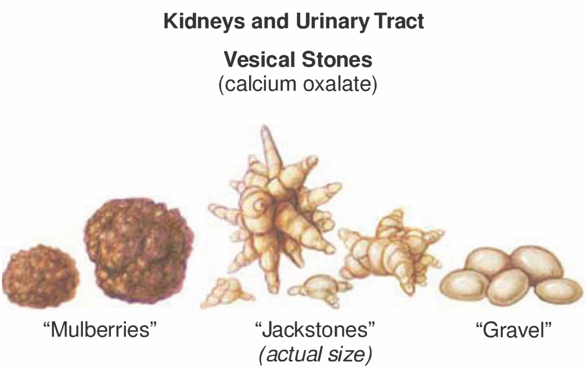

Figure 2-4. Vesicle Kidney Stones Labeled.

Kidney disease affects the quantity of fluids in the body and if those fluids are free from nitrogenous waste toxins. Among the important substances that the kidneys help control are sodium, potassium, chloride, bicarbonate (HCO_3^- measured indirectly as CO_2), blood pH, calcium, phosphate, and magnesium. Thus, kidney disease affects the body's critical acid–base status as well as many other functions related to critical electrolytes. Kidney disease often causes changes in urinalysis results that help the physician diagnose and monitor these patients. Other urinary tract injuries, chronic diseases, metabolic disorders, and genetic disorders also can affect urinalysis results.

Common Diseases of the Kidney: Vascular Disease and Diabetes

High blood pressure is a common condition that makes your heart work harder and over time damages the overworked blood vessels throughout the body. If the blood vessels of the kidney are damaged, the kidneys may stop removing nitrogenous wastes and excess fluids from the body. This raises blood pressure, creating a perpetuating cycle. Laboratory tests can assist in determining whether high blood pressure has damaged the kidneys. Measuring the blood nitrogen waste levels, such as by measuring a blood urea nitrogen (BUN) or serum creatinine level is helpful. Having too much creatinine in your blood is a sign that you have kidney damage. These values are elevated in kidney disease and this elevation of BUN in kidney disease is called **uremia** and the elevation of nitrogen waste byproducts is called **azotemia**. The physician should use the serum creatinine to estimate the main kidney function called **estimated glomerular filtration rate (eGFR)** or order a creatinine clearance test. Proteinuria is also associated with hypertension, heart disease, and blood vessel damage. Measuring protein in the urine is also helpful to assess hypertension.

In another common condition, diabetes, the small blood vessels of the body are injured. When kidney blood vessels

are injured, kidneys cannot cleanse the blood properly. The body retains water and salt, which can result in water weight gain and ankle swelling **(edema)**. Protein may be present in the urine. The finding of protein in urine is one of the key findings of kidney disease in general. Moreover, nitrogenous waste materials will build up in the blood as in hypertension above. In addition, diabetes may cause nerve damage which can cause difficulty in emptying the bladder. The pressure resulting from an overfull bladder can cause urine backup and injure the kidneys. If urine remains in your bladder for a long time, you can also develop an infection from growth of bacteria in urine enhanced by a higher sugar concentration. Diabetic patients are regularly screened for microalbuminuria. Measuring urinary protein levels, especially **microalbumin** can help detect diabetic kidney damage at an early stage. Microalbumin is not detected routinely with most urine dipsticks. Microalbuminuria may also be correlated with creatinine levels to assess significance. The onset of diabetic renal complications is first predicted by the detection of microalbuminuria. Monitoring the GFR helps track kidney disease progression. Detection of elevated urinary microalbumin is also associated with heart disease.

SCREENING

Diabetic and hypertensive patients should be regularly screened for kidney disease with two key tests: a GFR (or **estimated GFR (eGFR)**) and a urine microalbumin.

Creatinine is a nitrogenous waste from metabolism normally excreted by the nephron. Creatinine rises as GFR falls. The relationship between serum creatinine and GFR is not linear, so just following the serum creatinine level is not a precise way to estimate GFR. The older creatinine clearance test provides a good GFR in patients with normal kidney function. The test involves collecting a blood sample for serum creatinine and a 24-hour urine collection and urine volume. The results are reported in mL/min or mL/min/body surface area. The newer estimated GFR (eGFR) is determined using the patient's serum creatinine and a formula that includes age, race, and sex. It is important to remember that, however the GFR is measured, the kidney has an enormous functional reserve and the GFR remains within the normal range until extensive kidney damage has occurred. The American Diabetes Association and the National Institutes of Health recommend that the eGFR be calculated from serum creatinine at least once a year in all people with diabetes. Kidney disease is present when the eGFR is less than 60 mL/min.

Generally, urine microalbumin levels are assessed by comparing the amount of microalbumin to the urine creatinine level in a single urine sample. Healthy kidneys will yield large amounts of creatinine but almost no microalbumin. Even a small increase in the ratio of albumin to creatinine is a sign of kidney disease. The National Institutes of Health and the American Diabetes Association recommend annual assessment of urine microalbumin in patients with diabetes type 2 and those with diabetes type 1 for more than 5 years. Kidney disease is present if the urine contains more than 30 mg of albumin per gram of creatinine, with or without a decreased eGFR. Also see Chapter 1 for further explanation of these tests.

Diseases Affecting the Glomerulus

Glomerular diseases include those that present with **nephritic syndrome** and those that present with **nephrotic syndrome**. Characteristics of these syndromes are described below in Table 2-1. A trait they share is proteinuria (an increase in the amount of protein in the urine). Proteinuria is commonly seen with tubular injury and glomerular kidney injuries and so is

Table 2-1 Characteristics of Nephritic and Nephrotic Syndromes

GENERAL NEPHRITIC SYNDROME CHARACTERISTICS	GENERAL NEPHROTIC SYNDROME CHARACTERISTICS
Hypertension	Proteinuria >3.5 g/d in adults and >40 mg/kg in children (3+ to 4+ on urine protein dipstick)
Proteinuria (often <2 g/d in adults)	Low serum albumin
Red blood cell casts in the urine microscopic hematuria (blood visible in urine under the microscope but not necessarily visually) or gross hematuria (visible with the eye)	Elevated serum cholesterol, which is produced in response to proteinuria, by the liver
Many additional types of casts including broad and waxy casts if the condition is chronic (this is referred to as a telescopic urine with the presence of many casts)	Elevated urinary lipids, oval fat bodies, and fatty casts
	Edema, an accumulation of salt water that the kidneys have not excreted; with fluid initially accumulating in the legs with pitting edema

one of the key hallmarks and best predictors of renal disease. In adults, the kidneys normally excrete levels up to 150 mg/dL (0.15 g/dL) of protein. Glomerular diseases affect the glomerular filtration barrier and can increase protein excretion above 2 g/dL. Nephrotic syndrome proteinuria demonstrates protein excretion levels greater than 3.5 g/d for adults.

GLOMERULAR DISEASE CAUSES AND MECHANISMS

There are many types of glomerular diseases caused by both immune complexes or immunological processes and nonimmunological processes. They can have systemic disease origins or be a result of primary kidney disease or have genetic causes. Some glomerular diseases are acute and can resolve fairly quickly, whereas others are chronic and may lead to kidney failure. Acute glomerulonephritis can become chronic or a glomerulonephritis may just begin as a chronic condition. Glomerulonephritis can lead to nephrotic syndrome. Often, the various glomerular diseases have their own unique laboratory urine and serum values.

ACUTE GLOMERULONEPHRITIS DISEASES

Glomerulonephritis is a sterile, inflammatory condition affecting the glomerulus, resulting in protein, blood, and casts in the urine. There are multiple types of glomerulonephritis, and one type may change into another type over time, the condition may become chronic, and glomerulonephritis may also lead to nephrotic syndrome.

Acute Poststreptococcal Glomerulonephritis

Acute poststreptococcal glomerulonephritis is an autoimmune condition that may arise after a *Streptococcus pyogenes* (group A) infection of the throat or skin. This occurs approximately 7 to 12 days postinfection (the time needed to develop antibodies). This is a diffuse proliferative glomerulonephritis and this type of nephritis is also occasionally seen with other bacterial and viral agents. It is not arising from the organism but rather from an immunological poststreptococcal infection reaction. It causes an acute glomerulonephritis. The outlook is usually favorable, but there may be resulting permanent kidney damage. Common symptoms include edema (often around the eyes), hypertension, oliguria, hematuria, and fatigue.

Urinalysis findings include proteinuria, hematuria with RBCs and dysmorphic RBCs, WBCs, RBC casts, and possibly an occasional hyaline or granular cast. The patient may also have positive Anti-Streptolysin O serological results.

Rapidly Progressive (Crescentic) Glomerulonephritis

Rapidly progressive (crescentic) glomerulonephritis is a form of acute kidney disease that causes damage to the glomeruli and progressive loss of kidney function over weeks to months. This condition is also called necrotizing or crescentic glomerulonephritis (due to its pathologic appearance with crescent-shaped glomeruli). This glomerulonephritis is more common in adults aged 40 to 60 years and mini epidemics of this disorder have also occurred following a socioeconomic decline. This disease often progresses to **chronic glomerulonephritis**. This disease may show up as nephritic syndrome or unexplained kidney failure. The patient's laboratory values may also reflect another immunological condition, such as systemic lupus erythematosus, or show elevated fibrin degradation products.

Many conditions are known to cause or increase the risk for developing rapidly progressive glomerulonephritis. These include a variety of vascular, blood, or lymphatic disorders, **membranoproliferative glomerulonephritis,** a history of cancer, or exposure to hydrocarbon solvents. Symptoms are initiated by deposition of immune complexes in the glomerulus in another form of glomerulonephritis or in a systemic autoimmune disorder such as systemic lupus erythematosis.

Common symptoms include edema, hypertension, blood in the urine with dark or smoke-colored urine, decreased urine volume, deposition of IgA complexes in the glomerulus, and a general malaise.

Urinalysis findings include proteinuria, hematuria with RBCs and dysmorphic RBCs, WBCs, RBC casts, and possibly an occasional hyaline or granular cast.

GOODPASTURE SYNDROME

Goodpasture syndrome is an autoimmune disease with cytotoxic autoantibodies to collagen that are called antiglomerular basement membrane antibodies and with subsequent complement activation producing glomerular capillary destruction. This disease affects both kidneys and lungs. It usually involves rapidly progressive kidney failure that develops in days to weeks along with lung disease but some forms of the disease involve just the lung or the kidney, not both. The kidney disease resembles rapidly progressive glomerulonephritis.

Common symptoms include hemoptysis, hypertension, dyspnea, hematuria, proteinuria, and red blood cell (RBC) casts. These patients also often have antiglomerular basement membrane autoantibodies.

MEMBRANOUS GLOMERULONEPHRITIS

Membranous nephropathy is caused by pronounced thickening of the glomerular basement membrane. The glomerular basement membrane is a part of the kidneys that helps filter waste and extra fluid from the blood. This thickening results from deposition of immune complexes of immunoglobulin G. This glomerulonephritis is one of the most common causes of nephrotic syndrome. The condition may be a primary kidney disease of uncertain origin, or it may be associated with other conditions.

Common symptoms include microscopic hematuria, elevated protein and foamy urine, edema, weight gain, hypertension,nocturia, and a tendency of thrombosis.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Membranoproliferative glomerulonephritis type I is a kidney disorder arising from inflammation and resulting in changes in the microscopic structure of kidney cells and peripheral capillaries. This is also known primarily as mesangiocapillary glomerulonephritis type I. Deposits of antibodies are build up in a part of the glomerulus called the glomerular basement membrane. This disorder is often progressive and eventually results in chronic renal failure. Mesangiocapillary glomerulonephritis type II causes extremely dense deposits in the glomerular basement membrane.

Common symptoms include edema, hypertension, azotemia, hematuria, oliguria, and often a change in mental status.

CHRONIC GLOMERULONEPHRITIS

Chronic glomerulonephritis is the advanced stage of several kidney disorders, resulting in inflammation and slowly worsening destruction of glomeruli, with progressive loss of kidney function. Glomerulonephritis is among the leading causes of chronic kidney failure and end-stage kidney disease. Causes include diabetic nephropathy/sclerosis, **focal segmental glomerulosclerosis,** IgA nephropathy (Berger disease), lupus nephritis, **membranous glomerulonephritis,** mesangial proliferative disorder, nephritis associated with disorders such as amyloidosis, multiple myeloma, or immune disorders, including AIDS.

Specific symptoms include azotemia, hematuria (rust-colored urine), proteinuria, and foamy urine; chronic kidney failure symptoms that gradually develop may include the following: decreased alertness; drowsiness; somnolence; lethargy; confusion; delirium; coma; seizures; decreased sensation in the hands, feet, or other areas; decreased urine output; easy bruising or bleeding; frequent hiccups; general ill feeling (malaise); generalized itching; headache; increased skin pigmentation (skin may appear yellow or brown); muscle cramps; muscle twitching; nausea and vomiting; need to urinate at night; and unintentional weight loss.

Urinalysis findings include proteinuria, slight hematuria with few RBCs and dysmorphic RBCs, WBCs, mixed casts with many reflecting the chronic and severe nature of the disease, such as waxy and broad casts. Waxy casts indicated that casts have been present for a longer time to develop this waxy character. Broad casts reflect the severity of the disease as they are formed when many collecting ducts are affected as the larger duct where they pool is blocked. This mixed cast picture is often referred to as a "telescoped urinary sediment," a urinary sediment in which red cells, white cells, oval fat bodies, and all types of casts are found in equal amounts.

IMMUNOGLOBULIN A NEPHROPATHY (BERGER DISEASE)

In **Immunoglobulin A nephropathy (Berger disease),** patients chronically show increased serum levels of IgA and immune complexes containing IgA are deposited on the glomerular membrane. After an infection or strenuous exercise, these patients may exhibit gross blood in the urine. After this episode, they recover but still have asymptomatic chronic microscopic hematuria. Despite this, there is a continual progression to chronic glomerulonephritis. This disease is the most common cause of chronic glomerulonephritis.

DIABETIC NEPHROPATHY (KIMMELSTIEL–WILSON DISEASE)

In diabetes, the blood vessels of the kidney are damaged. There is continual damage to the glomerular membrane due to thickening and increased cellular proliferation and an accumulation of solid substances around the vascular tuft. This causes vascular sclerosis and can lead to end-stage renal disease. This chronic disease is currently the leading cause of end-stage renal disease. Laboratory values often resemble nephrotic syndrome.

MISCELLANEOUS DISEASES RELATED TO GLOMERULONEPHRITIS AND NEPHRON DAMAGE

A variety of systemic and vascular diseases are also associated with glomerular lesions and nephron damage. Systemic lupus erythematosis, Henoch–Schonlein Purpura, Wegener granulomatosis, bacterial endocarditis, diabetes, and amyloidosis have all been associated with glomerular lesions. Systemic vascular diseases, renal blood vessel injury, microangiopathies, and hypertension are all involved in diseases of the kidney. Classic childhood and adult hemolytic uremic syndrome are microangiopathic diseases that cause hematuria, severe oliguria, hemolytic anemia, thrombosis, and **acute kidney injury (acute renal failure)** (previously termed acute renal failure). These hemolytic uremic syndromes follow the consumption of verocytotoxin producing *Escherichia coli* or *Shigella* species.

NEPHROTIC SYNDROME AND RELATED DISORDERS

Nephrotic syndrome may occur over time as a complication of glomerulonephritis as mentioned above, or it may arise from circulatory shock with a decrease of blood flow to the kidney and decreased pressure in the glomerulus.

Nephrotic Syndrome

Nephrotic syndrome is caused by various disorders that damage kidneys, particularly those that damage the basement membrane

of the glomerulus causing abnormal glomerular permeability to plasma proteins and excretion of that protein in the urine. Membranous glomerulonephritis is the most common cause of nephrotic syndrome in adults. An alternate name for this condition is nephrosis. Nephrosis also occurs from circulatory problems that produce systemic shock. Treatment may be required for life for adults with this condition.

Nephrotic syndrome exhibits a group of symptoms including gross proteinuria of more than 3.5 g/d, along with low blood protein levels, high cholesterol levels, and edema. Symptoms include edema with weight gain, swollen abdomen, heavy proteinuria with foamy appearance of the urine, hypertension, and poor appetite.

Urinalysis usually shows 4+ proteinuria, and increased microscopic fat, which can include fat globules, oval fat bodies, or fatty casts. An oval fat body results from a renal tubular epithelial cell that has filled with fat sloughing off and degrading. This is another condition that often shows a telescoped urinary sediment with a variety of cells and casts similar to a chronic glomerulonephritis, as it often results as a complication following a previous glomerular disease.

Minimal Change Disease

Minimal change disease is not associated with much cellular change in the glomerulus, hence its name, although the podocytes appear to be less tightly fitting. In children, it is most common from ages 2 to 6. This disorder occurs slightly more often in males than in females. This disease has a much more favorable prognosis than the above adult nephrotic syndrome. It can also result from infections, allergic reactions, vaccinations, various autoimmune disorders, and various other glomerular diseases. Minimal change disease usually responds well to corticosteroids within a month. Symptoms and laboratory findings are similar to the adult nephrotic syndrome above.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis is caused by scar tissue that forms in areas of the glomeruli. Each kidney has thousands of glomeruli. "Focal" means that some of the glomeruli become scarred, while others have remained normal. "Segmental" means that only part of each individual glomerulus is damaged.

The causes of focal segmental glomerulosclerosis are generally unknown. A small number of cases result from reflux nephropathy. This condition can affect both children and adults. Males are affected slightly more often than females, and it also has been found to occur more frequently in African Americans. It is estimated that focal segmental glomerulosclerosis causes about 10% to 15% of all cases of nephrotic syndrome. Urinalysis findings often show proteinuria and microhematuria.

ALPORT SYNDROME

Alport syndrome is an inherited form of kidney inflammation that can cause symptoms ranging from mild hematuria to gross hematuria to nephrosis and end-stage kidney disease. It is caused by a mutation in a gene involved in the synthesis of a type of collagen. The disorder is uncommon and most often affects males more severely. However, women can transmit the gene for the disorder to their children. Risk factors include end-stage kidney disease in male relatives, family history of Alport syndrome, glomerulonephritis, or hearing loss before age 30.

Tubular Disorders

In addition to diseases of the glomerulus, diseases of the tubules affect urinalysis results. These disorders can be acquired or inherited. Some tubular diseases result from injury and damage to the tubule and some from metabolic conditions that damage the tubule.

ACUTE TUBULAR DISORDERS

Acute tubular necrosis is a kidney disorder involving damage to the tubule cells of the kidneys, resulting in acute kidney failure. Acute tubular necrosis is a condition with rapid onset caused by lack of oxygen to the kidney tissues (ischemia of the kidneys) or by exposure to materials that are nephrotoxic. The internal structures of the kidney, particularly the tissues of the kidney tubule, are damaged or destroyed. Acute tubular necrosis is one of the most common structural changes that can lead to acute renal failure and is one of the most common causes of kidney failure in hospitalized patients. In most people, acute tubular necrosis is reversible. The goal of treatment is to prevent life-threatening complications of AKI (renal failure) during the time the lesion is present.

Findings of this disorder include increased BUN and serum creatinine levels; altered excretion of sodium and urea; and the kidney biopsy may show acute tubular necrosis.

Urinalysis may show casts, kidney tubular epithelial cells, and RBCs; and despite high urine sodium, urine specific gravity and osmolarity may indicate relatively more dilute urine of a specific gravity around 1.010 with little ability to vary this specific gravity.

HEREDITARY AND ACQUIRED METABOLIC TUBULAR DISORDERS

Both genetic abnormalities and systemic conditions can affect the tubules via a buildup of metabolic byproducts that exceed the ability of the kidney tubules to reabsorb them.

Fanconi Syndrome

Fanconi syndrome is a tubular disorder in which certain substances normally reabsorbed into the bloodstream by the nephrons are released into the urine instead. This disorder results in the accumulation of various amino acids, glucose, phosphorus, sodium, and potassium in the urine. Fanconi syndrome can be caused by faulty genes, or it may result later in life due to kidney damage, or the cause may be unknown. Common causes of Fanconi syndrome in children are genetic **inborn errors of metabolism**. Urinalysis findings may include glycosuria, mild proteinuria, and in some instances cystine crystals.

Nephrogenic Diabetes Insipidus

Nephrogenic diabetes insipidus is a disorder in which a defect of the nephron results in the passage of large volumes of urine. Nephrogenic diabetes insipidus involves a defect in the kidney tubules which affects the ability of the kidneys to respond to antidiuretic hormone (vasopressin), which normally instructs the kidneys to make the urine more concentrated. As a result, the kidneys excrete an excessive amount of water into the urine, producing a large quantity of very dilute urine. Nephrogenic diabetes insipidus is a rare disorder. It may be present at birth as a result of an inherited defect that usually affects men, although women can pass the gene on to their children. Most commonly, nephrogenic diabetes insipidus is an acquired disorder. Factors that precipitate the disorder include drugs such as lithium, demeclocycline, and amphotericin, electrolyte disorders, and urinary blockage. Urinalysis results include a large volume of dilute urine with a low specific gravity.

Renal Glycosuria

A renal tubular disorder involving the reabsorption of glucose, this disorder is inherited as an autosomal recessive trait. These patients have increased urine levels of glucose with normal blood glucose levels. Patients with this disorder cannot reabsorb glucose to the typical capacity of 160 to 180 mg/dL. These patients will exhibit glycosuria and elevated specific gravity in their urinalysis.

Renal Tubular Acidosis

This is a group of primary or secondary disorders characterized by the impaired ability to secrete hydrogen ions in the distal tubule or to reabsorb bicarbonate ions in the proximal tubule leading to chronic metabolic acidosis. The resulting chronic metabolic acidosis causes potassium depletion and wasting, muscle weakness that can lead to paralysis, calcium loss in bone, elevated urine calcium loss, kidney stones, and renal failure.

Tubulointerstitial Disease

This term **tubulointerstitial disease** is used for infections and inflammatory conditions that affect both the interstitium and the tubules, which are in close proximity. UTIs can affect both the kidney (a tubulointerstitial disease) and the lower urinary tract and are the most common urinary tract diseases. Acute interstitial nephritis is an inflammatory tubulointerstitial disease.

ACUTE AND CHRONIC PYELONEPHRITIS

Pyelonephritis is an infection of the kidney and the ducts that carry urine away from the kidney (ureters). Pyelonephritis most often occurs as a result of UTI, particularly in the presence of occasional or persistent backflow of urine from the bladder into the ureters or kidney pelvis (vesicoureteric reflux) or obstructions. These infections most often arise as a result of ascending movement of bacteria from a lower UTI but may also be of a descending type, seeded from bacteria in the blood. See discussion of UTIs earlier in this chapter. Pyelonephritis can be further classified as follows: acute uncomplicated pyelonephritis (sudden development of kidney inflammation) or chronic pyelonephritis (a long-standing infection that does not clear). Chronic pyelonephritis is a more serious condition that can result in permanent damage to the kidney and chronic renal failure. **Cystitis** (bladder infection) is common; pyelonephritis occurs much less often. The risk is increased if there is a history of cystitis, kidney stones, renal papillary necrosis, vesicoureteric reflux, or obstructive uropathy. The risk is also increased when there is a history of chronic or recurrent UTI and when the infection is caused by a particularly pathogenic type of bacteria. Acute pyelonephritis can be particularly severe in the elderly and in people who are immunosuppressed (e.g., those with cancer or AIDS).

ACUTE INTERSTITIAL NEPHRITIS

Interstitial nephritis is a kidney disorder in which the spaces between the kidney tubules become inflamed. The inflammation can affect the kidneys' ability to filter waste. Interstitial nephritis may be acute or it may be chronic and gets worse over time. This condition is most often caused by an allergic reaction to a drug (acute interstitial allergic nephritis—side effect of medications such as some antibiotics, nonsteroidal anti-inflammatory drugs, furosemide, and thiazide diuretics). The acute form of interstitial nephritis is common. This disorder may be more severe and more likely to lead to chronic or permanent kidney damage in elderly people.

Renal Failure

Renal failure can occur rapidly or over time posing a serious or even life-threatening risk to the patient. This situation requires measures such as dialysis or kidney transplant to rid the body of toxic nitrogenous wastes that the kidney is no longer able to remove.

ACUTE KIDNEY INJURY (ACUTE RENAL FAILURE)

AKI is the sudden loss of the ability of the kidneys to remove waste and concentrate urine without losing electrolytes. There are many possible causes of kidney damage that precipitate this condition. They include decreased blood flow, which may occur with extremely low blood pressure caused by trauma, surgery, serious illnesses, septic shock, hemorrhage, some medications associated with crystalluria, toxins, radiology contrast dye, burns, or dehydration; acute tubular necrosis; infections that directly injure the kidney such as acute pyelonephritis or septicemia; urinary tract obstruction (obstructive uropathy); autoimmune kidney disease such as interstitial nephritis or acute nephritic syndrome; disorders that cause clotting within the small blood vessels of the kidney; transfusion reaction; and many more. These patients accumulate urea and other nitrogenous waste products and have decreased glomerular filtration rates. The patient's kidney disease status may be categorized as per the "RIFLE" categories below in Table 2-2. This classification system was developed by an expert panel of the Acute Dialysis Initiative group. These patients may display a variety of other conditions as well including loss of appetite, dehydration, fatigue, headache, nausea, vomiting, fluid imbalances, hypertension, elevated potassium levels, elevated BUN:serum creatinine ratio, low urine sodium, and oliguria. Mortality among ICU patients with AKI is high. Due to the high mortality and the importance of rapid disease detection in AKI, there is an ongoing search for better biomarkers for this disease. In September 2014, the U.S. Food and Drug Administration (FDA) permitted the marketing of a new commercial test, NephroCheck, which detects the presence of urinary insulin-like growth factor–binding protein 7 and tissue inhibitor of metalloproteinases-2.

Table 2-2 RIFLE Criteria for Acute Kidney Injury (Developed by a Panel of the Acute Dialysis Initiative Group)

	GFR CRITERIA	URINE OUTPUT
Risk	Serum creatinine ↑ 1.5× GFR ↓ >25%	<0.5 mL/kg/hr × 6 hrs
Injury	Serum creatinine ↑ 2.0× GFR ↓ >50%	<0.5 mL/kg/hr × 12 hrs
Failure	Serum creatinine ↑ 3.0× or GFR ↓ 75% Or serum creatinine ≥4 mg/mL	<0.5 mL/kg/hr × 24 hrs or Anuria for 12 hrs
Loss	Persistent acute renal failure Loss of kidney function >4 wks	
ESKD	End-stage kidney disease Loss of kidney function >3 mo	

HEPATORENAL SYNDROME

This syndrome usually occurs with fulminant hepatitis or advanced cirrhosis of the liver with ascites fluid buildup. Hepatorenal syndrome has an acute onset in these patients with severe liver disease. This condition is characterized by a progressively intense vasoconstriction which leads to oliguria, elevated BUN and creatinine, and renal failure. Despite renal failure, little renal morphologic changes can be seen in biopsy. The kidneys are still able to produce a smaller amount of hypertonic urine. This urine is very low in sodium due to hyperaldosteronism. The characteristic proteinuria of kidney disease is absent and the urine does not contain abnormal sediment. If the hepatic function can be restored, the kidney function can also improve.

CHRONIC RENAL FAILURE

The rate of **chronic renal failure** progression varies from several months to many years. This progression occurs in four stages: (a) diminished renal reserve (GFR drops to about 50% of normal), (b) renal insufficiency (GFR drops from 20% to 50%, azotemia, anemia, and hypertension begin), (c) renal failure (GFR is less than 20%, kidneys cannot regulate volume and solute concentration, and metabolic acidosis, edema, and hyperkalemia develop), and (d) end-stage renal disease (GFR less than 5% of normal, glomerular scarring and reduction of renal capillaries, tubular atrophy and fibrosis, and loss of kidney mass and dialysis or transplantation may be required for survival. Critical renal functions are lost.)

END-STAGE RENAL DISEASE—DIABETES

Currently, diabetes mellitus and related **diabetic nephropathy (Kimmelstiel–Wilson disease)** are the most common cause of end-stage renal disease. These patients can start out with mild kidney disease that over time begins to resemble nephrotic syndrome and ends with Kimmelstiel–Wilson glomerular nodules and kidney failure. Monitoring patients with diabetes for the presence of microalbuminuria is important for early detection of the onset of diabetic nephropathy. Control of blood sugar and blood pressure is critical for patients with diabetes to control damage of the kidney. Laboratory findings for this condition and other urinary tract disorders may be found in Table 2-3. Also, see Chapter 1 for information on the stages of chronic kidney disease and their associated GFR rates.

Tips for Categorizing Urinary Tract Diseases

The history and signs and symptoms will guide you and the urinalysis can provide valuable clues to the patient's disease. A chronic inability to vary the urine specific gravity along with

Table 2-3 Laboratory Findings in Urinary and Kidney Diseases

DISEASE/DISORDER	TYPICAL URINALYSIS FINDINGS	OTHER LABORATORY FINDINGS
Lower urinary tract infection	WBCs, RBCs, bacteria, mild proteinuria, increased pH	+ Urine cultures
Renal stones	RBCs, crystals, pH changes	
Acute poststreptococcal glomerulonephritis	Gross hematuria, proteinuria, RBC casts, hyaline, granular casts	+ Antistreptolysin O serology
Rapidly progressive glomerulonephritis	Gross hematuria, proteinuria, RBC casts	↑BUN, ↑creatinine, ↓creatinine clearance
Goodpasture syndrome	Gross hematuria, proteinuria, RBC casts	Antiglomerular basement membrane antibodies
Membranous glomerulonephritis	RBCs, proteinuria	Antinuclear antibodies, hepatitis B antigen, FTA-ABS
Membranoproliferative glomerulonephritis	RBCs, proteinuria	Serum compliment levels
Chronic glomerulonephritis	RBCs, proteinuria, glucose, cellular, granular, waxy, and broad casts	↑BUN, ↑creatinine, ↓creatinine clearance
Berger (IgA nephropathy)	Hematuria—as disease progresses findings resemble chronic glomerulonephritis	↑Serum IgA
Hemolytic uremic syndrome		Oliguria, ↑BUN, ↑creatinine, ↓creatinine clearance
Nephrotic syndrome	Heavy proteinuria, RBCs, RTEs, oval fat bodies, fat droplets, fatty and waxy casts	Decreased serum albumin, increased serum lipids
Minimal change disease	Heavy proteinuria, RBCs, RTEs, fat droplets	Decreased serum albumin, increased serum lipids
Focal segmental glomerulosclerosis	Proteinuria, RBCs	Drug testing, genetic testing
Alport syndrome	Heavy proteinuria, RBCs, RTEs, oval fat bodies, fat droplets, fatty and waxy casts	
Diabetic nephropathy	Microalbuminuria	Blood glucose, ↑BUN, ↑creatinine, ↓creatinine clearance, polyuria
Acute tubular necrosis	Proteinuria; microhematuria; RTE cells; RTE casts; hyaline, granular, waxy, broad casts	Hemoglobin, hematocrit, cardiac enzymes
Fanconi syndrome	Glucose, cystine crystals	Amino acids in urine, altered electrolytes
Nephrogenic diabetes insipidus	↓Specific gravity	↓Osmolarity Antidiuretic hormone Polyuria
Renal glycosuria	Glucose	
Renal tubular acidosis	Glucose	↑Urine calcium, kidney stones, ↑blood potassium
Acute interstitial nephritis	RBCs, proteinuria, WBCs, eosinophils, WBC casts	Eosinophils in urine may require staining ↑BUN, ↑creatinine, ↓creatinine clearance
Acute pyelonephritis	WBCs, RBCs, bacteria, proteinuria, increased pH, WBC casts	↑BUN, ↑creatinine, + urine cultures, + blood cultures
Chronic pyelonephritis	WBCs; RBCs; bacteria; proteinuria; increased pH; WBC casts; granular, waxy, broad casts	↑BUN, ↑creatinine, ↓creatinine clearance, + urine cultures, + blood cultures
Acute renal failure	Proteinuria, hemoglobinuria, RTEs and RTE casts, WBC and other casts, crystals	↓urine output with inability to vary specific gravity, ↑BUN, ↑creatinine, ↓creatinine clearance
Chronic renal failure	Proteinuria, hemoglobinuria, waxy and broad casts, crystals	↓Urine output with inability to vary specific gravity, ↑BUN, ↑creatinine, ↓creatinine clearance

Table 2-4 Urinalysis Casts and Renal Disease

CAST TYPE	LIKELY RENAL DISEASE/CONDITION
Red Blood Cell Cast	Acute glomerulonephritis
White Blood Cell Cast	Pyelonephritis, interstitial nephritis
Hyaline Cast	Strenuous activity, fever, diuretics, concentrated urine-dehydration
Renal Tubular Cast	Acute tubular necrosis, interstitial nephritis
Fatty Cast	Nephrotic syndrome, minimal change disease
Broad, Waxy Cast	Chronic renal disease, chronic renal failure
Granular Cast	Nonspecific, often contains cells that have broken down/degenerated or contains aggregates of plasma proteins, seen more often in chronic conditions, but not exclusively

1. A common finding in the urinalysis of a patient with urolithiasis is:
   - a. WBC casts
   - b. Azotemia
   - c. Microalbumin
   - d. RBCs or small amount of blood
2. This test is considered to be a hallmark of kidney disease the best urinalysis test for renal disease.
   - a. Creatinine
   - b. Protein
   - c. Microscopic crystal examination
   - d. BUN
3. The following urinary tests are helpful in examining patients with diabetes for kidney disease:
   - a. Microalbumin
   - b. GFR
   - c. Creatinine
   - d. All of the above are helpful
4. A urinalysis and blood work performed on a 13-year-old girl yield the following results:
   The above are MOST consistent with:

Specific gravity	1.015	WBC/HPF	5–10
pH	7.0	RBC/HPF	25–50
Protein	2+	Casts/LPF	
Glucose	Negative	Hyaline	0–2
Ketones	Negative	RBC	1–5
Bilirubin	Negative	Epithelial	0–1
Blood	3+	Coarse granular	0–1
Nitrite	Negative	Bacteria: rare	
Leukocyte	Positive	Uric acid crystals: moderate	
Urobilinogen	0.1 Ehrlich units/dL		
Blood tests: Anti-Streptolysin O titer = elevated.			

   - a. Yeast infections
   - b. Pyelonephritis
   - c. Acute glomerulonephritis
   - d. Renal failure
5. Which of the following casts is more associated with a chronic glomerulonephritis than with an acute glomerulonephritis?
   - a. WBC cast
   - b. RBC cast
   - c. Waxy cast
   - d. Hyaline cast
6. Which of these systemic diseases contribute to kidney damage and disease?
   - a. Diabetes mellitus
   - b. Systemic lupus erythematosis
   - c. Hypertension
   - d. Amyloidosis
   - e. All of the above
7. WBC casts are more likely to be indicative of which of these?
   - a. Cystitis
   - b. Urethritis
   - c. Pyelonephritis
   - d. Aminoaciduria
8. A 3-year-old girl has edema that is noticeable in her eyelids. Urinalysis and blood tests reveal the following results:
   Serum albumin: decreased
   Serum cholesterol: elevated
   Serum urea nitrogen: elevated

Urinalysis: protein 4+; hyaline, granular, and fatty casts

This is most compatible with:

a. Acute poststreptococcal glomerulonephritis
b. Minimal change glomerular disease
c. Acute pyelonephritis
d. Diabetes mellitus

9. Renal failure would most likely *not* be associated with which of these:

a. Systemic shock with drop in blood pressure
b. Transfusion reaction
c. Urine with a high specific gravity
d. ↓GFR

10. Kimmelstiel–Wilson disease is related to:

a. Alport disease
b. Acute glomerulonephritis
c. Diabetes
d. Urolithiasis

CASE STUDIES

Case 2-1 A 60-year-old man with a 17-year history of type 2 diabetes mellitus visits a clinic because of increasing swelling in his lower extremities. The swelling began approximately 7 months previously and has worsened over the past 6 to 8 weeks. The patient reports no recent illnesses and specifically denies any fever, chills, arthralgias, joint swelling, or skin rash. In addition, he reports no visual changes, epistaxis, hemoptysis, or cough. The patient's medical history is significant for hypertension, type 2 diabetes mellitus, and an appendectomy.

His medications include glyburide 10 mg daily, amlodipine 10 mg daily, and ibuprofen occasionally. He does not abuse alcohol, tobacco, or illicit drugs. No family history of kidney disease is present, although several of his family members have diabetes mellitus and heart disease. His physical examination reveals a healthy-appearing man in no acute distress. He has 3+ pitting edema up to the midcalf. His glomerular biopsy shows the lesion below (Fig. 2-5).

Blood tests: Hemoglobin A_{1c}, 8.1% ↑
Urinalysis findings:
Protein 4+
RBCs, 10–15/hpf
Renal tubular epithelial cells, few oval fat bodies, few Hyaline casts, few
GFR, 31 mL/min ↓ microalbumin 310 mg/24 hrs ↑

1. What is the most likely cause of the patient's renal disease?

a. Diabetic nephropathy
b. Focal and segmental glomerulonephritis
c. Acute glomerulonephritis
d. Membranous glomerulonephritis
e. Minimal change disease

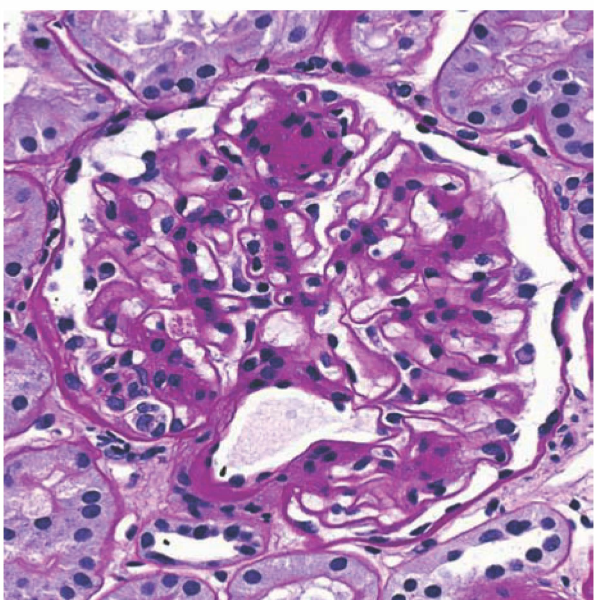

Figure 2-5. Glomerulus with single well-developed Kimmelstiel–Wilson nodule at 12 o'clock. Also note uniform thickening of GBM. (PAS, ×390.)

2. What is the most appropriate treatment?

a. Administer more amlodipine for better control of blood pressure
b. Begin insulin therapy for better glycemic control
c. Initiate therapy with an angiotensin-converting enzyme inhibitor
d. Restrict dietary protein to 0.6 g/kg body weight per day

3. In what stage of chronic kidney disease is this patient?

a. 2
b. 3AI
c. 3B
d. 4
e. 5

Case 2-2 A 39-year-old female suddenly notices her urine is a dark smoke color and she feels general malaise. Her urine output is decreased and she has edema with puffy eyelids. Her blood pressure has become elevated. She has a history of systemic lupus erythematosis, but her urinary symptoms, edema, and hypertension are new; so she goes to see her physician. The physician orders a BUN, creatinine, creatinine clearance test, and a urinalysis. Her results are below:

BLOOD TEST RESULTS	URINALYSIS RESULTS
↑BUN, ↑creatinine	Dipstick: 4+ protein, 4+ blood, 1+ leukocyte esterase
Also, her creatinine clearance shows a greatly decreased GFR	Other dipstick tests are negative or normal
	Microscopic examination: RBCs 25–50/HPF, many dysmorphic, RBC casts 5–10/LPF, hyaline cast 0–1/LPF

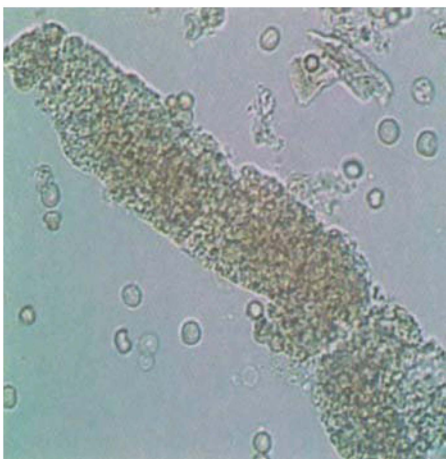

Figure 2-6. RBC cast: urine sediment; 400×. (University of Washington Department of Laboratory Medicine, with permission.)

1. Is this condition acute or chronic?
2. What do you suspect is her condition?
3. What are other conditions in this group?
4. What further tests could you do to confirm this?

Case 2-3 A 65-year-old female has a long history of microscopic hematuria and has had oliguria for a few years. She has been feeling fatigued and run down this past year and feels like she has been getting worse for a few years. She visits her physician and he orders a BUN, creatinine, creatinine clearance test, serum phosphorus, serum IgA, and a urinalysis. Her results are below:

BLOOD TEST RESULTS	URINALYSIS RESULTS
↑BUN, ↑creatinine, ↑serum phosphorus, ↑serum IgA	Dipstick: SG 1.010, 3+ protein, 4+ blood, 1+ leukocyte esterase, glucose trace
Also, her creatinine clearance shows a markedly decreased GFR	Other dipstick tests are negative or normal
	Microscopic examination: RBCs >100/HPF, WBCs 5–10/HPF, mixed casts—granular casts 5–10/LPF, hyaline cast 0–1/LPF, waxy casts 5–10/LPF, mixed cell casts 0–1/LPF, broad waxy casts 0–1/LPF
	Urine culture is no growth

1. Is this condition acute or chronic?
2. What do you suspect is her condition?
3. What are the other conditions in this group?

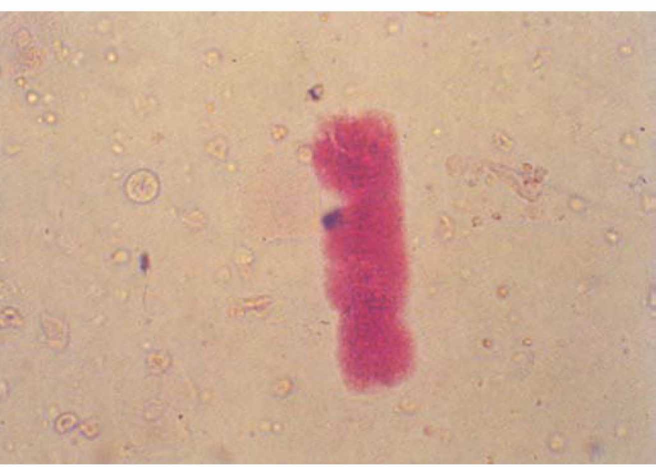

Figure 2-7. Broad finely granular cast becoming waxy cast with mixed cellular background. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott, 1998.)

4. Is the specific gravity significant in this case?
5. What additional problems does this urinalysis suggest?
6. What is the significance of broad and waxy casts?
7. Why might this patient have glucose in her urinalysis findings?

Case 2-4 A 67-year-old female has a history of kidney and circulatory problems and now has oliguria and marked edema. She visits her physician and he orders a BUN, creatinine, creatinine clearance test, blood lipid and albumin levels, a urinalysis, and a urine culture and sensitivity. Her results are below:

BLOOD TEST RESULTS	URINALYSIS RESULTS
↑BUN, ↑creatinine, ↑serum lipids, ↓serum albumin	Dipstick: SG 1.010, 4+ protein, 2+ blood, 1+ leukocyte esterase, glucose trace
	Other dipstick tests are negative or normal
Also, her creatinine clearance shows a decreased GFR	Microscopic examination: RBCs 20–25/HPF, WBCs 0–5/HPF, oval fat bodies, cholesterol crystals
	Mixed casts: granular casts 0–5/LPF, hyaline cast 0–1/LPF, waxy casts 0–1/LPF, mixed WBC/RTE casts 0–1/LPF, fatty casts 0–5/LPF
	The urine culture is no growth

1. Is this condition acute or chronic?
2. What do you suspect is her condition?

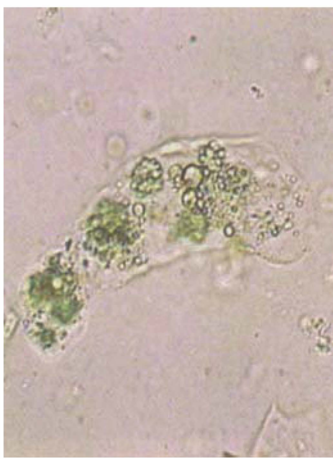

Figure 2-8. Fatty casts, urine sediment; 400×. (University of Washington Department of Laboratory Medicine, with permission.)

Figure 2-9. Fatty casts; polarized light (POL); urine sediment; 400×. (University of Washington Department of Laboratory Medicine, with permission.)

3. Is the specific gravity significant in this case?
4. What additional problems does this urinalysis suggest?
5. What is the significance of waxy casts?
6. Are renal tubular epithelial cells seen in this condition? Why?
7. What are oval fat bodies?
8. What type of microscopy helps visualize fatty casts?
9. Why might this patient have glucose in the urinalysis findings?

Case 2-5 A 5-year-old female has a history of previous lower UTIs. She suddenly becomes very ill and develops rust-colored urine, burning, back pain, and fever. The physician orders a BUN, creatinine, urinalysis, and a urine culture and sensitivity. Her results are below:

BLOOD TEST RESULTS	URINALYSIS RESULTS
↑BUN, ↑creatinine	Dipstick: 2+ protein, 1+ blood, 4+ leukocyte esterase, 4+ nitrate
	Other dipstick tests are negative or normal
	Microscopic examination: WBCs 25–50/HPF, RBCs 5–10/HPF
	WBC casts 5–10/LPF, hyaline cast 0–1/LPF, many bacteria
	Urine culture: >100,000 colonies/mL of *E. coli*, sensitivity pending

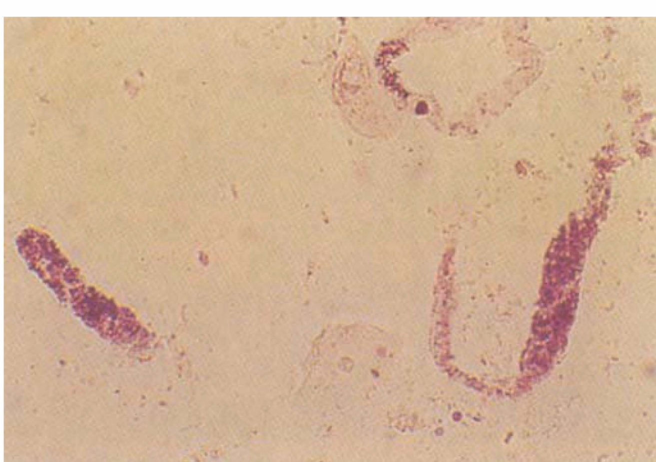

Figure 2-10. Granular casts, urine sediment. SM stain; 200×. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott, 1998.)

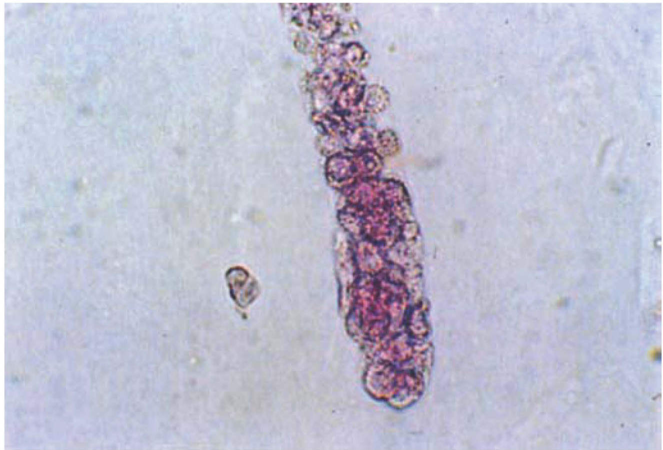

Figure 2-11. WBC cast, urine sediment. SM stain; 400×. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott, 1998.)

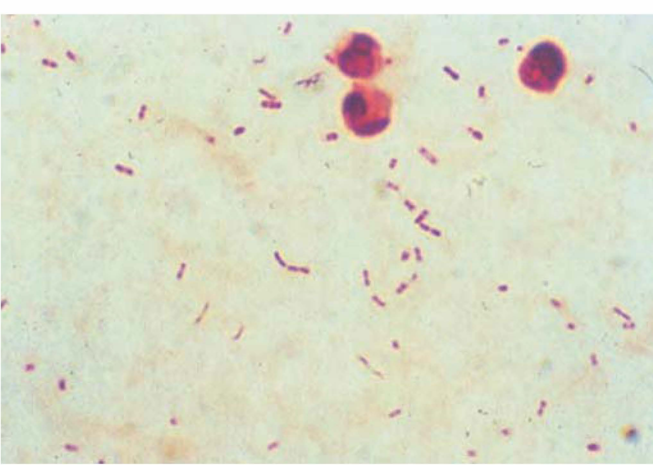

Figure 2-12. Direct Gram stain of urine showing gram-negative rod bacteria and three WBCs (neutrophils); 1000×. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott, 1998.)

1. What is this patient's condition?
2. What dipstick findings and what microscopic findings support this decision?
3. Is the patient's history significant?
4. If the child were taking vitamins and extra vitamin C, could this interfere with the test results?

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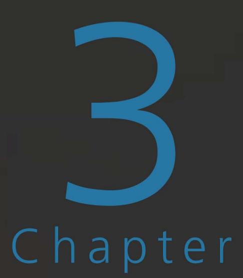
Metabolic Diseases and Related Urinalysis Findings



KEY TERMS

Albinism Alkaptonuria
Aminoaciduria
Cystinosis Cystinuria
Fatty Acid Oxidation Disorders
Galactosuria
Hartnup Syndrome
Homocystinuria
Inborn Error of Metabolism
Lesch–Nyhan Disease
Maple Syrup Urine Disease
Newborn Screening
Phenylketonuria
Porphyrinuria
Purines
Tyrosinemia

LEARNING OBJECTIVES

1. Describe the inborn errors that can result from deficiencies in metabolic pathways.
2. Discuss the current status of newborn screening methods in the laboratory.
3. List abnormal characteristics such as urinary odors, colors, crystals, and other laboratory findings.
4. Define and list the various types of aminoacidurias.
5. Identify the two most common fatty acid oxidation disorders.
6. Describe the metabolic deficiency in phenylketonuria and the dietary implications for the patient.
7. Describe the metabolic pathway deficiency resulting in tyrosinemia and albinism.
8. Differentiate between cystinuria, cystinosis, and homocystinuria.
9. Describe the synthesis of heme and its significance to the porphyrias.
10. List the causes for the major porphyrias.
11. Discuss how to differentiate between porphobilinogen and urobilinogen.
12. Describe a carbohydrate disorder that justifies testing for urinary reducing substances in newborns.
13. Name a metabolic disorder of purine metabolism.

mebooksfree

The term "inborn error of metabolism" (IEM) has historically been the descriptor used for explaining abnormal findings in infants. In metabolic diseases a variety of chemical substances are found in the blood or urine, reflecting alterations in amino acid, carbohydrate, protein, or other metabolic pathways. After birth, and especially after the infant is exposed to milk or foods, the biochemical abnormalities become detectable often before there is any clinical evidence of the disease. It is therefore important that diagnosis be made early and treatment be initiated, if possible. Blood spot newborn screening in the United States has expanded greatly from the initial work done by Guthrie in the early 1960s. A universal mandate of newborn screening applies to all states although the number of conditions screened for differ from state to state.

Genetic alterations are often grouped as aminoacidurias due to transport or metabolism dysfunction, carbohydrate metabolism error, fatty acid oxidation disorders, and porphryias. These inherited conditions may be due to an enzyme deficiency resulting in or causing accumulated metabolic byproducts that may damage organs of the body. Advances in laboratory methods provide the opportunity for a wide variety of conditions to be tested for by public health, private, or reference laboratory technologists. The current practice of using tandem mass spectrometry is described in the section on newborn screening methods. Although there are a number of possible inherited metabolic disorders, for which testing is performed such as endocrine disorders, hemoglobinopathies, and severe combined immune deficiency. This chapter focusses on the most common screening of metabolic disorders.

Newborn Screening

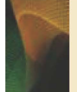
Newborn screening in North America can be documented in the early 1960s. Introduction of tandem mass spectrometry as a method of testing in the 1990s has greatly increased the number of conditions for which screening is available. Currently in the United States, state public health laboratories have evolved as the most cost-effective way to provide newborn screening for as many as 29 core conditions related to inborn errors of metabolism. Private laboratories also offer additional tests for these disorders. The National Newborn Screening and Genetics Resource Center (NNSGRC) maintains an information website concerning these disorders (see reference list). Genetic testing methods are continually being developed for these disorders.

Blood tests for newborn disorders are drawn from newborns by heelstick before the baby leaves the hospital. The blood is applied to filter paper, allowed to dry and then sent to the corresponding state department or regional health department. Use of blood samples is preferred to urine, because these substances from metabolic pathways are elevated in the blood before their elevation is detected in urine. Early detection is essential for follow-up and treatment for positive newborn screens. In many cases, diet modifications can help alleviate much of the damage from metabolic disorders.

Each State has a list of screening tests that it requires for infants born in that State. An example of information provided from the State of Illinois can be found on their website (see reference list).

Aminoaciduria

Aminoacidurias are inborn errors of metabolism that result in excess amino acids in the excreted urine. This group of metabolic diseases is frequently inherited in an autosomal recessive manner and are usually caused by the absence or inactivity of a specific enzyme required for normal metabolic activity. The types of aminoaciduria are often described as primary, which results from an enzyme defect in the pathway by which the amino acid is metabolized. A secondary aminoaciduria occurs when the defect relates to renal tubule transport or dysfunction. These disorders may result in abnormal urine colors, odors, and crystals (explained in Chapters 7 and 9).



Disturbances of Aminoacid Transport

CYSTINURIA

Cystinuria is an inherited renal tubular disorder. Transport by the carrier protein of the amino acids arginine, cytosine, lysine, and/or ornithine is defective. Because the amino acids cannot be reabsorbed from the glomerular filtrate, they build up and are excreted in the urine. Patients with this condition most likely have the pathological urinary crystal cystine, seen as a colorless hexagonal crystal in acid urine. Formation of renal calculi composed of cystine may also occur. In the past a cyanide–nitroprusside test was positive but current testing utilizes chromatography methods.

CYSTINOSIS

In cystinosis, the aminoaciduria is more generalized and not limited to arginine, cysteine, lysine, and ornithine. Cystine crystal deposits are found in the kidneys, eyes, bone marrow, liver, spleen, and macrophages. Thus this condition is considered a lysosomal storage disorder resulting from a defect in the transport passage of cysteine across lysosomal membranes. Cystine stone formation is rare in cystinosis.

Depending on the time of onset of symptoms, this condition may be life-threatening. When proximal tubular defects results in aminoaciduria along with glycosuria, phosphaturia, and proteinuria, the condition is referred to as Fanconi

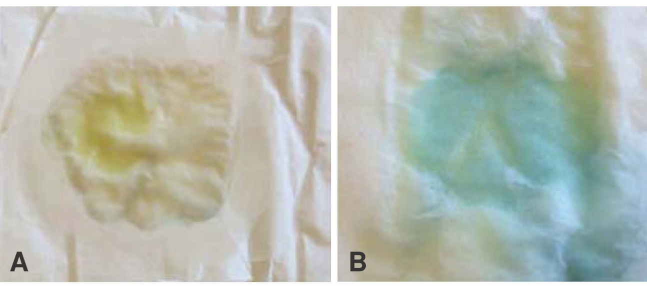


Figure 3-1. **A:** Diaper with urine from normal infant. **B:** Diaper with urine from infant with Hartnup syndrome.

syndrome. These crystal deposits in cells can lead to early renal failure. The milder adult forms of cystinosis have no kidney involvement. These patients have polyuria and positive urine tests for reducing substances. Their urinalysis tests show a lack of ability to concentrate urine resulting in a constant specific gravity known by the term isosthenuria.

HARTNUP SYNDROME

Hartnup syndrome is a relatively rare disorder that results in general difficulty for the body to absorb a variety of amino acids. As an example, the lack of reabsorption of tryptophan in newborns results in excess production of indole that is absorbed by the intestines and converted to indican which is excreted in the urine. Once the indican is exposed to air (oxidized) it changes to a blue color that is noted in the infant's diaper (Fig. 3-1). The malabsorption of tryptophan which is a precursor for nicotinamide, may also result in a skin rash usually resulting from exposure to sunlight. Some other symptoms associated with this condition include short stature, sensitivity to light, an unsteady gait, anxiety, rapid mood swings, hallucinations, delusions, speech difficulties, and abnormalities in muscle tone. Urine screening for the presence of amino acids is necessary to diagnose and suggest treatment for this condition. See the reference list for website information regarding Hartnup syndrome.

Disturbances of Aminoacid Metabolism

PHENYLKETONURIA

Phenylketonuria (PKU) is an IEM disease characterized by the absence or deficiency of the enzyme phenylalanine hydroxylase. This liver enzyme is needed to convert phenylalanine to tyrosine in the pathway demonstrated in Figure 3-2. When the enzyme is not available, the result is an excessive accumulation of phenylalanine and its metabolites in the body fluids. Figure 3-3 shows the normal metabolites of phenylalanine that become present in abnormal concentrations in PKU. PKA gets its name from the presence of high levels

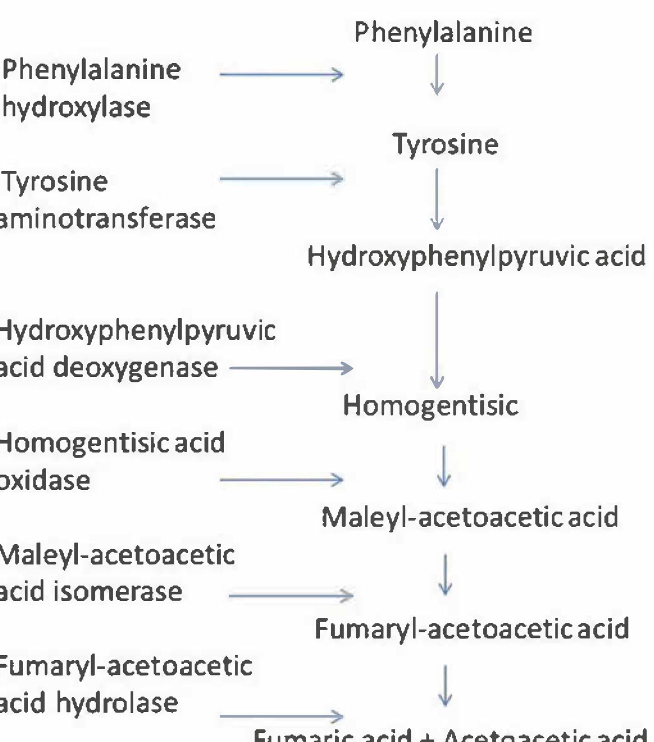


Figure 3-2. The normal metabolic pathway of phenylalanine metabolism.

of phenylketones in the urine, especially phenylpyruvic acid, a metabolite which gives the urine the characteristic "musty" or "mousy" odor.

This disease is inherited as an autosomal recessive gene and it occurs in greater than 1 in 25,000 newborns. If the disease is left untreated, the excessive levels of phenylalanine in the blood will cause brain damage resulting in severe mental retardation. Other characteristics of this disease include lighter skin and hair color, seizures, and susceptibility to eczema. Patients with PKU appear normal at birth but can become severely afflicted by age 1 if untreated. The treatment for PKU is a low phenylalanine diet. Screening of infants for PKU is included in all state newborn screening programs.

Because milk contains phenylalanine, an infant with PKU will show a rise in plasma phenylalanine within 1 or 2 days after the first few feedings. Urine levels of phenylalanine and phenylpyruvic acid do not become elevated until the infant is 1 to 6 weeks old.

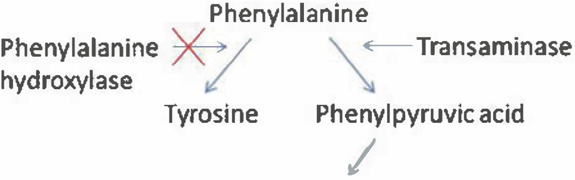


Figure 3-3. Increased formation of phenylalanine metabolites resulting from the deficiency of phenylalanine hydroxylase.

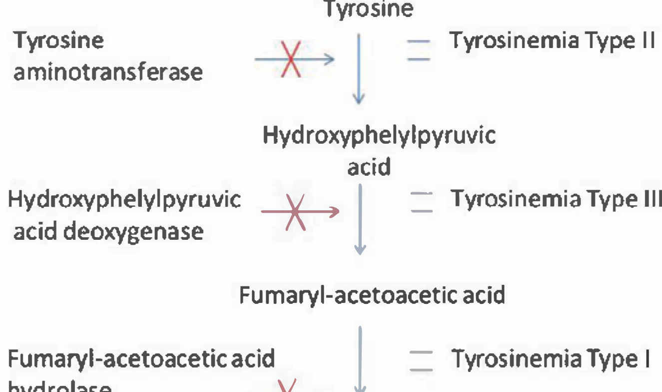


Figure 3-4. Types of tyrosinemia depend on the enzyme that is deficient.

Because the plasma phenylalanine level increases first, the required screening test is performed on blood before the newborn is discharged from the hospital, provided that the infant has been on milk feedings for at least 24 hours. Otherwise, the baby is tested on future outpatient physician visits. Older screening methods for this disorder include the Guthrie test and Phenistix, but these screening tests have largely been replaced by tandem mass spectroscopy of dried blood samples. Follow-up of a positive screening test often includes a repeat of the original sample or a request for a new sample from the infant. In addition, the primary care provider should conduct a clinical evaluation along with consultation with a genetic specialist.

TYROSINEMIA

Inherited disorders of tyrosine metabolism can result in three types of **tyrosinemia,** each caused by deficiency of a different enzyme (Fig. 3-4). The most severe form is Type I found in about 1 in 100,000 births and is caused by low levels of the enzyme fumarylacetoacetate hydrolase needed to break down tyrosine. Symptoms include failure to thrive, diarrhea, vomiting, jaundice, and possible liver and kidney failure. Type II is rarer and is caused by the deficiency of the enzyme tyrosine aminotransferase. Symptoms of mental retardation, photophobia, and eye pain may occur in these individuals. Only a few cases have been reported of Type III caused by the deficiency of the enzyme 4-hydroxyphenylpyruvate dioxygenase. Diagnosis of all types includes an elevated tyrosine level in blood using tandem mass spectroscopy. Treatment is a low protein diet.

ALBINISM

Albinism is a congenital disorder that results in the absence of melanin. Melanin is a pigment which occurs normally in the skin, hair, and in the choroid of the eye. It is derived from tyrosine (Fig. 3-5) and is normally not present in the urine.

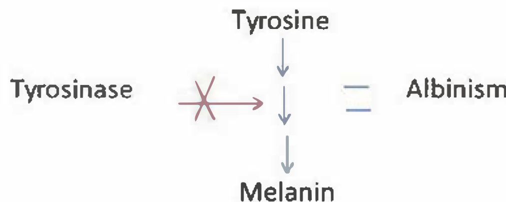


Figure 3-5. Tyrosine also has another metabolic pathway, the production of melanin. Albinism results from a deficiency in tyrosinase.

A deficiency in melanin production is associated with lack of color in hair skin and eyes and is referred to as albinism. There are different types of albinism.

Oculocutaneous albinism type 1 (OCA1 or tyrosinase-related albinism) results from a genetic defect in an enzyme called tyrosinase. This enzyme helps the body to change the amino acid tyrosine into pigment. There are two subtypes of OCA1. In OCA1A, the enzyme is inactive and no melanin is produced, leading to white hair and very light skin. In OCA1B, the enzyme is minimally active and a small amount of melanin is produced, leading to hair that may darken to blond, yellow/orange or even light brown, as well as slightly more pigment in the skin.

Oculocutaneous albinism type 2 (OCA2 or P gene albinism) results from a genetic defect in the P protein that helps the tyrosinase enzyme to function. Individuals with OCA2 make a minimal amount of melanin pigment and can have hair color ranging from very light blond to brown.

Oculocutaneous albinism type 3 (OCA3) is rarely described and results from a genetic defect in TYRP1, a protein related to tyrosinase. Individuals with OCA3 can have substantial pigment.

Oculocutaneous albinism type 4 (OCA4) results from a genetic defect in the SLC45A2 protein that helps the tyrosinase enzyme to function. Individuals with OCA4 make a minimal amount of melanin pigment similar to persons with OCA2. More information regarding albinism can be found at the website listed in the references.

ALKAPTONURIA

Alkaptonuria is a rare disease characterized by the excretion of homogentisic acid in the urine. It is due to the congenital lack of the enzyme homogentisic acid oxidase, which mediates an essential step in the catabolism of phenylalanine and tyrosine (Fig. 3-6).

The absence of homogentisic acid oxidase results in the accumulation and excretion of homogentisic acid (2,5-dihydroxyphenylacetic acid) in the urine which is normally not present. Visible darkening in urine that contains homogentisic acid may occur if allowed to stand up to 12 to 24 hours. This darkening is the result of the formation of polymerization products of homogentisic acid, and the process begins at the surface of the urine and gradually spreads throughout. Presence of ascorbic acid in the urine will interfere with

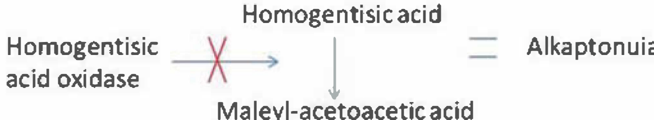


Figure 3-6. Alkaptonuria results from a deficiency in homogentisic acid oxidase.

this darkening process. Infants may have darkly stained diapers with a strong odor. Later in life, adults may present with arthritis and dark pigmentation of the cartilage (ochronosis).

In addition to lack of melanin production, patients with a tumor such as malignant melanoma excrete melanin or its colorless precursor, melanogen, in their urine. Upon exposure to air, melanogen is readily oxidized to the colored compound melanin, and urine that contains large quantities of melanin will become dark brown or black after standing for several hours (example shown in Chapter 7).

MAPLE SYRUP URINE DISEASE

Maple syrup urine disease (MSUD) results in an accumulation of branched chain amino acids such as valine, leucine, and isoleucine. The disease is caused by a deficiency of the enzyme α-ketoacid dehydrogenase. Symptoms occur soon after birth and a strong sweet-smelling urine odor resembling the smell of maple syrup is detected by 48 to 72 hours after birth. Medical nutrition therapy is used to rapidly reduce toxic metabolites by restricting dietary branched chain amino acids.

HOMOCYSTINURIA

Another inherited autosomal recessive disorder of amino acid metabolism is **homocystinuria** due to the lack of the enzyme cystathionine β-synthase necessary for metabolism of methionine. Increased plasma and urine homocysteine and methionine are found. The incidence is about 1 in 200,000 births and early symptoms are indistinct. Findings in late childhood include osteoporosis, mental retardation, dislocated lenses in the eye and thrombosis. Diet modification with reduction in methionine (low protein) and high doses of vitamin B6 may help prevent some of these adverse effects.

Disorder of Carbohydrate Metabolism and Transport

GALACTOSEMIA

A cellular deficiency of enzymes that catalyze the reaction in which galactose is converted to glucose is known as **galactosemia.** This results in an increase of galactose in the blood and urine. These enzyme deficiencies result in mental retardation that becomes evident within the first year of life. Galactose must be removed from the diet to prevent irreversible complications. Galactose is a reducing sugar that is undetected by urine multistix chemical reactions but will test positive with the copper reduction urine test known as the Clinitest. It is a standard laboratory practice to screen for reducing sugars in children 2 years old and younger.

Fatty Acid Oxidation Disorders

Fatty acid oxidation disorders are a group of inherited metabolic conditions that lead to an accumulation of fatty acids, and a decrease in cell energy metabolism. Each fatty acid oxidation disorder is associated with a specific enzyme defect in the fatty acid metabolic pathway and affects utilization of dietary and stored fat.

Newborn screening in some States includes testing for a panel of acylcarnitines. In some cases, an elevated level of a particular acylcarnitine may indicate the possibility of one of several different fatty acid oxidation disorders; the specific disorder cannot be determined without diagnostic further testing. More information regarding State of Illinois newborn screening can be found at the website listed in the references. The most numerous cases of the fatty acid group are the medium–chain-acyl-CoA dehydrogenase (MCAD) enzyme deficiency and the short–chain-acyl-CoA dehydrogenase (SCAD) enzyme deficiency.

Affected infants can be diagnosed in the neonatal period. Children with MCAD have a significant risk of death during the first, or subsequent clinical episode of hypoglycemia. In most cases, the first episode arises following illness or fasting, and occurs in infancy or early childhood. Fatty acid oxidation disorders can cause recurrent episodes of hypoglycemia. Clinical findings may include lethargy, hypotonia, failure to thrive, persistent vomiting, hepatomegaly, rhabdomyolysis, and Reye syndrome-like episodes.

Pophyrinurias

PORPHYRIAS

Porphyrins are complex iron-free cyclic substances which are intermediates in the biosynthetic pathway of heme (Fig. 3-7). They consist of four pyrrole rings linked by methane bridges to form a large ring structure (tetrapyrrole ring). The various types of porphyrins differ in the side chains which are present at the eight available positions on the pyrrole rings. The main sites of porphyrin production are the bone marrow and the liver. Porphyrins formed in the bone marrow are intermediates in the synthesis of hemoglobin, whereas porphyrins formed in the liver and other tissues are intermediates for other heme proteins such as myoglobin.

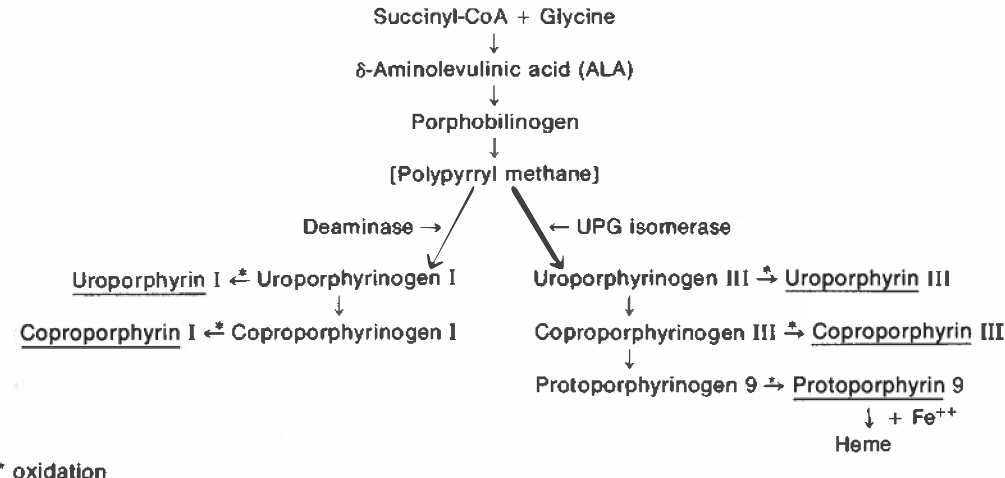


Figure 3-7. Biosynthesis of heme. (Porphyrins are underlined.)

Overproduction of porphyrin intermediates and/or their precursors by either the bone marrow or the liver causes increased urinary and fecal excretion of these substances as well as tissue accumulation. There are a variety of disorders related to porphyrin metabolism, some of which are inherited (e.g., congenital erythropoietic porphyria) and some of which are acquired (e.g., lead intoxication). Depending upon the disease, various porphyrins or precursors become elevated in the urine, blood, and/or feces. These disorders may have neurological symptoms, cutaneous symptoms, or both. Table 3-1 lists some of the major porphyrias and corresponding urinary findings.

A variety of porphyrins are present in urine. When there is an increase, particularly a large increase in total urine porphyrins, it is often useful to determine the individual porphyrin present. It is seldom important to do this if the total urine porphyrins is normal.

A common method for separating the individual porphyrin is high performance liquid chromatography which may be performed in the chemistry department.

Porphobilinogen (PBG) and the porphyrinogens (uro-, copro-, and proto-) are colorless, nonfluorescent substances, whereas the oxidized forms of the porphyrins have red pigments which exhibit fluorescence when viewed under an ultraviolet light (Fig. 3-8) such as a Wood lamp. Urine samples which contain large amounts of porphyrins may have a port-wine or burgundy color (Fig. 3-9), or it may become dark red only after standing. The urine color depends on the type of porphyrin disorder.

Table 3-1 Porphyrin and Porphyrin Precursors in the Urine

	URINARY FINDINGS			
DISORDER	**ALA**	**PBG**	**CP**	**UR**
Inherited				
Congenital erythropoietic porphyria (Gunther disease)	N	N	↑ (I)	↑↑ (I)
Acute intermittent porphyria (acute attack)	↑↑	↑↑	↑	N or ↑
Variegate porphyria (chronic)	N	N	N or ↑	N or ↑
Variegate porphyria (acute)	↑↑	↑↑	↑	↑
Hereditary coproporphyria (acute)	N or ↑	N or ↑	↑ (III)	N
Acquired				
Lead intoxication	↑↑	N or sl ↑	↑↑ (III)	N or sl ↑
Acquired porphyria cutanea tarda (symptomatic porphyria)	N	N	↑	↑↑

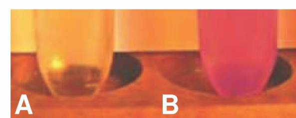


Figure 3-9. **A:** Normal urine. **B:** Urine containing porphyrins.

1. Many metabolic byproducts from inborn errors appear first in:
   a. Blood
   b. Urine

c. Spinal fluid
d. Mucus

2. The common method for analysis of blood samples from newborns is:
a. HPLC
b. Gas chromatography
c. Tandem mass spectrometry
d. Nepholmetry

3. Hartnup syndrome is due to the lack of absorption of which amino acid?
a. Arginine
b. Ornithine
c. Cysteine
d. Tryptophan

4. Albinism results from a deficiency of producing which substance?
a. Melanin
b. Cysteine
c. Tyrosine
d. Purine

5. Porphyrias result when there is a breakdown in the synthesis of:
a. Bilirubin
b. Heme
c. Proteins
d. Amino acids

6. Cystinuria and cystinosis are conditions that result from the disturbance of amino acid:
a. Transport
b. Absorption
c. Metabolism
d. Excretion

7. The metabolic condition that results in a medium–chain-acyl-CoA dehydrogenase (MCAD) enzyme deficiency is found in which group?
a. Aminoaciduria
b. Porphyria
c. Fatty acid oxidation disorder
d. Purine disorder

8. Which of the following aminoacidurias is most common and results in urine that has a "mousy" odor?
a. Indicanuria
b. Melaninuria
c. Phenylketonuria
d. Fanconi disease

9. Which of the following diseases results in the production and excretion of large amounts of homogentisic acid?
a. Melanuria
b. Tyrosyluria
c. Alkaptonuria
d. Maple syrup urine disease

10. A carbohydrate metabolic disorder found in newborns following introduction of milk in the diet is known as:
a. Diabetes insipidus
b. Maple syrup urine disease
c. Galactosemia
d. Fructosuria

11. In which of the following disorders can patients have urine that has a sweet odor?
a. Fanconi disease
b. Maple syrup urine
c. Phenylketonuria
d. Tyrosinuria

12. In which of the following disorders can patients have urine that darkens upon standing? (choose all that apply)
a. Alkaptonuria
b. Maple syrup urine
c. Melaninuria
d. Porphyria

13. In which of the following disorders can infants have urine that contains uric acid crystals, which appears as orange sand in their diapers?
a. Fanconi disease
b. Lesch–Nyhan disease
c. Maple syrup urine
d. Phenylketonuria

14. A patient whose urine is port-wine in color may have:
a. Fanconi disease
b. Hartnup disease
c. Lesch–Nyhan disease
d. Porphyria

15. In which of the following disorders can infants have urine that becomes blue upon exposure to air?
a. Fanconi disease
b. Hartnup disease
c. Lesch–Nyhan disease
d. Porphyria

16. Oculocutaneous albinism type 1 is caused by a defect in:
a. P protein
b. SLC45A2 protein
c. TYRP1 protein
d. Tyrosinase

CASE STUDIES

Case 3-1 A 42-year-old man presented to the emergency room with hematuria and pain. He reported a history of kidney stones going back to his teen years and said he had been told he had a metabolic disorder. A urinalysis gave the following results.

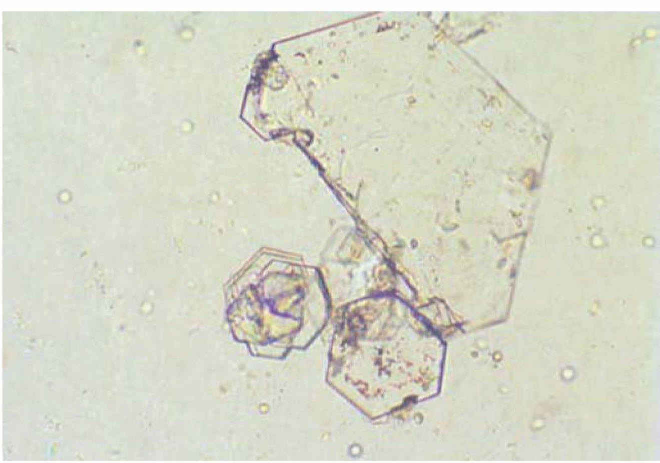


Figure 3-11. Image for Case study 3-1.

Color:	Yellow
Appearance:	Hazy
Specific gravity:	1.010
pH:	6.0
Protein:	2+
Glucose:	Negative
Ketone:	Negative
Bilirubin:	Negative
Blood:	4+
Urobilinogen:	1.0 EU
Nitrite:	Negative
Leukocyte est.:	1+
WBCs:	5–10/HPF
RBCs:	>100/HPF
Bacteria:	2+
Casts:	5–10/LPF (mixed)
Crystals:	Many (see Fig. 3-11)

1. What metabolic disorder does this patient most likely have?
2. Are these typical findings for kidney stones?
3. What causes this disorder?
4. Name a disorder with which this may be confused.
5. Name three major differences between these two disorders.
6. What further tests could be done?

Case 3-2 A 37-year-old female was seen by her physician because she was having nausea and weight loss. No abnormalities were found upon physical examination, however the patient's history include the recent employment with a company that performs lead-abatement for various facilities. The patient does not experience any photosensitivity but her urine does become reddish upon standing.

1. What is the disorder that this patient displays?
2. What further testing may be performed and what are the expected results in this case?
3. Why is this patient not sensitive to sunlight?

Case 3-3 A 4-month-old infant was brought to the emergency department because of vomiting and diarrhea. The physical examination revealed an enlarged liver and spleen. Urinalysis testing gave the following results.

Color:	Yellow
Appearance:	Cloudy
Specific gravity:	1.010
pH:	7.0
Protein:	1+
Glucose:	Trace
Ketone:	Negative
Bilirubin:	Negative
Blood:	Negative
Urobilinogen:	0.2 EU
Nitrite:	Negative
Leukocyte est.:	Negative
WBCs:	0–5/HPF
RBCs:	none seen
Bacteria:	none seen
Casts:	Rare hyaline
Crystals:	Moderate (see Fig. 3-12)

1. The symptoms and urine findings shown by this infant may indicate which diagnosis?
2. What is the most common form of this disorder and what is the cause?
3. What additional test would be helpful in establishing a diagnosis and what are the expected results?

Figure 3-12. Image for Case study 3-3.

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Urinalysis Clinical Laboratory Operations

4
Chapter

KEY TERMS

Centers for Disease Control and Prevention (CDC)
Centers for Medicare & Medicaid Services (CMS)
Chain of Custody
CLIA '88
Clinical Laboratory Standards Institute (CLSI)
College of American Pathologists (CAP)
Commission on Office Laboratory Accreditation (COLA)
Critical Values
Delta Check
Department of Health and Human Services (HHS)
Department of Transportation (DOT)
Environmental Protection Agency (EPA)
Food and Drug Administration (FDA)
Globally Harmonized System (GHS)
Hazardous Materials (HAZMATs)
Hazard Statement
Health Insurance Portability and Accountability Act (HIPAA)
Levels of Laboratory Testing
Nuclear Regulatory Commission (NRC)
Occupational Safety and Health Administration (OSHA)
Personal Protection Equipment (PPE)
Physician Office Laboratories (POLs)
Pictogram
Point of Care Testing (POCT)
Precautionary Statement
Preexamination, Examination, and Postexamination Errors
Proficiency Testing
Public Health Service Act
Quality Assessment (QA)
Quality Assessment and Process Improvement (QAPI)
Quality Control (QC)
Safety Data Sheets (SDS)
Signal Word
Standard of Care
Standard Precautions
The Joint Commission (TJC)

LEARNING OBJECTIVES

Clinical Laboratory Regulation and Management

1. Define compliance and discuss how it relates to urinalysis and body fluid analysis.
2. List the four categories of clinical laboratory testing under CLIA '88 and list which personnel may perform laboratory tests in these categories.
3. Write a synopsis of the federal regulations and federal regulatory agencies that govern activities of the clinical laboratory and state their corresponding jurisdictions.
4. Discuss external accreditation and CLSI standards and their importance in laboratory management and compliance.
5. Describe legal and ethical concerns related to the clinical laboratory.
6. Write a summary of the scope of and importance of quality assessment.
7. Explain how the components of quality assessment are implemented in the clinical laboratory.

Safety in the Clinical Laboratory

8. Explain the responsibility of laboratories to develop and publicize safety policies and procedures.
9. Identify and describe six types of safety risks that exist in the clinical laboratory.
10. Discuss the effective management of safety concerns in the clinical laboratory.
11. Comply with standard precautions; proper use of PPEs, handling of hazardous materials, and disposal of sharps in the laboratory.
12. Compare chemical labeling systems.
13. Interpret the meaning of various safety symbols and pictograms.

Urinalysis and body fluid analysis are performed in the clinical laboratory, which is a part of the healthcare organization. Both healthcare organizations and clinical laboratories have differing organizational configurations and offer a variety of services to physicians and patients with the goal of providing the best possible patient care. Providers of healthcare services must continually assess, update, and adjust their services to achieve optimal outcomes for the patients they serve. This requires managers and leaders in laboratory medicine to have knowledge and expertise in scientific, medical, and technical matters as well as comprehension of related government regulations and safety issues. It is part of the duties of laboratory managers and leaders to disseminate this knowledge to the laboratory staff and to monitor and ensure compliance to government regulations and adherence to established institutional policies and procedures.

Regulations and Regulatory Agencies Governing the Clinical Laboratory

Various federal, state, county, and city regulations apply to the clinical laboratory. In addition to federal and national professional groups that inspect the clinical laboratory, states have additional laboratory inspections as well as their own penalties for noncompliance to their regulations.

FEDERAL REGULATIONS AND REGULATORY ORGANIZATIONS

1. **CLIA '88**—Clinical Laboratory Improvement Amendments of 1988.
   Most hospital laboratories, **physician office laboratories (POLs)**, and reference laboratories for clinical testing as well as **point of care testing (POCT)** are regulated by CLIA '88. An exception is that federal laboratories, such as veteran's hospitals and medical centers, are not covered by these requirements. The regulations govern how clinical laboratories perform their work. These regulations were put in place to ensure that laboratory test results are of high quality regardless of where the tests are performed. Included are mandates for quality control, proficiency testing, quality assessment, external inspections, site visits, consultations, and minimum personnel requirements. Also established are regulations that vary with the level of testing. All clinical laboratory testing is divided into one of the following **levels of laboratory testing**:
   - **Provider-Performed Microscopy (PPM)**—This category includes brightfield or phase microscopy tests performed by physicians, dentists, or other midlevel practitioners under physician supervision. Included are wet mounts, KOH preps, fern tests, postcoital examinations, urine sediment examinations, and microscopic examinations for WBCs. PPM allows physicians to obtain results on labile samples that must be tested immediately.
   - **Waived Testing**—These tests are approved by the Food and Drug Administration (FDA) for home use and are designed to minimize testing errors and pose no reasonable risk of harm to patients when performed inaccurately.
   - **Moderate Complexity Testing**—These tests are more difficult to perform than the waived tests in the POL. Instrument calibration, training documentation, proficiency testing, and onsite inspections are required under CLIA '88. In the hospital setting, both MCT and WT must adhere to moderate complexity test standards. Most hematology, clinical chemistry, and automated or semiautomated urinalysis and urine microscopic analysis fall into this category.
   - **High-Complexity Testing (HCT)**—These tests require a high degree of interpretative knowledge and skill and must be performed by more experienced personnel and/or more complex instrumentation. Many tests performed in the cytology, immunology, immunohematology, microbiology, and molecular diagnostics departments fall into this category.
2. **Public Health Service Act.** To receive payment from Medicare or Medicaid, a laboratory must be licensed under this act. This act mandates adherence to CLIA '88.
3. The **Centers for Medicare & Medicaid Services (CMS)** is under the **Department of Health and Human Services (HHS)**. This federal agency has established regulations to implement CLIA '88 and has also established **Commission on Office Laboratory Accreditation (COLA)** for accrediting POLs. COLA-accredited laboratories are surveyed every 2 years.
4. **Occupational Safety and Health Administration (OSHA)**. This government agency regulates issues of worker safety for the clinical laboratory. As a laboratory worker you have the right to a safe working environment and can report unsafe work practice concerns to OSHA. The employer is not to retaliate in any way for such reporting and will be penalized for any such actions.
5. **Environmental Protection Agency (EPA)**. This agency ensures that healthcare providers follow the Medical Waste Tracking Act. The act defines medical waste and establishes acceptable practices for treatment and disposal of this waste.
6. **Food and Drug Administration (FDA)**. This governmental agency is responsible for the approval of medical and diagnostic equipment, pharmaceuticals, reagents, and diagnostic tests before these can be marketed. The FDA also regulates content labeling requirements. Prior to product approval, the FDA evaluates the safety, efficacy, and medical need for medical products and devices.

7. **Centers for Disease Control and Prevention (CDC)**. This agency implements public health regulations and reporting requirements for the clinical laboratory and other healthcare providers. CDC is responsible for categorizing newly developed laboratory tests as WT, MCT, or HCT and also performs CLIA-related studies.
8. **Department of Transportation (DOT)**. This agency has requirements for the safe packaging and transport of biologically hazardous and other hazardous materials (HAZMATs).
9. **Nuclear Regulatory Commission (NRC)**. This agency regulates handling and disposal of radioactive materials. Although the clinical laboratory tries to minimize the use of these agents, there are still some tests involving these substances.

More information about regulations and practices mandated by CLIA '88 can be found at the government web site http:www.cms.hhs.gov/CLIA.

EXTERNAL ACCREDITATION AND INSPECTION

In order for a healthcare organization to engage in and receive payment from federal Medicare or Medicaid programs, it must be certified by CMS as complying with the Conditions of Participation set forth in federal regulations. CMS may grant the accrediting organization "deeming" authority so it may "deem" an accredited healthcare organization as meeting the Medicare and Medicaid certification requirements. This healthcare organization would then have "deemed status" and would not be subject to the Medicare survey and certification process.

The three main external laboratory accreditation agencies are as follows:

- **College of American Pathologists (CAP)**—This professional organization has deemed status to provide this service for the federal government.
- **Commission on Office Laboratory Accreditation (COLA)**—This commission is administered through the CMS. This office is under the HHS.
- **The Joint Commission (TJC)**—formerly Joint Commission on Accreditation of Healthcare Organizations (JCAHO), this organization also has status to provide this service for the federal government.

Other organizations that inspect or accredit laboratories include state agencies, American Society for Histocompatibility and Immunogenetics (ASHI), American Association of Blood Banks (AABB), and American Osteopathic Association (AOA).

These organizations provide a valuable service to laboratories by regular assessment, through the inspection process, of compliance to regulations and evaluation of an individual laboratory's policies and practices.

Laboratory Standards

TECHNICAL STANDARDS

The **Clinical Laboratory Standards Institute (CLSI)** is a nonprofit, private educational organization that develops and publishes national and international laboratory standards on a variety of clinical laboratory testing procedures and policies. These guidelines assist clinical laboratories in the development of acceptable procedures and policies for their institutions. CLSI recommendations and standards follow the CLIA '88 mandates and assist the clinical laboratory in adhering to federal regulations.

LEGAL AND ETHICAL CONCERNS

In addition to laws regulating clinical laboratories, laws also protect patients' rights. Beyond established law, healthcare professionals have ethical obligations to treat patients as they would like to be treated if under their care. Most often, minor problems will not result in legal action. Nonetheless, laboratory professionals have a moral and ethical obligation to treat patient with respect and incorporate compassion and concern for the patient into your decisions and actions. In addition, laboratory personnel should keep informed of and follow established procedures and policies at their institution. In case an event occurs that might have legal or ethical implications for patients or employees, an incident report is usually completed to document the event.

INFORMED CONSENT

The laboratory has an obligation to ensure that the patient understands the testing to be performed and that the patient gives consent to this testing. The patient has the right to refuse testing. If the patient does not speak English, efforts should be made to find an interpreter, or a guardian may be needed for minors or patients with certain disabilities. For certain complex procedures or procedures with important risks, a written informed consent form may be required.

STANDARD OF CARE

Laboratory employees have the responsibility to know and follow the accepted **standard of care** defined as the care that a reasonable laboratory professional should provide. Implied in this definition is the knowledge and use of acceptable procedures and patient care. If a laboratory provider does not provide this standard of quality care and serious complication or death results, medical negligence may be charged. Continuing education is important for laboratory personnel to keep abreast of changes in acceptable practices for the laboratory.

CONFIDENTIALITY

The **Health Insurance Portability and Accountability Act (HIPAA)** of 1996 mandates the privacy of patient information. Patient information, the tests they are having done and their laboratory results, must be kept strictly confidential. This confidential information is not to be shared with insurance companies, lawyers, or relatives of the patient unless they are authorized to have this information.

SPECIMENS FOR LEGAL CASES

When a specimen is collected for a case that may involve litigation, special safeguards are recommended to protect the rights of all those involved. Laboratory workers are required to know and adhere to the established policies of their facility for these specimens for legal cases. These types of specimens include blood-alcohol levels, specimens from rape cases, specimens in paternity cases, and medical examiner's specimens. If a laboratory professional does not know the policies for these samples, he or she will be negligent in his or her duty to the patient. With specimens for legal cases, **chain of custody** must be maintained. This means that the specimen must be collected and handled in a particular manner with the names of all individuals obtaining, handling, and testing the specimen documented. These specimens should be kept in a locked or secure refrigerator to prevent specimen tampering.

Quality Assessment

CLIA '88 mandates that **quality assessment** (monitoring of laboratory testing and reporting accuracy) activities be a continual process in the laboratory and that these efforts be documented. Results from quality assessment activities must be evaluated and communicated in order to reduce medical errors and improve patient outcomes.

The Clinical and Laboratory Standards Institute (CLSI) has categorized laboratory performance standards into three phases: **preexamination** (pretesting), **examination** (testing), and **postexamination** (post-testing). This current terminology was adopted by CLSI in 2010 to align its documents with the preferences of the International Organization for Standardization (ISO).

VARIABLES AFFECTING LABORATORY TEST QUALITY

Errors can occur throughout the testing process and include errors from preexamination, examination, and postexamination sources. Quality control is used to monitor the examination (or testing) process. This is critical to ensure the accuracy and precision of laboratory test results. It is not sufficient, however, as steps must also be taken to reduce these. Table 4-1 provides examples of behaviors that can lead to preexamination, examination, and postexamination errors.

QUALITY ASSESSMENT PROCESS

Quality assessment should include a process of maintaining qualified personnel, establishment of written policies, a procedure manual with appropriate methods, establishment of procedures for specimen collection and handling, an equipment maintenance program, established quality control and quality assurance programs, and methods to ensure accurate test ordering and reporting. Issues of patient service and wait times are other examples of quality issues that may be studied in quality assessment.

Essentially, effective management of communication, of adherence to policies, and of documentation must govern laboratory practice. This consists of clearly written laboratory policies and procedures and established policies that are

Table 4-1 Examples of Laboratory Testing Errors

PREEXAMINATION ERRORS	EXAMINATION ERRORS	POSTEXAMINATION ERRORS
Patient identification errors	Technologist error	Computer result entry error
Improper patient preparation	Instrument calibration error	Test interpretation errors
Inappropriate test orders	Reagent deterioration	Illegible report
Incorrect container/additives	Pipetting errors	Failure to deliver report
Specimen labeling errors	Instrument bias or failure	Incorrect patient information
Improper specimen collection or	Test procedure steps not followed	Transcription errors
Improper timing of collection	Timing errors while running	Delayed report
Hemolyzed or contaminated specimen	Instrument not operated correctly	Failure to phone critical results

known and followed by all. Proper laboratory result reporting requires establishing and rapid reporting of critical values. The documentation of results that are telephoned to the physician is also required. To avoid errors in laboratory result reporting, the **delta check** (comparing current test results with previous test results) is used to monitor changes in individual patient results and to assess whether these changes are biologically possible. The practice or results review prior to release, and cosigning serves to reduce reporting errors. Most laboratories have established a **critical values** list, with test results that are important enough to be called to the physician immediately. Despite the greatest efforts, errors will still occur. It is important that errors are acknowledged promptly, properly documented, and follow-up measures are taken.

PROCESS IMPROVEMENT

Laboratories must have a system in place that looks at how laboratory processes affect the quality of laboratory results and ultimately patient outcomes. This system is usually referred to as **quality assessment and process improvement (QAPI)**. When an event occurs that may affect quality, various forms of documentation are used to record the event. As stated earlier, errors may occur at any point in the laboratory testing process, preexamination, examination, and postexamination. An audit of these records is conducted to discover weaknesses in the process that was being followed at the time of the occurrence. A committee or individuals may perform these audits and make recommendations for improvements to the process. Once changes are implemented, results are monitored to see if the process problems were eliminated. Efforts to improve processes may require better training or retraining of staff. The ultimate goal of quality assessment and process improvement is to ensure the quality of care for our patients.

QUALITY CONTROL

Quality control (QC) is a set of procedures and practices that monitor the testing process and those procedures that verify the reliability, accuracy, and precision of testing. Standards and controls are used in this process. Standards contain a known amount of the analyte being tested and are used to calibrate the test.

Controls are materials of the same matrix as the sample (composed of serum for serum tests and urine for urine tests) that have an established acceptable range for the analyte being tested. The controls are always run with the test, and control values are monitored statistically to assess the validity of the test results. If the controls do not fall in acceptable range, the test results may be invalidated. By monitoring the control values daily or with each shift, the accuracy and precision of the test method can be observed.

Controls are usually in the normal patient level and in the clinically significant abnormal level(s) (usually high and possibly also low levels). Quality control must be recorded and analyzed to be of any benefit. Abnormal quality control results must be noted by the technologist performing it and the supervision must be notified as well. The supervisor and laboratory administrators also have the obligation to review the quality control records to look for both random problems and trends or repeat problems. Most urinalysis and body fluid procedures are qualitative or semiquantitative, but if quantitative testing is performed, monitoring with a systematic statistical analysis such as through the use of Westguard rules should be performed as well.

Other components of quality assessment include validation of new procedures, new lots of reagents, and new shipment of current reagent lots. In addition, correlation of an individual patient's results among all laboratory tests should be made to ensure quality patient care.

PROFICIENCY TESTING

External **proficiency testing** is mandated by CLIA '88. To conduct proficiency testing, agency such as College of American Pathologists (CAP) provides unknown samples for each of the tests that your laboratory issues. The laboratory is to run these samples as it would a patient sample and then report the results to the issuing agency. The laboratory results of each participating agency are compared with the results of designated reference laboratories. Internal proficiency testing is also useful to detect problems within your laboratory. A supervisor may include an internal sample without the knowledge of the laboratory staff and check the results against known results or results from another laboratory. These exercises point out areas of testing deficiencies for the participating laboratories.

Safety in the Clinical Laboratory

Regulations at all levels of government and employer policies mandate safe practices to protect everyone involved in health care—employees, patients, and visitors. It is critical to familiarize yourself with potential risks in your laboratory. Such risks should be identified whenever possible. One way to achieve this is through labeling of potential hazards through the use of signage. Laboratory workers should recognize common safety symbols (Fig. 4-1).

Laboratories must also develop their own safety policies and must create safety manuals that are accessible to all personnel. A designated safety officer is integral to the implementation of a laboratory safety program. The safety officer holds responsibility for compliance with existing safety regulations and adherence to safety policies. Employees should file an incident report if there is any event involving safety of a patient or for themselves.

Figure 4-1. Common safety symbols. (Courtesy of McBride L. *Textbook of Urinalysis and Body Fluids.* Philadelphia, PA: Lippincott; 1998.)

Physical Hazards

As in many workplace environments, the laboratory contains many mechanical devices which cause accidents if they malfunction or are used improperly. Commonsense precautions also apply to the laboratory; avoid running or rushing, watch for wet floors, a void dangling jewelry, tie back long hair, operate laboratory equipment as recommended by the manufacturer, and maintain an organized and clean workspace. It is also important to get enough help when lifting heavy items and remember to bend your knees when lifting anything awkward or heavy. Try to create a workspace that is ergonomically friendly to avoid long-term health problems.

Electrical Hazards

Electrical burns, shocks, and electrocution are avoided by the prevention of electrical potentials across laboratory personnel. Fuses, circuit breakers, and ground fault interrupters are used to prevent overloaded circuits that could cause fire or explosion. Three-pronged grounded plugs provide protection from a possible short between one side of the power line and the instrument or the person touching the instrument. Do not use equipment that has just had liquid spilled on it or with wet hands. If equipment is damaged, malfunctions, smells unusual, or makes a loud noise, it should be turned off. In addition, electrical cords should not be stretched and should not be used if frayed or damaged.

Fire and Explosive Hazards

Every effort should be made to prevent fire and explosion. Circuit overload, misuse of chemicals, lack of training, and carelessness are causes of fires and explosions in the laboratory. A safety committee should be formed to set policies and to form an evacuation plan in case of fire. Employees need to be trained in the proper use of chemicals and equipment and they need to know hospital policies in case of fire.

Fire extinguishers must be readily available and employees must also be trained in their use (see Table 4-2 on classes of fire extinguishers and their uses). In case of fire, remember to rescue those who need immediate help, pull the alarm or phone in the alarm, contain the fire as much as possible, and extinguish if possible. All should be evacuated from the area of the fire quickly. Participation in fire drills assist in speeding the process in the event of a real fire. Remember to "RACE" (Rescue, Alarm,Contain,and Extinguish) and to evacuate as needed.

Chemical Hazards

Chemicals found in clinical laboratories can be hazardous and employees must be aware of proper use, storage, and handling of these chemicals. OSHA document 29 CFR 1910 and State "right to know documents" contain standards for communication that informs employees about chemical hazards present in their work environment. OSHA requires employers to

Table 4-2 Classes of Fire Extinguishers and Their Uses

CLASS	USE	WATER EXTINGUISHER	DRY EXTINGUISHER
A	Ordinary combustible materials, paper	Yes	Yes
B	Flammable liquids and gases	No (spreads liquid and fire)	Yes
C	Electrical equipment	No (risk of shock)	Yes
D	Combustible metals	No (intensifies fire)	No (sand or special extinguishing agents)

Remember "PASS" when using the extinguisher—Pull, Aim, Squeeze, and Sweep the base of the fire.

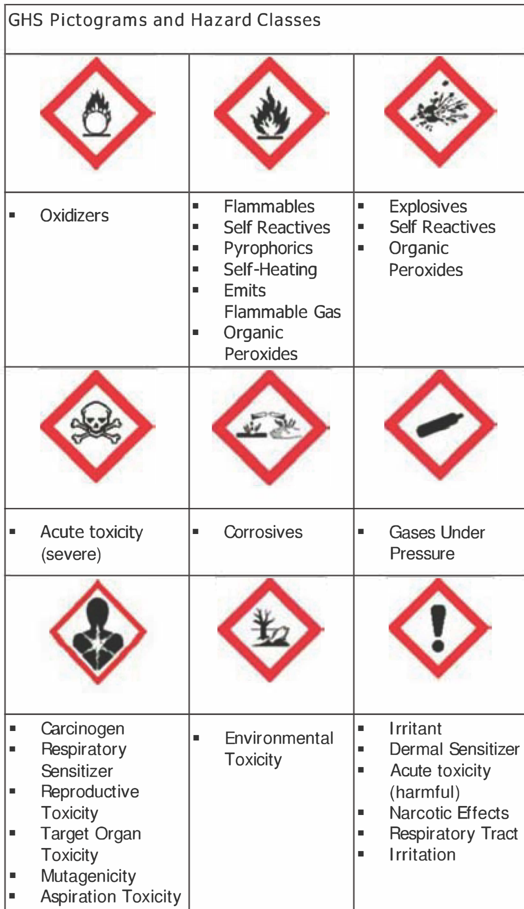

Figure 4-2. Global Harmonization System pictograms used for chemical hazard communication (as found on the OSHA web site).

make available **safety data sheets (SDS)** provided by manufacturers of chemicals. These SDSs contain requirements for proper chemical handling and storage, specifics about hazards associated with these chemical, first-aid measures, personal protection required, and other relevant information.

CHEMICAL LABELING

Chemicals need to be properly labeled with the contents of the container, the date of purchase or preparation, the expiration date, and the initials of the preparer. OSHA recommends that all chemically hazardous materials be labeled with each hazardous component designated and marked regarding the level of risk with a hazard symbol.

The OSHA Hazard Communication Standard (HCS) includes the internationally accepted **Globally Harmonized System (GHS).** According to the GHS a chemical or reagent label must include the identity of the chemical and hazard warnings using the harmonized signal word, pictogram, and hazard statement. A **pictogram** is a symbol that conveys specific information about the hazards of a chemical. The GHS has nine pictograms. See Figure 4-2 for these pictograms. A **signal word** (either "danger" or "warning") indicates the level

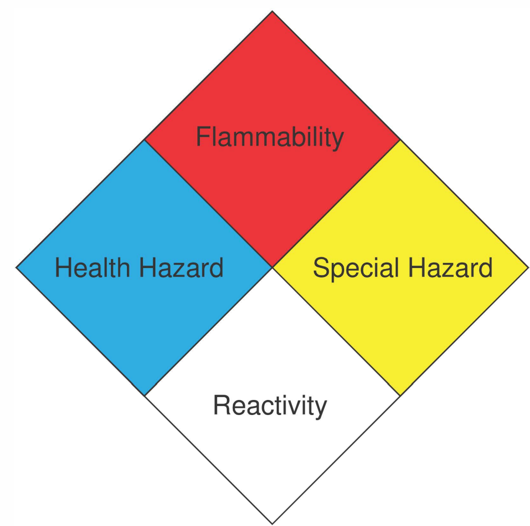

Figure 4-3. National Fire Protection Agency (NFPA) symbol.

of severity of hazard. A **hazard statement** describes the nature and degree of the chemical's hazard(s). A **precautionary statement** explains how to prevent adverse effects due to exposure to a hazardous chemical. Labeling systems that are in use may partially or completely meet the requirements of OSHA.

Two such systems that may be encountered in the clinical laboratory setting include the hazards identification system developed by the National Fire Protection Agency (NFPA) and the American Coating Association, Inc. (ACA). Each facility must determine which system or combination of systems is most appropriate for its needs.

The hazards identification system developed by the National Fire Protection Agency (NFPA) has been commonly used and is recognized by laboratory personnel. In the NFPA system, a diamond is the most commonly used symbol (Fig. 4-3). The red quadrant of the diamond indicates the degree of flammability hazard of the chemical. The blue quadrant indicates the level of hazard the chemical poses to health. The yellow quadrant indicates the chemical's reactivity or stability at certain temperatures. The white quadrant may contain symbols that refer to addition hazards. The degree of hazard in each quadrant is indicated by numbers ranging from 0 (not harmful under normal circumstances) to 4 (most severe risk). The white diamond may contain abbreviations for special risks such as COR for corrosive, OXY for an oxidizer, or W for do not add water. In addition, a radioactive symbol will be present if the chemical also has a radioactivity hazard. The NFPA diamond is designed to provide quick access to information about the effects of short or acute chemical exposure in emergency situations.

Using color codes similar to the NFPA diamond, the ACA developed a Hazardous Materials Identification System (HMIS) that is being adopted by some healthcare facilities. The HMIS color label (Fig. 4-4) is used to convey broad health warning information and is not intended for emergency handling information.

HMIS labels display similar colors as NFPA labels and are used to convey similar information. The top bar is blue,

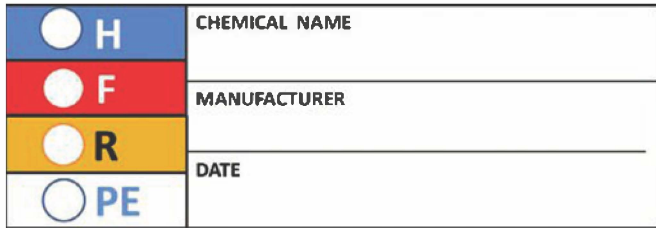

Figure 4-4. Hazardous Materials Identification System (HMIS) label.

indicating the level of health hazard. The second bar is red and indicates the chemical's degree of flammability. The third bar can be yellow or orange and indicates the chemical's level of physical hazard. The main difference is the bottom white bar the white bar of the HMIS label, which is used to provide information about appropriate PPEs.

CHEMICAL HANDLING

When acid is to be added to a reaction, it should be added to water and not water added to acid, to avoid sudden splashes. Use glassware of appropriate size for careful handling. Pipetting by mouth is unacceptable in the laboratory.

Personal protection equipment (PPE) (combinations of gown, apron, goggles, face mask, or face shield) and engineering controls, such a working behind a shield (Fig. 4-5), should be used as needed. State and federal regulations must be observed in the storage of and in the disposal of chemicals. Compressed gas cylinders must be chained to the wall and chained properly in a handcart if transported. Great care must be taken to avoid dropping gas cylinders as they can have explosive pressure.

Figure 4-5. A table-top safety shield is one engineering device that can be used to protect personnel when handling specimens or chemicals.

Figure 4-6. A chemical spill cleanup kit contains the materials needed to clean up various types of chemical spills.

CHEMICAL SPILLS

If an accident causes chemical contact with skin or eyes, the best first aid is immediate flushing with large amounts of water. For this reason, it is important to know the location of emergency showers and eye washes. Contaminated clothing should be removed as soon as possible. Chemical spill kits should be available to quickly neutralize and minimize exposure to chemical spills on surfaces in the laboratory (Fig. 4-6).

CHEMICAL EXPOSURE LIMITS

Many toxic, carcinogenic, and teratogenic chemicals currently have exposure limits set forth in OSHA regulations. These are designated as threshold limit values (TLVs) and permissible exposure limits (PELs). TLVs are designated limits of safe maximum exposure set by federal regulation. PELs are regulatory limits on the concentration of a substance in air or on skin, set to protect workers from toxic chemical exposures. Formaldehyde, benzene, and xylene are examples of such regulated substances.

Biohazard Risks

Many of the risks related to analysis of urine and other body fluids fall into the category of biological hazards. As stated previously, OSHA mandated that healthcare organizations have an exposure control plan that is reviewed annually by all employees as well as by all new employees upon hire, with the goal of reducing workplace exposures to infectious agents.

STANDARD PRECAUTIONS

In 1996, the CDC issued the currently used **standard precautions** guidelines. These guidelines stress safe work practices to prevent disease transmission that include the following as well as guidelines on handling biological waste.

1. PPE/barrier protection
   a. Wearing gloves and gowns and the use of bandages on cuts or abrasions are proposed to prevent direct contact with infectious agents. Gloves should be changed between patients. Nonlatex gloves must be available f or employees or patients who are allergic to latex. Generally these allergies are mild, but they can be life threatening and exposure to latex should be avoided with these allergies.
   b. Facial barriers (splash shields) are used for protection against splashes to mucosal surfaces of the face and mouth.
   c. Respiratory protection in the form of fitted masks is required in some circumstances to prevent the inhalation of airborne pathogens.
2. Hand washing is of critical importance to break the chain of infection and halt the spread of organisms throughout the healthcare facility. Hands should be washed frequently, after any accidental exposure, between patients, and upon leaving your work area.
3. Decontamination of work surfaces and instruments must be frequently and whenever contamination occurs with an antimicrobial liquid such as 10% sodium hypochlorite. The EPA recommends the use of registered products for decontamination agents as they have demonstrated performance as disinfectants, rather than chlorine bleach from the grocery store.
4. Specimens containing infectious agents must be properly labeled regarding the hazards they contain through use of a biological hazard label.
5. Spills of infectious samples must also be decontaminated with care. Use proper PPE as the samples may be mixed with broken glass. Do not handle glass directly, but rather scoop up the material with cardboard or special spill kits scoopers. Remove as much of the contaminated material as possible and then decontaminate the area with a disinfectant.
6. Pipetting aids and other engineering controls must be used to prevent direct contact or ingestion of infectious material.
7. Immunizations, tests for antibody titer levels, and monitoring tests such as the PPD for exposure to *Mycobacterium tuberculosis* are used to protect both employees and their patients.
8. Employees must be cognizant of the need for protection from the aerosolization of infectious material in order to block droplet exposure to infectious agents. In some cases, *Mycobacterium tuberculosis* might be in a specimen or specimens may even contain suspected bioterrorism agents and in these instances special protective measures are needed to avoid the risk of inhaling these organisms.
9. Exposures to infectious agents must be dealt with promptly as preventative measures and prophylactic treatment can be administered, so report all exposures promptly.
10. Specimen transport and shipping must be done properly to avoid public hazards. Samples must be properly packaged and labeled. When shipping samples, DOT packaging and labeling guidelines must be employed to be compliant with federal regulations.

A good practice that most laboratories have in place is the use of separate sinks for hand washing and specimen handling, processing, and disposal (Fig. 4-7).

BIOLOGICAL WASTE

Sharps hazards are an omnipresent safety risk for the clinical laboratory. Percutaneous injury gives infectious organisms immediate access to the blood and tissues. Rigid, puncture-proof, red plastic containers must be available in all patient rooms and in all laboratory work areas for sharps disposal. These containers are marked with the biohazard label and they must not be overfilled. Moreover, please keep children away from these containers. Needle stick must be prevented in practice both by use of engineered safety devices such as onehanded needle covering devices or retractable needles and through employee practice policies. Of course, recapping of needles is not permitted.

POLICIES FOR HAZARDOUS MATERIALS

Each healthcare facility must develop and disseminate policies and plans for handling **hazardous materials (HAZMATs)**. Hospitals are environments that have many very hazardous chemicals, organisms, materials, procedures, and equipment. Regulatory agencies require that personnel be oriented to and educated about this environment. There are many levels of training required for hospital personnel depending upon their employment duties and employment risks. For routine tasks, hospital will have policies and practices established for their personnel to follow. With larger incidents, a HAZMAT team

Figure 4-7. Sinks designated as **(A)** "clean" for hand washing and **(B)** "dirty" for processing of specimens should be clearly marked and their uses enforced.

may need to be called into handle a large spill or an exposure affecting many people. Special policies, training, and exercises are developed for community HAZMAT exposures as well.

EXPOSURE CONTROL PLAN

The exposure control plan is designed to protect workers from potential pathogens and to guide them in safe management of biohazardous waste. The OSHA-mandated Occupational Exposure to Bloodborne Pathogens program was enacted in 1992.

OTHER SAFETY ISSUES SPECIFICALLY RELATED TO URINALYSIS AND BODY FLUIDS

Many infectious agents, including but not limited to human immunodeficiency virus, hepatitis C virus, and hepatitis B virus, can be transmitted through the handling of blood

1. All are reasons for participating in a proficiency testing program ***except:***
   a. To ensure the best quality of laboratory results
   b. To compare your laboratory's results with other laboratories' results
   c. It is mandated by CLIA '88
   d. It will justify higher charges for laboratory analyses
2. This government agency is responsible for oversight of employee safety:
   a. HHS
   b. HIPAA
   c. OSHA
   d. CMC
3. CLIA '88 delineates the following categories of laboratory testing ***except:***
   a. Waived testing
   b. High-complexity testing
   c. Low-complexity testing
   d. Physician-performed microscopy
4. A control sample should be all of the following ***except:***
   a. Material of the same matrix as your test samples
   b. Used to calibrate the test
   c. Have an established acceptable range
   d. Be run along with your test samples and monitored statistically
5. TLVs are:
   a. Exposure levels permitted for employees
   b. Tracing lower volume
   c. A biohazard risk
   d. Threshold limit values
6. Complete the table below identifying the color and meaning that appears in each quadrant of the NFPA diamond.

QUADRANT	COLOR	MEANING
Upper		
Left		
Right		
Bottom		

7. Complete the table below identifying the color and meaning that appears in each bar of the HMIS labels.

BAR	COLOR	MEANING
Top		
Second		
Third		
Bottom		

CASE STUDIES

Case 4-1 A new instrument is purchased for the hospital's clinical laboratory. This instrument needs a compressed nitrogen gas tank attached to it. The laboratory manager is planning the space for this new instrument adjacent to the urinalysis bench.

The laboratory manager will be ordering the nitrogen tanks and is deciding where to store the spare tank as there is limited space in the instrument area.

1. How must the nitrogen tank that will be in use and the backup nitrogen tanks be stored?
2. What precautions must be taken when handling and changing tanks?
3. What dangers are associated with compressed gas tanks?

Case 4-2 As a technologist was opening a rubber-stoppered urine collection tube, the specimen splashed into the face of the technologist and the student with her. Embarrassed, the technologist noticed that the student was busy and had not even noticed the splash and she said nothing to the student. They continued working without addressing the splash.

1. What possible infectious agents might this technologist and student now be exposed to?
2. What are the proper steps for handling this incident?
3. What should have been done to prevent this incident from happening?
4. What ethical issues were not addressed by the technologist?

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Microscopy

5

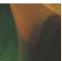
Chapter

KEY TERMS

Aberration
Achromat
Apochromat
Birefringence
Brightfield
Compensated Light
Condenser
Confocal Microscopy
Darkfield
Differential Contrast
Dry Preparations
Field Diaphragm
Fluorescence
Interference Contrast
Iris Diaphragm
Kohler Illumination
Microscope
Modulation Contrast
Nelson Illumination
Objective
Ocular
Optical Tube
Parfocality
Phase Contrast
Plan Achromat
Polarizing Microscopy
Scanning Electron Microscopy (SEM)
Stage
Supravital
Transmission Electron Microscopy (TEM)

LEARNING OBJECTIVES

1. Identify the parts of a compound microscope.
2. Explain the function of each microscope component.
3. Differential among the basic principles of microscopy techniques presented in this chapter.
4. Suggest appropriate use of microscopy techniques.
5. Explain methods used to adjust illumination.
6. Explain methods used to improve image contrast.
7. Outline proper handling and care of a microscope.



The Microscope

With sight we can rapidly perceive various characteristics of objects around us. The capability of our eyes has been extended to the very remote with telescopes and to the very small with microscopes. The word **microscope** is derived from the Greek *mikros,* small, + *kopein,* to look. Resembling a simple camera, the eye has a lens in front and a light-sensitive layer (the retina) covering its interior, rear portion. The structure of the lens includes an outer, transparent cornea; the iris, which changes the pupil diameter; and the lens proper. The lens is unique because its focal length can be varied (within certain limits) without being repositioned relative to the retina. When the shape of the lens is altered, the focal length is changed. Within certain limits, objects appear larger as they are brought closer to the eye. The practical limit for this is the ability of the lens to focus the object clearly; this ability varies from person to person. This limit can be overcome by the use of an instrument we call the microscope.

Components of a Microscope

A simple microscope consists, minimally, of a single, short-focus, positive (magnifying) lens. The ray diagram for this simple arrangement is shown in Figure 5-1.

In its basic form, the compound microscope consists of a lens (or group of lenses) called the **objective** (the lens closest the specimen) and a second lens (or group of lenses) known as the **ocular** (eyepiece). The function of these lenses is shown in Figure 5-2. The objective lens produces an image of the sample, and the upper or "eye lens" of the eyepiece focuses the image of the specimen on the retina of the eye. This information is perceived by the eye and brain to be an enlarged image of the sample located about 250 mm below the eyepiece. This is a virtual image. The corresponding real image, which can be observed on a screen or recorded in photomicrography, is projected from the eyepiece at a distance of about 250 mm above it. These relationships are shown in Figure 5-3.

Early microscopes consisted of a tube with a simple objective lens mounted at one end and a simple eyepiece lens mounted at the other end. The ray diagram for this arrangement is shown in Figure 5-4. Because of **aberrations** (image errors), a lens does not produce a perfect image of an object. Aberrations only minimally affect the image produced by a single lens, but they are of critical importance in the compound microscope. The errors become more obvious as more lenses are included in the system.

The major lens errors are spherical aberration, chromatic aberration, and curvature of field. Spherical aberration

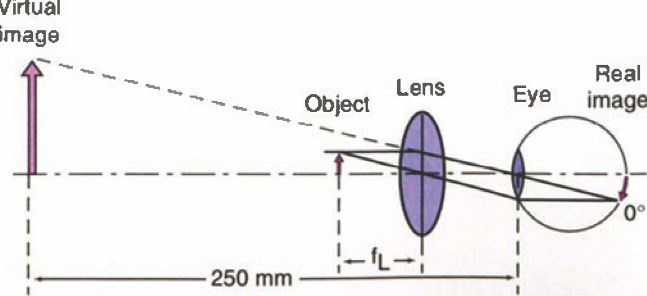


Figure 5-1. Ray diagram of a simple microscope. The object is imaged on the retina of the eye by the lens. This appears to the eye as if it were from an object at the position of the virtual image. (From Richardson H. *Handbook for the light microscope: A user's guide*. Park Ridge, NJ: Noyes Publications; 1991.)

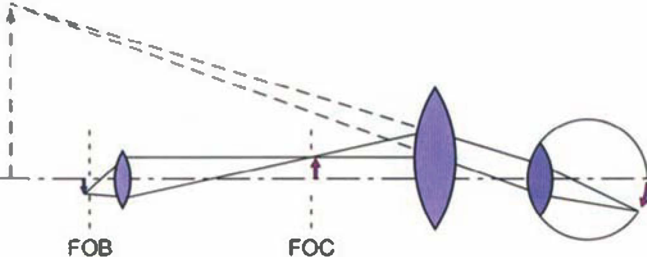


Figure 5-2. Ray diagram of a compound microscope; the object is just outside of the first focal plane of the objective (FOB); the intermediary image is just within the focal plane of the eyepiece FOC; compare with Figure 5-1. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

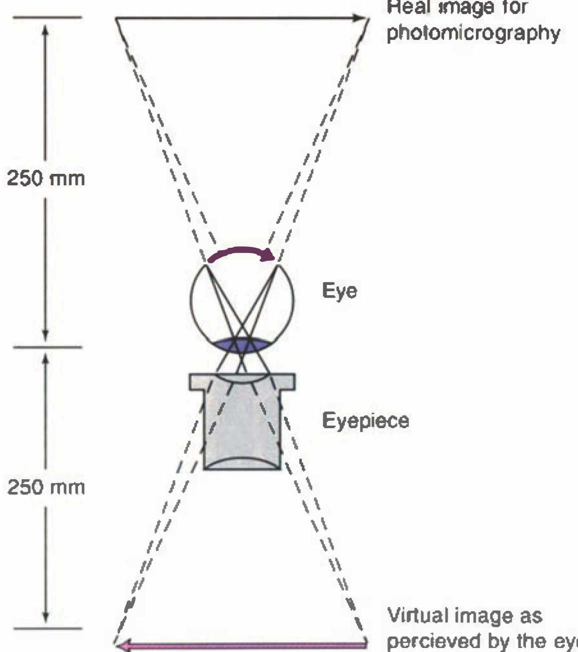


Figure 5-3. Schematic relationship between the virtual image as perceived by the eye and the real image that exists above the eyepiece; this latter image is the one used for photomicrography. (From Richardson H. *Handbook for the light microscope: A user's guide*. Park Ridge, NJ: Noyes Publications; 1991.)

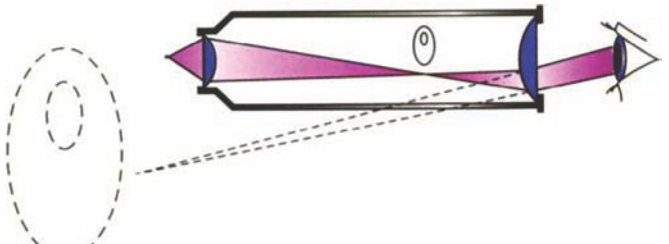


Figure 5-4. Ray diagram of a compound microscope. (From James J, Tanke HJ, eds. *Biomedical Light Microscopy*. Dordrecht: Kluwer Academic Publishers; 1976.)

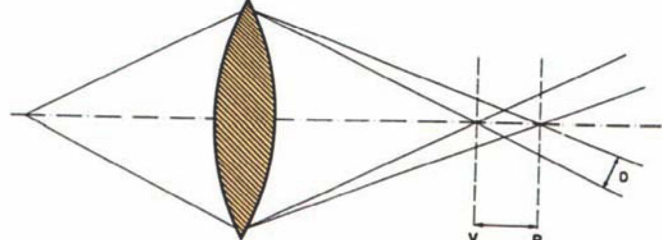


Figure 5-6. Chromatic aberration. As a consequence of different refractions of rays of various wavelengths coming from a luminous point emitting mixed light, dispersion occurs for which the distance D is a measure. On the optical axis, a spectrum of image points will be formed, with points for violet (V) and red (R) at the extremities. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

(aperture error) occurs because rays that pass through the outer portion of a lens come to a different focal point than rays that pass through the central area of a lens (Fig. 5-5). This results in an image that is blurred and cannot be sharply focused.

Chromatic aberration occurs because the refractive index of the lens material is not the same for different wavelengths of light. The focal point is nearer to the lens with shorter wavelengths, as illustrated in Figure 5-6. As a result, color fringes appear around very fine structures.

Curvature of field means the image of a flat plane perpendicular to the optical axis appears curved rather than flat. This is especially disturbing for photomicrography. Toward the end of the 18th century, various combinations of positive and negative lenses made with different types of glass were used to produce lenses at least partially corrected for aberrations. These achromatic microscopes spherically corrected for green light and chromatically corrected for red and blue-green light.

The components of any microscope are mounted on a stand (or frame and body). For the special application of viewing the bottom of culture vessels or Petri plates, an inverted stand is used. The more common arrangement for work with transmitted light is the upright stand. The stand serves to hold the basic components of the microscope in place. They are the same regardless of the stand: a source of illumination, a condenser to focus the light onto the specimen, a stage to hold the specimen for viewing, and the optical tube (or body tube) with its eyepieces and objectives. A general diagram is shown in Figure 5-7.

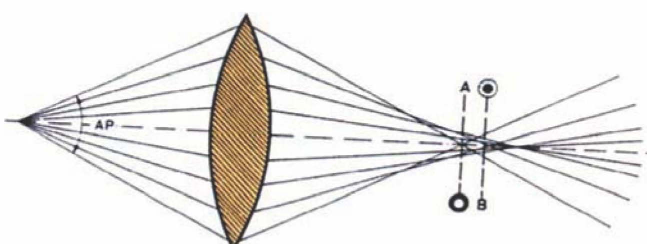


Figure 5-5. Spherical aberration. When the object is a luminous point emitting monochromatic light, a bright circle with darker border will be seen on a screen held in the object space at A; at plane B a bright ring with a darker center will be seen. AP—angular aperture of the lens. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

ILLUMINATION SYSTEM

The light source for modern instruments is usually mounted in the base together with a power transformer and controls to regulate brightness. There are two types of the most widely used source of illumination: a low-voltage incandescent lamp.

One type is an ordinary tungsten filament enclosed in an inert gas atmosphere by a soft glass envelope. After several hours of use, these sources gradually emit less light because the tungsten evaporates from the filament, deposits on the glass envelope, and gradually darkens it. This darkening is avoided in the second type of incandescent lamp by the addition of a small amount of a halogen (such as bromine or iodine) admixed with the inert gas atmosphere. When the tungsten evaporates from the filament, it reacts to form a tungsten halide gas, which decomposes at the hottest part of the filament to reform the metal and release the halogen to react again. This prolongs the useful life of the lamp. The tungsten halogen lamps have a smaller sized, quartz glass envelope. Light from the lamp is collimated by a collector or condensing lens (or system of lenses) and directed upward through the **field diaphragm** to the substage condenser. The field diaphragm serves to limit the diameter of the light beam entering the substage condenser as well as to reduce stray light.

SUBSTAGE CONDENSER SYSTEM

The purpose of the substage **condenser** system (or simply the condenser) is to distribute evenly and focus the light from the illumination source onto the specimen. The condenser is usually arranged on a focusing and centerable mount so the illumination can be properly aligned to the optical axis of the microscope. Proper illumination of the specimen is critical for the sharpness and general character of the image. It is the condenser that provides the necessary means to adjust the

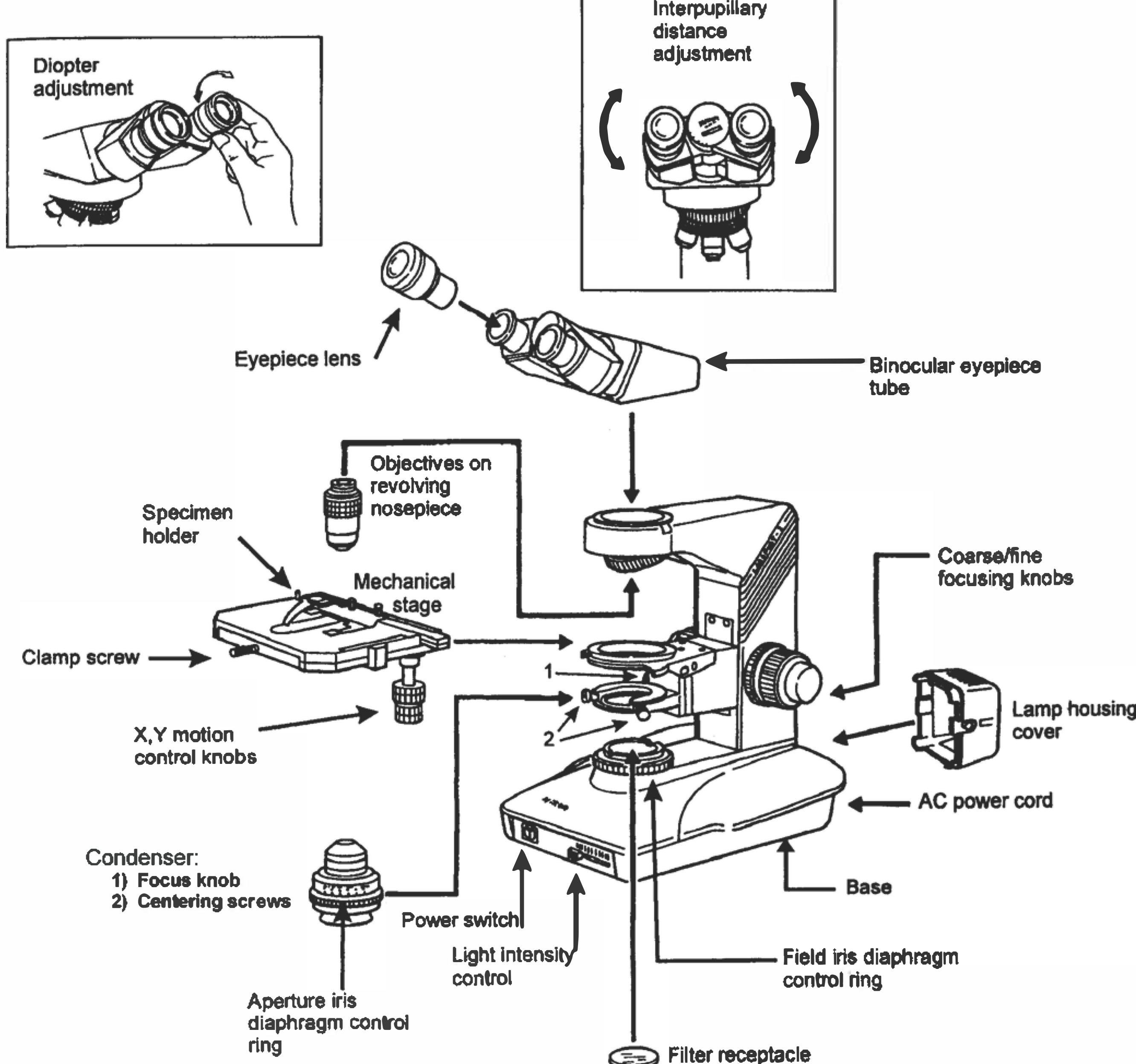


Figure 5-7. Basic components of a compound light microscope. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

illumination of the sample. The condenser consists of at least two lenses, an **iris diaphragm** (or aperture diaphragm), and a carrier or holder for a removable filter.

The lenses of the condenser collect the light from the illumination system below and produce a cone of light converging at the sample plane. The angle of this cone of light is controlled and adjusted by the aperture diaphragm. Proper adjustment of the aperture diaphragm is critical in establishing the potential numeric aperture (N.A.) of the system, in minimizing glare and stray light, and in adjusting the contrast in the specimen. Refer to the section on "The Objective Lens," later, for a brief discussion of N.A.

MECHANICAL STAGE

The **stage** supports the specimen to be viewed at right angles to the optical axis of the instrument. For transmitted light, the stage of course has an opening in the center to permit light to reach the specimen. In simpler designs, the stage is not moveable and the glass microscope slide on which the specimen is mounted is held in place on the stage by two metal clips. The slide is then moved around manually.

Laboratory and research-grade microscopes have a mechanical stage with a quick-release spring clip device to hold the microscope slide firmly in place. The stage itself, with

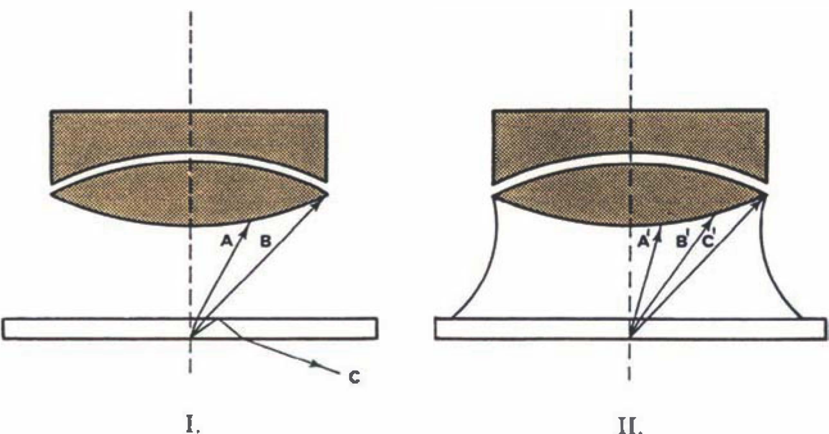


Figure 5-8. The effect of oil immersion. In situation I, without immersion, of three rays with an ever increasing angle toward the optical axis, only ray A reaches the objective in such a way that it can be presumed to take part in image formation, whereas ray C does not even reach the upper part of the object space, being totally reflected at the surface of the cover glass. In situation II, the same three rays reach the object space (virtually without being refracted) and both rays A′ and B′ probably can take part in image formation C′, reaching at least the upper part of the object space. (It should be noted that the angles of ray A and B with the optical axis are greater than those of their counterparts A′ and B′). (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

the mounted slide, can then be moved orthogonally in each direction by means of separate or coaxial control knobs. Some stages are equipped with vernier scales along one horizontal edge and one vertical edge to aid in relocating a particular field of view. For crystallographic and metallurgic studies, a round, revolving stage is used.

OPTICAL TUBE

The **optical tube** (or body tube) provides a light-tight support and correct spacing for the objective lenses and the eyepieces. To focus the specimen, either the tube is moved up and down in reference to the stand or the stage is moved while the optical tube remains stationary. Focusing is by means of coarse and fine single or coaxial adjustment knobs that drive gear mechanisms.

The length of the tube, or the mechanical tube length, must match the optical parameters of the objective design. Mechanical tube lengths vary from 140 to 250 mm. Some research model instruments have an adjustable, graduated draw tube that permits “fine tuning” the length of the optical tube to match the requirements for any specific objective lens.

The simplest eyepiece arrangement is on a monocular tube. More commonly, a binocular body is provided for two eyepieces to permit the use of both eyes simultaneously. In addition, the optical tube may be trinocular to permit the mounting of a camera. The binocular body has two adjustments, one is the interpupillary spacing adjustment, the other is the interpupillary acuity adjustment. These adjustments are referred to later in reference to microscope adjustments.

OBJECTIVE LENS

Objective lenses are usually mounted in a revolving “nosepiece” to facilitate their selection and change during viewing. Advantages of this system include convenience and speed, as well as protection from dust.

This **objective** lens produces the magnified primary image of the specimen and thus is perhaps the most important part of the entire system. Several parameters must be considered in achieving the optimum image for a given objective and instrument, including N.A., magnification, optical corrections, tube length, immersion fluid, coverglass (coverslip) thickness, flatness of field, parfocality, and working distance. In most instances, certain of these parameters are engraved on the barrel of the lens, including tube length required, coverglass thickness needed, magnification of the intermediate image, lens corrections, and the N.A.

A measure of the amount of light a lens can collect is the N.A., defined as: N.A. = η sin a, where η is the designated refractive index of the material between the sample and the lens and a is the half angle of the most oblique rays entering the lens. For an objective, “lens” means the front lens. The effective N.A. of the objective depends on the N.A. of the condenser. For maximum resolution with the system, the N.A. of the condenser should equal or at least be close to that of the objective.

The refractive indices of the materials in the light path between the objective lens and the sample also have a strong effect on the N.A. The controlling factor is the material that has the lowest refractive index. If air is a part of the light path, its refractive index of 1 controls the effective N.A. of the total system regardless of how high the N.A. of the lenses may be. Immersion media can be placed in between the objective lens and the coverglass/sample to improve the effective N.A. Media include water (η = 1.33), glycerin (η = 1.440), and oil (η = 1.5–1.6). The water immersion objective is essentially obsolete because the performance of oil immersion objectives does not depend on the coverglass thickness, and they also produce a more brilliant image. The effect of oil immersion is shown in Figure 5-8.

As the N.A. of a lens increases, thereby increasing its potential resolving power, the actual resolving power that can be realized depends in large measure on the lens corrections. Gradually, over the years, lens designs have been produced that are increasingly successful in correcting lens aberrations. Lenses have been produced to correct simultaneously for spherical, chromatic, and curvature of field errors. An example of how two different types of glass are combined to correct for chromatic aberration is shown in Figure 5-9.

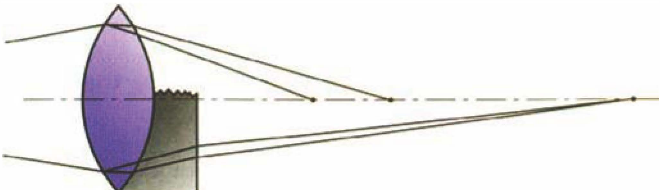


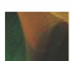
Figure 5-9. Correction of chromatic aberration for two colors by the combination of a positive and a negative lens of materials with different dispersion; both image points come to coincide, but it entails an increase in the focal distance of the refractive complex. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

"**Achromat**" objectives are spherically corrected for green light, and chromatically corrected for red and blue-green light. The semiapochromatic or fluorite objectives are spherically and chromatically corrected for two colors. The objectives providing the best contrast and resolution are designated "**apochromat**" and are corrected chromatically for three colors and spherically for two. An objective lens corrected for curvature of field has the designation "**plan achromat**."

Parfocality means that the distance between the sample and the intermediate image is made the same for each member of the objective lens set. Therefore, it is not necessary to refocus very much (if at all) when the objectives are changed one to another.

OCULAR

The **ocular** (eyepiece) is a self-contained unit and is designed to fit into the upper end of the microscope body tube. The ocular has a lens at either end. The one at the upper end is the eye lens and the one below is the field lens. The ocular is designed to form a magnified image of the intermediate image produced by the objective lens.

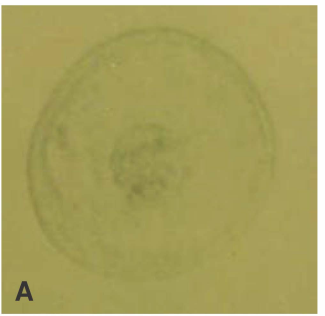


Types of Microscopy

BRIGHTFIELD

In **brightfield** microscopy, the image of the specimen appears dark against a bright background (Fig. 5-10A). Brightfield microscopy presents difficulties in visualizing translucent objects such as hyaline casts, mucus, and some cells. Contrast can be improved by adjusting the light intensity using the lamp voltage control built into the power supply. Contrast may also be adjusted by slightly opening or closing the aperture diaphragm on the substage condenser (Fig. 5-10B). However, adjustment should be used with discretion because closing the aperture down too far is detrimental to the effective N.A. of the system.

Once adjusted for Kohler illumination (discussed later), the condenser should be left in that position to maintain maximum resolution in the system. It is a poor technique to rack the condenser up and down to alter contrast. By combining light source adjustments with slight changes to the aperture diaphragm, adequate contrast can usually be obtained.



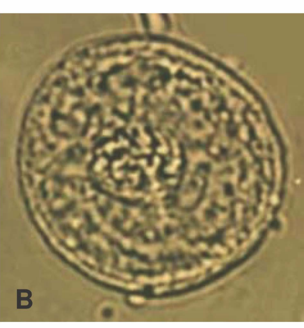


Figure 5-10. **A:** Brightfield does not allow for viewing of cellular details, however **(B)** adjusting the aperture does allow for observation of details. (Modified from Hoffman R, Drewry J. *A Comparison of Four Types of Light Microscopy*. 5th ed. Dayton, OH: Educational Materials for Health Professionals; 2002.)

PHASE CONTRAST

The structural elements of the specimens we wish to resolve with the microscope differ only slightly in refractive index and therefore exert only a negligible influence on the light they transmit. This slight change is in the phase of momentary vibration state, which cannot be detected by the eye. Conventional brightfield illumination does not reveal brightness differences between the structural details of the specimen and its surroundings. The image lacks contrast and details remain invisible.

With **phase contrast** microscopy subtle differences in refractive index and the subtle changes in phase these produce are converted into clearcut variations of light intensity and of contrast (Fig. 5-11). Many laboratory scientists prefer to examine the urine sediment unstained with phase contrast microscopy. However, certain highly refractive objects such as fat or some crystals are more easily identified using brightfield rather than phase contrast microscopy.

Objects viewed under the microscope retard light waves and produce phase changes to different degrees depending on their unique shape, refractive index, and absorbance properties. When some of the light waves are slowed while passing through an object, the intensity observed is lowered. Waves that are retarded exactly one-half of a wavelength completely cancel out an unaffected light wave. The best contrast is achieved when light retardation is one-fourth of a wavelength. Components added to a brightfield microscope achieve this in phase contrast microscopy. Areas of the specimen appear light and dark with haloes of various intensities. With phase contrast, unstained specimens, especially living cells and components that have a low refractive index, are imaged in more detail than is possible with brightfield microscopy. On the other hand, certain objects and structures produce such bright haloes that visualization of detail and dimension is actually worsened.

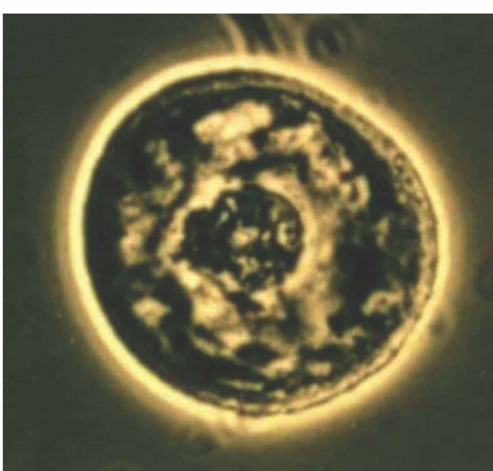


Figure 5-11. Phase contrast microscopy intensifies subtle contrasts. (Modified from Hoffman R, Drewry J. *A Comparison of Four Types of Light Microscopy*. 5th ed. Dayton, OH; Educational Materials for Health Professionals; 2002.)

The components necessary to convert a brightfield instrument to phase contrast must be added to both the condenser and the objective. For a particular objective, a corresponding annular diaphragm, resembling a target, is fitted to the condenser and produces a ring of light. The specimen is illuminated by this ring of light. The objective is fitted with a phase-shifting element that is the reverse of the condenser ring—that is, the central ring retards light by one-fourth of a wavelength, producing a ring of reduced intensity. The light and dark rings must be centered for maximum contrast. This can be achieved by removing an eyepiece and looking down the optical tube at the back lens of the objective (a focusing telescope helps here) and adjusting the condenser ring (the light ring) with the centering screws until the image shows the rings are centered that is, the light ring is superimposed on the dark ring. Alternatively, one can view the object under phase contrast and simply adjust the centering screws until maximum contrast is achieved for a suitable test object (e.g., a hyaline cast). Each combination of light and dark rings needs to be adjusted separately for proper centering and maximum viewing contrast. A schematic view of a phase contrast system is shown in Figure 5-12.

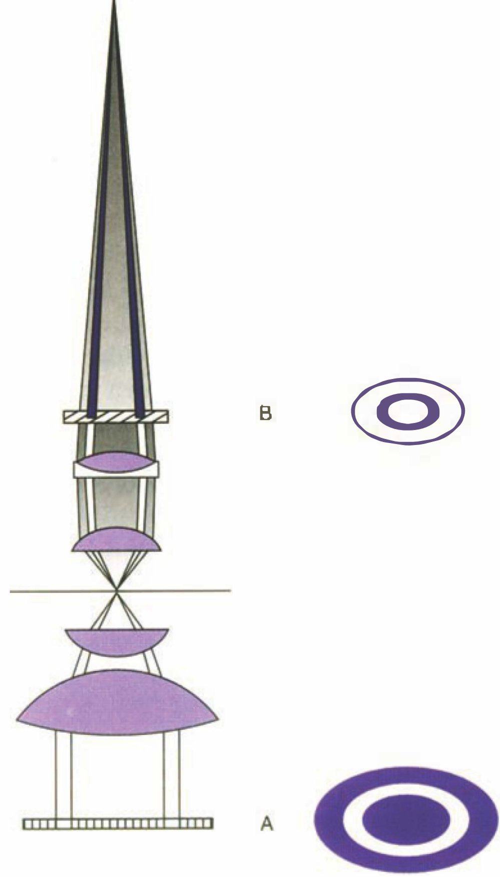


Figure 5-12. Schematic view of the practical realization of the phase contrast principle; **(A)** phase annulus under the condenser, **(B)** the corresponding phase plate in the objective. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

POLARIZED AND COMPENSATED LIGHT

Polarization

Polarizing microscopy has widespread applications in the clinical laboratory, as well as in pharmaceuticals, forensics, pathology, geology, and other fields. For the end view of a beam of unpolarized light, the wave theory of light propagation holds that vibration is occurring in all directions perpendicular to the direction of travel. Plane-polarized light vibrates in only one plane. Materials that produce plane-polarized light are termed polarizers.

To convert a brightfield microscope to a polarizing one, two filters are required. One, called the polarizing filter, is located at some point below the condenser. If not built into the instrument, it can simply be perched as needed on top of the light port in the base of the microscope. The polarizer is constructed to permit light vibrating in an east–west direction perpendicular to the light path to pass to the specimen. The other filter, the analyzer, is mounted in the microscope between the objective and the eyepiece. It is constructed to permit the passage of light that is vibrating in a north–south direction perpendicular to the light path. When the two filters are "crossed," the field appears black unless an optically active material is present to rotate the plane of the polarized light (Fig. 5-13A).

Compensation

Compensated light microscopy is achieved by placing a filter composed of highly linear polymers between the two crossed filters of a polarizer. With compensated light,

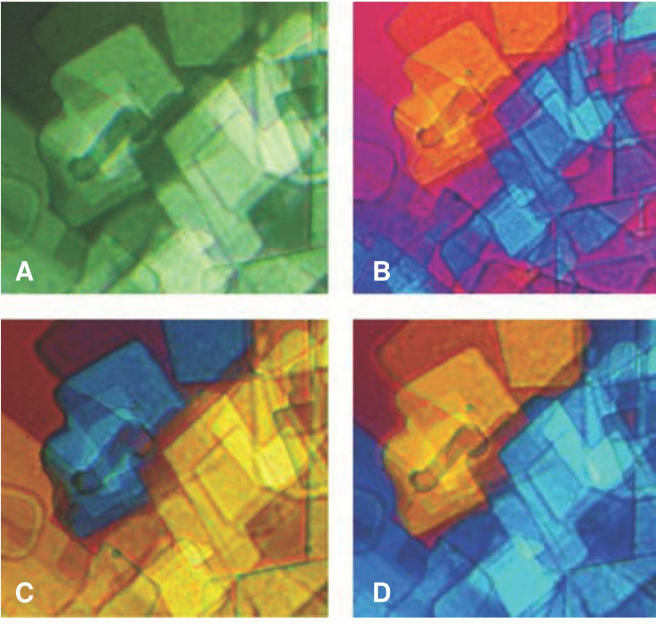


Figure 5-13. **A:** Using a polarizing filter produces a blackened field of view; materials that polarize allow light to pass. **B:** A first order red compensation applied after polarization produces a red field of view, while polarized materials appear yellow or blue depending upon the orientation of the compensation filter. In this image the orientation of the compensator is in a neutral position. **C:** After adjusting the axis of the compensator filter, crystals aligned with the compensation filter appear yellow if negatively birefringent while crystals lying perpendicular to the filter appear blue, showing their positive birefringence. **D:** When the axis of the filter is flipped, the colors of the crystals reverse.

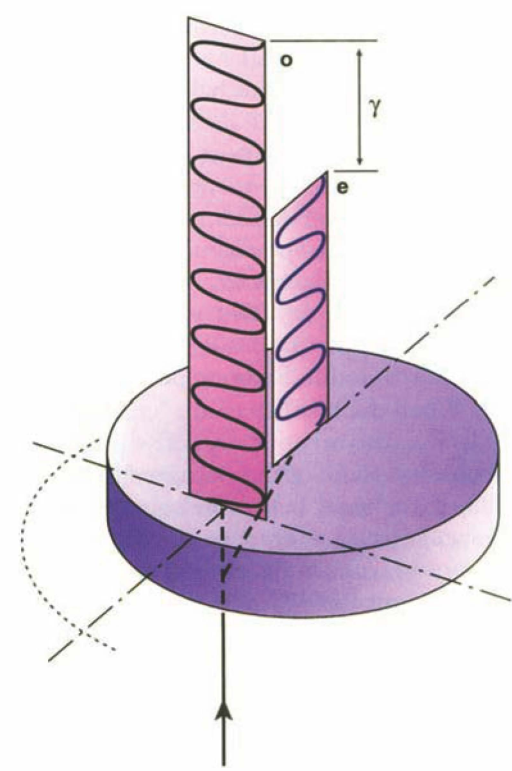


Figure 5-14. Schematic view of the development of a path difference when a light ray traverses a doubly refracting material; o, ordinary ray; e, extraordinary ray; γ, path difference. (From James J, Tanke HJ, eds. *Biomedical Light Microscopy*. Dordrecht: Kluwer Academic Publishers; 1991.)

some substances display **birefringence** (the ability to refract light in two directions, one following the original light path, the other rotated 90 degrees to the original). This relationship is pictured in Figure 5-14. Many such birefringent or optically active substances are found in the clinical laboratory, such as crystals, fibers, bones, or minerals. Various compensator filters are used to demonstrate birefringence of a substance. The most common one in the clinical setting is the first order red compensator. When this filter is placed between crossed polarizers, the field is no longer black but red-violet, as seen in Figure 5-13B. The filter splits plane-polarized light into two rays, slow and fast. The direction of the vibration of the slow ray is inscribed on the filter plate.

Birefringence can be negative or positive. Negative birefringence is rotation to the left or counterclockwise (when looking toward the light source); positive birefringence is rotation to the right or clockwise. Crystals found in clinical specimens that are negatively birefringent include monosodium urate crystals. Those that are positively birefringent include calcium pyrophosphate crystals. Others, such as cholesterol plates demonstrate both positive and negative birefringence (Figs. 5-13B to D).

INTERFERENCE CONTRAST

With **interference contrast** microscopy, differences in the optical path through the specimen are converted to intensity differences. Specimen images of high contrast and resolution without the haloing seen with phase contrast microscopy are achieved. Optical sectioning is possible with interference contrast methods because the image at each depth of field level is unaffected by material above or below the plane of focus. Images have a three-dimensional appearance. The technique is excellent for unstained specimens and is especially good for wet mounts.

Modulation Contrast

Modulation contrast takes place when light intensity varies above and below an average value. Three modifications of brightfield microscopy are required for modulation contrast: a special slit aperture placed below the condenser, a polarizer to control contrast placed below this slit aperture, and a special amplitude filter, called a modulator, placed in the back of each objective. A diagram illustrating this system is shown in Figure 5-15.

By removing the slit aperture, the instrument can be converted back to brightfield, darkfield, polarizing, or fluorescence

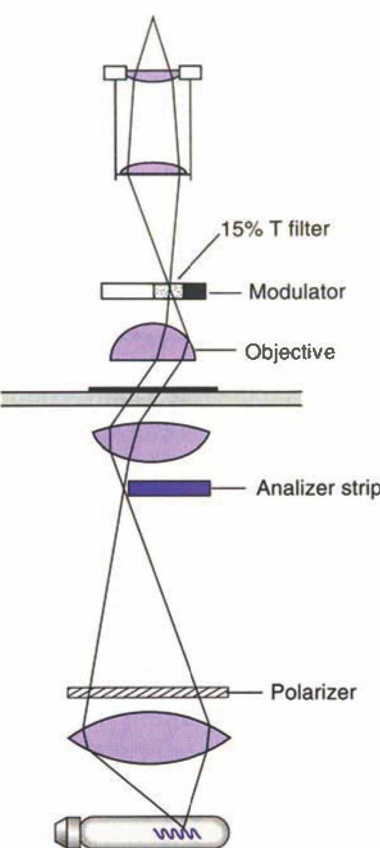


Figure 5-15. Schematic representation of the ray path in a light microscope adapted for modulation-contrast microscopy after Hoffman. (From James J, Tanke HJ, eds. *Biomedical Light Microscopy*. Dordrecht: Kluwer Academic Publishers; 1991.)

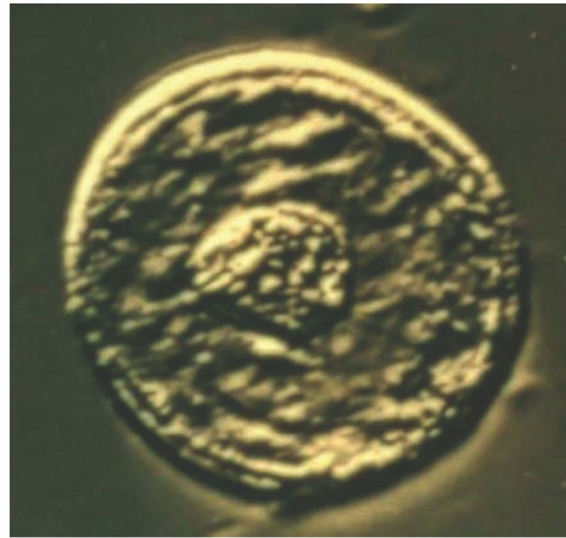


Figure 5-16. Modulation contrast microscopy creates a three-dimensional image. (Modified from Hoffman R, Drewry J. *A Comparison of Four Types of Light Microscopy*. 5th ed. Dayton, OH: Educational Materials for Health Professionals; 2002.)

techniques. Light from the first polarizing filter passes through the slit aperture, which is partially covered by the second polarizing filter. Rotating the slit aperture with its polarizer achieves variations in contrast and a reduction of scattering effects such as flare and fringes at specimen edges. The light proceeds to the specimen and interacts with it, and the location at which the light enters the objective depends on diffraction within the specimen. The light passes through different parts of the modulator located at the back of the objective. The modulator is divided into three regions of different size and light transmission. The modulator determines the intensity gradients of light to dark observed in the three-dimensional image but does not effect a change in light phase (Fig. 5-16).

Differential Contrast

With **differential contrast** microscopy intensity differences in the specimen image are produced through the use of birefringent crystal prisms as beam splitters. The prism placed before the specimen plane splits the light into two beams. These beams follow different paths through the specimen; a second prism placed after the objective recombines the two beams of light into one. The light then passes into a polarizing (or analyzer) filter before entering the eyepieces. It is the analyzer that produces the interference image observed by changing the direction of vibration of the recombined rays so that they interfere with each other. With this technique, it is possible to change the background field of view from black or dark gray to various colors such as yellow, blue, and so forth. Because in reality two images are formed but the eye does not resolve them, the specimen image observed appears to be in relief, or three-dimensional. The light path for this method is illustrated in Figure 5-17.

DARKFIELD

Darkfield microscopy produces a bright specimen image against a dark or black background (Fig. 5-18). This procedure is used with unstained specimens. In the clinical laboratory it has been the preferred approach for the identification of spirochetes under the microscope. Darkfield uses a special condenser that directs the light through the specimen only from oblique angles by means of a "darkfield stop" in the base of the condenser. Light passing through the specimen interacts with it (by refraction, reflection, or diffraction), which results in light entering the objective. The image is of a shining specimen on a black background. If there is no specimen, a black field of view is observed because no light is entering the objective.

FLUORESCENCE

In **fluorescence**, light of a selected wavelength is presented to the specimen. If a fluorescent substance is present that

Figure 5-17. Scheme of the course of interfering rays in differential interference contrast. W I and W II, Wollaston prisms; C, condenser; OB, object; 0, objective; EP, eyepiece. A polarizer in front of W I and analyzer behind W II are not shown. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

absorbs that particular excitation illumination, it emits a portion of that energy after a finite but short period of time as light (fluorescence) at a different, longer wavelength. The emitted light is transmitted to the eyepiece for viewing.

The procedure requires two filters. The first, called the excitation filter, selects the wavelength of the excitation light presented to the specimen. The second filter, called the barrier or emission filter, permits a specific wavelength of fluorescent light from the specimen to pass to the eyepiece. Some biologic materials are naturally fluorescent, but most applications of this technique require staining the specimen with fluorescent dyes called fluorophores. Each fluorescent substance or fluorophore has a unique excitation as well as emission wavelength.

Two types of optical systems are available. One method is transmitted fluorescence microscopy, in which the excitation light is presented to the condenser, focused, and passed through the specimen to continue on up the optical

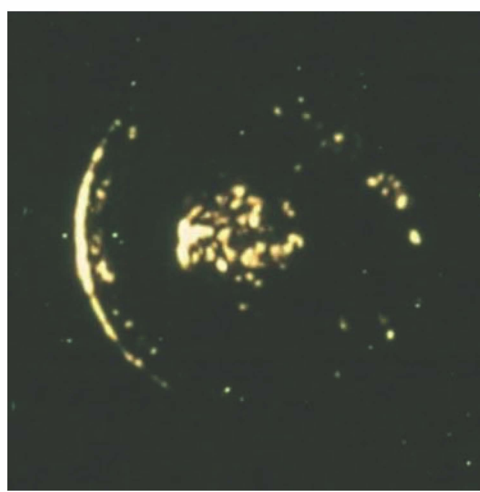


Figure 5-18. Darkfield microscopy produces a bright image on a dark background. (Modified from Hoffman R, Drewry J. *A Comparison of Four Types of Light Microscopy*. 5th ed. Dayton, OH: Educational Materials for Health Professionals; 2002.)

tube to the analyzer and eyepieces. These systems require that an immersion medium be placed between the top lens of the condenser and the bottom of the microscope slide. A more convenient and recent modification is the reflected illumination system (or epi-illuminator), where the light impinges on the specimen from above through the objective lens.

This is shown in Figure 5-19. A beam-splitting dichroic mirror in the system has a high reflectance for the light passed by the excitation filter. However, the mirror is transparent to

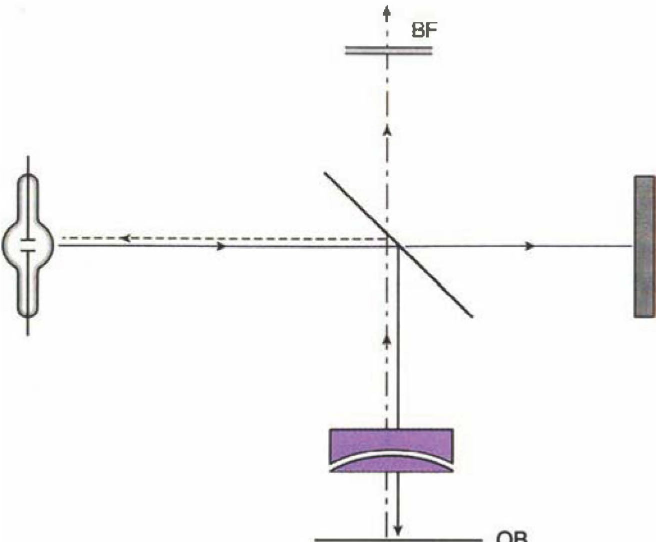


Figure 5-19. Schematic view of the course of the rays in a vertical fluorescence illuminator with dichroic mirror. OB, object plane; BF, barrier filter; at right, a dark layer absorbing unused excitation light that has passed the dichroic mirror. (From James J. *Light Microscope Techniques in Biology and Medicine*. Dordrecht: Kluwer Academic Publishers; 1976.)

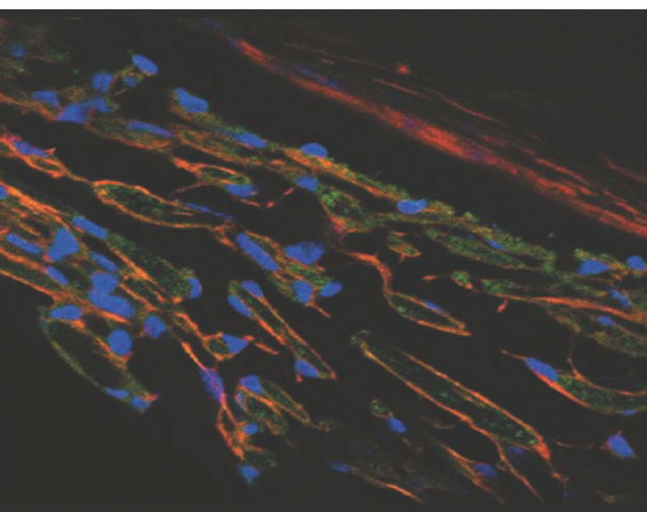


Figure 5-20. Images show a high level of contrast when confocal microscopy is used in conjunction with fluorescence techniques.

the fluorescence coming from the specimen and allows it to pass upward through the barrier filter to the eyepieces. Fluorescence microscopy is very sensitive to small quantities of fluorescent dyes or fluorophores attached to antibodies, antigens, bacteria, viruses, and the like. Thus, the method is frequently used with exquisite selectivity and sensitivity in microbiologic and immunologic procedures in the clinical laboratory.

CONFOCAL MICROSCOPY

In **confocal microscopy** both the illumination and detection optics are focused down on a single volume element of the specimen—that is, they are confocal. The illumination beam diverges above and below the plane of focus so that elements away from the focal plane receive a much lower flux of illumination. A complete image is built up by sequentially adding the volume elements within the focal plane. A confocal microscope is particularly effective when operating in fluorescence (Fig. 5-20).

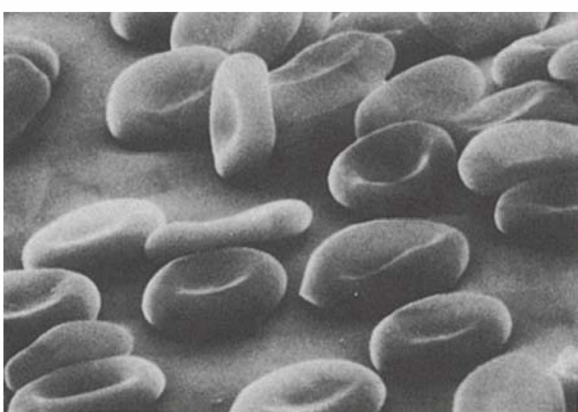


Figure 5-21. Red blood cells as seen using a scanning electron microscope.

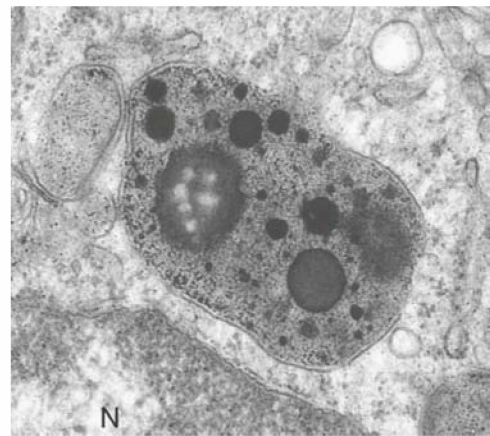


Figure 5-22. White blood cell as seen using a transmission electron microscope (call out shows this cell type viewed in brightfield microscopy with Wright stain).

ELECTRON MICROSCOPY

Scanning Electron Microscopy

Scanning electron microscopy (SEM) produces images by scanning specimens with a focused beam of electrons, that interact with atoms in the specimen. Signals are produced that contain information about the specimen's surface topography and composition. SEM is able to achieve a 1-nm resolution and allows specimens to be observed in high or low vacuum, in wet conditions, and at a wide range of temperatures. Figure 5-21 shows red blood cells using SEM.

Transmission Electron Microscopy

In **transmission electron microscopy (TEM)** a beam of electrons interacts with a specimen as they are transmitted through the specimen. An image of this interaction is magnified and focused onto a fluorescent screen or photographic film. TEM allows for examination of fine detail as seen in Figure 5-22.

Adjustments to Illumination

NELSON ILLUMINATION

Nelson illumination, also called critical illumination, produces an image of the lamp filament (or the ground glass in front of the lamp condenser) superimposed on the image of the specimen. With this system, it is necessary to rack the substage condenser just slightly downward to spread out the image of the filament and make it more diffuse so as to avoid interfering with the specimen image. Uneven illumination from the image of the coiled filament/ground glass can be a problem.

KOHLER ILLUMINATION

Kohler illumination is the preferred system throughout the world, was developed by Kohler early in the 19th century and bears his name. The object of Kohler illumination is to permit the use of coiled filament lamps (the standard type) while avoiding uneven illumination.

With Kohler illumination, adjustments are made so that the image of the lamp filament does not appear in the field of view but is actually focused in the plane of the aperture diaphragm. This produces a bright, evenly lit field of view against which the detail of the specimen is plainly recognizable. Kohler illumination also results in as wide a cone of illumination as possible from the condenser to achieve maximum resolution of fine detail. In Kohler illumination, the field diaphragm controls the area of the focused specimen being viewed, but has no effect on intensity or resolution. The procedure for adjusting Kohler illuminiation are outlined in Box 5-1.

While viewing a specimen, minor adjustments of the aperture diaphragm can be used advantageously to reduce stray light and glare, as well as to enhance contrast. However, the aperture diaphragm should not be used to reduce the brightness of the image field. This results in a loss of resolving power. It is better to adjust the brightness of the image field by means of the lamp voltage control, or, for photomicrography, by means of neutral density filters.

BOX 5-1 Adjusting Kohler Illumination

1. Turn on the microscope lamp, and adjust the intensity to a comfortable level with the voltage control.
2. Bring the X 10 objective into position, and secure a specimen slide on the mechanical stage. Position the specimen under the objective with the stage adjustment knobs.
3. Adjust the interpupillary distance until binocular vision is obtained.
4. Focus the specimen with the coarse and fine adjustment knobs (Fig. 5-23A).
5. Perform the diopter adjustment by first bringing the specimen into sharp focus while viewing only with the right eye. Now, using only the left eye, focus the specimen by rotating the diopter adjusting ring located on the left eyepiece. If the left eye is dominant, reverse eyepieces and the procedure.
6. To obtain Kohler illumination, first close down the field diaphragm, then move the substage condenser up or down by means of the height adjustment knob to bring the image of the field diaphragm into sharp focus onto the already-focused image of the specimen. Once adjusted in this manner, the condenser should be left in that position to maintain Kohler illumination (Fig. 5-23B).
7. By means of the condenser centration knobs, adjust the image of the field diaphragm so that it occupies the center of the field of view (Fig. 5-23C).
8. Open the field diaphragm until its image just disappears from the field of view (Fig. 5-23D).
9. Lift out an eyepiece, and while observing the image of the aperture diaphragm at the back of the objective, adjust the diaphragm until it is two-thirds to three-fourths open. Then replace the eyepiece.
10. Readjust both the field and the aperture diaphragms each time an objective is changed.

Methods to Increase Contrast

Many specimens, unless stained with **supravital** stains (stained in the living state) or **dry preparations** (fixed and stained), are very difficult to study because not enough contrast is produced by the object (e.g., a hyaline cast) or components in the object (e.g., cellular features). Here the refractive indices involved are too similar to the surrounding medium as well as the glass microscope slide and cover glass for a good quality image to be produced without reducing the light intensity to almost the vanishing point. Some methods to increase contrast include supravital methods (staining or altering living cells) and dry preparation stains.

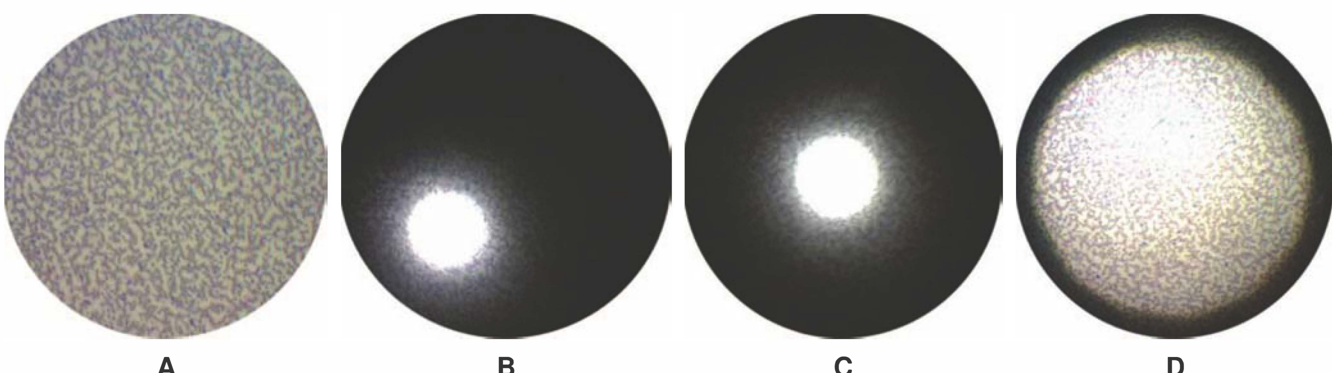


Figure 5-23. Adjusting Kohler illumination **(A)** slide in focus for the objective to be used **(B)** misalignment of the condenser is made obvious when the field diaphragm is closed **(C)** properly centered condenser with closed field diaphragm **(D)** when the field diaphragm is opened, the light coming through a properly centered condenser fills the field evenly.

SUPRAVITAL METHODS

Acetic Acid

Acetic acid may be used to aid in the identification of white blood cells. Small WBCs with nuclei and granulation not readily apparent may be difficult to differentiate form RBCs and certain epithelial cells. Adding one or two drops of 2% acetic acid enhances the nuclear pattern of WBCs and epithelial cells and lyses the RBCs.

Lipid Stains

Globules of neutral fat or triglycerides floating free in the urine or contained within cells or casts may be difficult to distinguish from other objects but stain orange or red with lipid stains such as Sudan III or Oil Red O. Only neutral fats stain with these stains. Cholesterol and cholesterol esters do not stain but can be confirmed with polarizing microscopy.

Sternheimer–Malbin Stain

Sternheimer–Malbin stain, the most commonly used stain in the clinical laboratory, is a mixture of crystal violet and safranin. One or two drops of the stain are mixed with the concentrated urine sediment before examination. The stain is absorbed well by WBCs, epithelial cells, and casts, providing better delineation of structure. Nuclei and cytoplasm are stained contrasting colors. Sternheimer–Malbin stain is supplied under various trade names by clinical and biologic supply houses.

Toluidine Blue

A 0.5% solution of toluidine blue is a good supravital stain to differentiate between the nucleus and cytoplasm in various cells. For example, it aids in distinguishing between cells of similar size, such as leukocytes and small renal collecting duct cells.

DRY PREPARATION METHODS

Gram Stain

Gram stain is usually performed in the microbiology section of the medical laboratory. A microscope slide with a dried preparation of urine sediment is heat fixed, then placed on a staining rack. The slide is flooded with crystal violet, followed by iodine (used as a mordant to fix the stain). The specimen is decolorized using alcohol or acetone and counterstained with safranin or carbol fushin.

Wright Stain

Wright stain is primarily used to differentiate among white blood cells in blood and body fluids. Wright stain might also be used to assess the presence of eosinophils in urinary sediment. Increased numbers of eosinophils are seen in urine of patients with medication induced acute interstitial nephritis.

Hansel Stain

Hansel stain is superior to Wright stain in staining eosinophils in urine and other body fluids. Hansel stain consists of eosin-Y and methylene blue, which allows for the visualization of reddish-orange eosinophils against a blue background.

Care and Preventive Maintenance

Modern clinical microscopes are precision instruments and should be handled and used with care and respect. A microscope should be carried firmly with both hands (one underneath the base). Routine cleaning (Fig. 5-24) and maintenance ensures long-term mechanical and optical performance. Dust, dirt, or other particulate matter should be removed from lenses with a soft, "camel hair" brush, or blown away with an ear or nose syringe. Some residues can be removed with lens paper after breathing on the lens surface to deposit a thin film of moisture. Manufacturer-recommended lens cleaners can also be used with the lens paper.

After using an oil immersion objective, the immersion oil should be wiped off with lens paper followed by lens cleaner and additional lens paper. Gauze, facial tissue, or laboratory wipes should not be used to clean lens surfaces because scratching can occur. Proper covering and storage of the microscope minimizes the accumulation of dust. The National Committee for Clinical Laboratory Standards

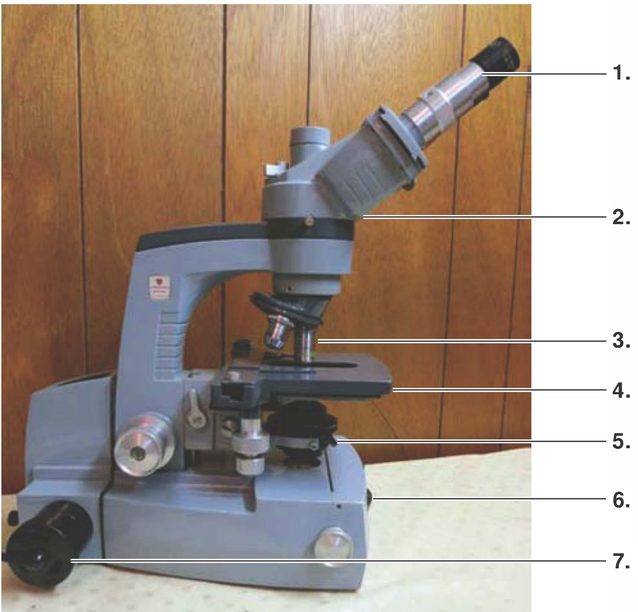
Figure 5-24. Proper microscope maintenance includes cleaning of lenses of the **(A)** oculars, **(B)** objectives, and **(C)** substage condenser with manufacturer recommended lens cleaner and lens paper.

Document POLl-T214 contains a section on general microscopy as well as an excellent summary of the care of the microscope.

STUDY QUESTIONS

Label this image of a compound microscope.

Match each microscope component listed below with its function.



8. _____ Controls the angle of light

9. _____ Controls diameters of light rays

10. _____ Focuses light onto specimen

11. _____ Moves the specimen

12. _____ Produces primary image

13. _____ Produces secondary image

14. _____ Provides illumination

A. Aperture diaphragm
B. Condenser
C. Field diaphragm
D. Light source
E. Mechanical stage
F. Ocular
G. Objective

Place the following methods of improving contrast into their appropriate category.

A. Supravital
B. Dry preparation

15. _____ Acetic acid

16. _____ Gram stain

17. _____ Hansel stain

18. _____ Oil Red O

19. _____ Sternheimer–Malbin

20. _____ Toluidine blue

21. _____ Wright stain

22. Which of the following lenses produces the primary image in brightfield microscopy?
a. Condenser
b. Iris
c. Objective
d. Ocular

23. Which of the following types of microscopy uses fluorescence?
a. Bright light
b. Confocal
c. Darkfield
d. Interference

24. Which technique(s) would you use in order to determine if a urinary crystal demonstrates birefringence?
a. Compensated light
b. Darkfield
c. Fluorescence
d. Polarization

25. A specimen will appear three-dimensional when using which technique(s)?
a. Differential contrast
b. Modulation contrast
c. Scanning electron microscopy
d. Transmission electron microscopy

Place these steps in order for adjusting a microscope for Kohler illumination.

26. _____ Adjust the substage condenser.

27. _____ Adjust the interpupillary distance.

28. _____ Center the condenser.

29. _____ Close field diaphragm.

30. _____ Focus the specimen.

31. _____ Open the field diaphragm.

32. _____ Perform diopter adjustment.

33. _____ Properly position sample.

34. _____ Turn on microscope and adjust lamp intensity.

35. _____ View the image with ocular removed and adjust diaphragm; replace ocular.

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The Urinalysis

SECTION

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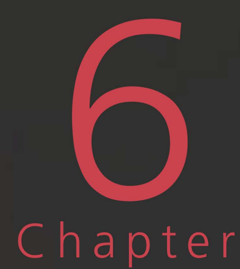
Collection and Preservation of Urine



KEY TERMS

24-Hour
Boric Acid
Catheterization
Chloroform
Chlorhexidine
Clean-Catch
Diurnal Variation
First-Morning
Formalin
Postprandial
Preservative Tablets
Random
Suprapubic Aspiration
Three-Glass Collection
Thymol
Timed Specimen
Toluene
Urea-Splitting Bacteria
Urine Collection Bags

LEARNING OBJECTIVES

1. Compare methods of urine collection.
2. Suggest appropriate urine collection method depending on testing ordered.
3. Compare urine preservation methods.
4. Select appropriate types of urine preservation depending on testing ordered.
5. Explain what changes occur in unpreserved urine over time.

Urine is the most conveniently obtainable specimen used in laboratory testing. Accuracy of these test results depend on the collection and handling of specimens. Several techniques and preservatives are used in the collection of urine, which should be used appropriately to allow for the most accurate results.

Specimen Collection Methods

The performance of an accurate urinalysis begins with the proper collection technique. Typically, 15 mL of urine is collected for routine testing. However, situations exist—such as in pediatric patients or patients producing a low volume—that dictate modification of methods used for testing. Routine physical, chemical, and microscopic tests are described in subsequent chapters. There are several methods available, depending on the type of specimen needed.

Nonsterile Urine Collection Methods

The first important step is the use of a clean, dry container. Disposable containers provided by most laboratories are the container of choice, because they avoid the possibility of contamination from improperly washed patient provided containers. One method frequently used is that of collecting the entire voided sample. The problem with this method is that the specimen cannot be used for bacterial examination. Moreover, specimens from female patients may be contaminated with vaginal discharge.

Sterile and Near Sterile Urine Collection Methods

CLEAN-CATCH

Samples that are to be cultured must be collected into sterile containers. **Clean-catch**, or clean-voided midstream, specimen is usually the method of choice for obtaining noncontaminated specimens. It is easy to perform and it provides a sample that can be used for bacteriologic examination as well as for routine urinalysis. Prior to collection, the external genitalia are thoroughly cleansed with a mild antiseptic solution. Most brands of urine collection kits include a cleansing pad (Fig. 6-1).

During the collection the initial portion of the urine stream is allowed to escape while the midstream portion is collected into a sterile container. Women should spread the labia apart while voiding. The final portion of the urine flow is also discarded. This procedure can be modified if the specimen is not needed for bacterial examination. The midstream collection, without prior cleansing or the use of a sterile container, provides a satisfactory sample for routine urine testing. If a specimen for culture is being collected into a bedpan first, the bedpan must also be sterile.

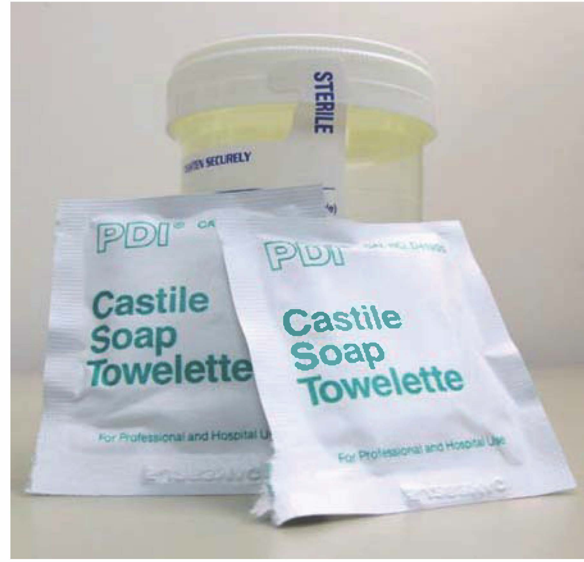


Figure 6-1. Clean-catch urine collection kits include cleansing wipes.

THREE-GLASS

Three-glass (using three containers) **collections** are similar to the clean-catch collection and are used to determine prostate infection. In the three-glass collection, all portions, beginning, middle, and final portion of the void, are collected in three separate containers. The prostate is massaged prior to collection in the third container. Urinary tract infections will show increased white blood cell counts and bacteria in the second and third containers, while prostate infections will demonstrate white blood cell counts and bacteria higher in the third container than in the second.

CATHETERIZATION

Catheterization (insertion of a sterile tubing) of the bladder is sometimes necessary to obtain a suitable specimen. This method may be used if the patient is having difficulty voiding. It can also be used in a female patient to avoid vaginal contamination, especially during menstruation. However, since this procedure carries with it the possibility of introducing organisms into the bladder which may, in turn, cause infection, it should not be routinely used for the collection of culture specimens.

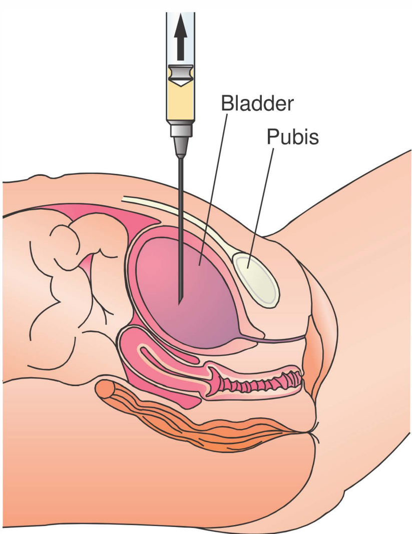


Figure 6-2. Suprapubic aspiration of urine directly from the bladder.

SUPRAPUBIC ASPIRATION

Suprapubic aspiration (Fig. 6-2) of the bladder is sometimes used in place of catheterization for obtaining a single sterile urine sample. This procedure involves the insertion of a needle directly into the distended bladder. This technique avoids vaginal and urethral contamination and can also be useful in getting urine from infants and small children. The specimen obtained by this method can also be used for cytology studies.

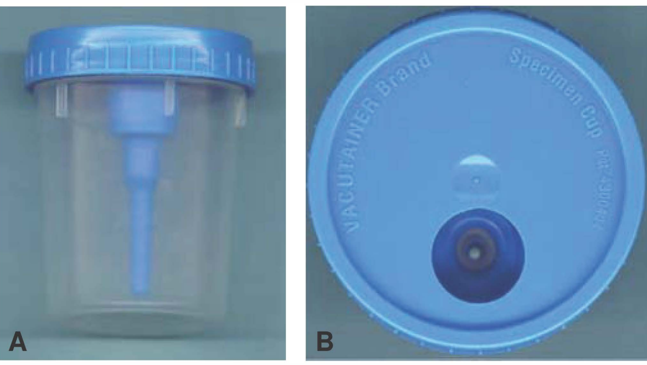


Figure 6-3. The transfer of urine from collection containers **(A)** to testing tubes is made easy with the inclusion of a stopper-piercing port **(B and C).** Always ensure the transfer tube is labeled with the same patient identifiers as the primary specimen.

Figure 6-4. Transfer of urine from collection cups not having a port in their caps is made easier with the use of a sterile transfer device.

Urine Collection Systems

ADULT URINE COLLECTION SYSTEMS

Whether clean-catch or nonsterile, most urine collection uses a collection cup from which the urine may need to be transferred to urine testing tubes. Most sterile cups have a port through which urine may be transferred (Fig. 6-3). A sterile urine aspiration device (Fig. 6-4) may be used with cups that do not have a specimen transfer port in their caps. The choice of tube to be used depends on the required tests. Urine preservative tubes that do not aspirate specimens must have urine transferred to them using a sterile pipet and technique (Fig. 6-5). Figure 6-6 shows some urine testing tubes.

Figure 6-5. Use appropriate PPEs when transferring specimens from primary container to aliquot tubes. Always label the aliquot with the same patient identifiers as the primary container before transferring specimens.

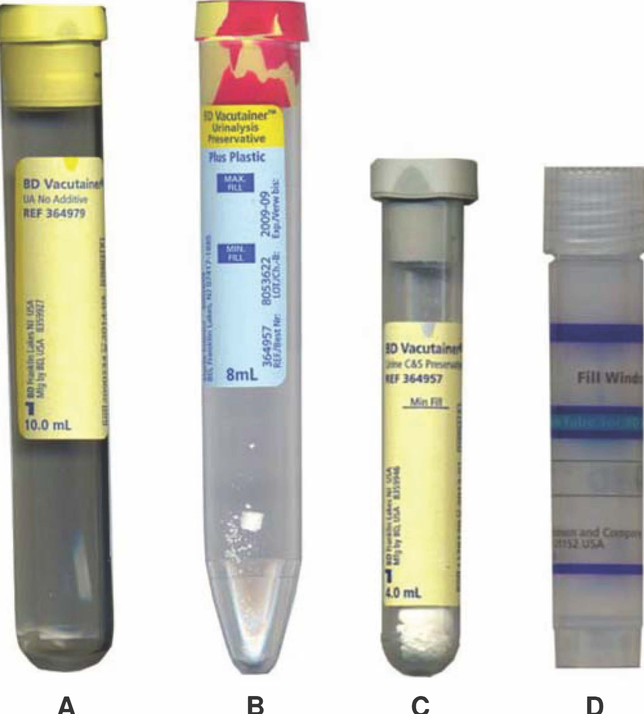


Figure 6-6. Becton Dickinson urine tubes: **(A)** no additive urine tube; **(B)** chlorhexidine, along with other chemicals, is used to stabilize urine for routine testing for up to 72 hours; **(C)** boric acid is used for urine cultures; **(D)** special urine preservative tube (UPT) containing trademarked preservative suitable for molecular testing.

INFANT URINE COLLECTION SYSTEMS

To obtain suitable specimens from infants and small children, pediatric **urine collection bags** (Fig. 6-7) are attached to the genitalia. These collection bags are soft and pliable and cause little discomfort to the patient. The bag opening, at the point of attachment varies according to its use for male or female infants. As in all urine collections, care must be taken to a void fecal contamination.

Figure 6-7. Male infant urine collection bag.

Unacceptable Urine Collection Methods

Unacceptable urine collection techniques include collecting the sample into a container that may still have detergent residue or bleach, or one that has not been adequately cleaned. Urine collected in a bedpan that also contains feces is not an acceptable specimen, nor is urine that has been squeezed out of a diaper. A sample from a diaper contents consists of filtered urine and diaper fibers (Fig. 6-8); most of the important sediment structures remain in the diaper.

Timing of Collection

RANDOM COLLECTION

A **random** sample is collected at any time convenient for the patient and is usually sufficient for performance of most urinary screening tests. However, specimens collected randomly during the day are sometimes so dilute due to increased fluid consumption that they tend to give a false picture of the patient's health.

FIRST MORNING

The **first-morning** urine is usually the most concentrated and is the preferred specimen of choice.

POSTPRANDIAL

Postprandial specimens are those collected 2 to 3 hours after eating. Postprandial specimens are often used screen for carbohydrate metabolism disorders.

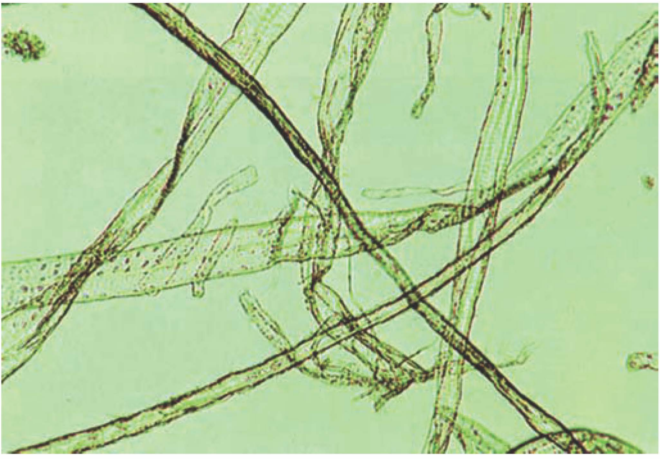


Figure 6-8. Cloth fibers (160×).

TIMED

Timed specimens are those that are collected at a particular time of day. Some substances, such as urobilinogen, demonstrate **diurnal variation** (concentration varies according to time of day). For example, urobilinogen is best evaluated in a specimen collected from 2 to 4 o'clock PM.

24-HOUR

Because urinary substances are excreted in varying concentrations throughout the day, it is necessary to collect timed specimens to accurately quantitate some substances such as creatinine, glucose, total protein, electrolytes, hormones, and urea. The most commonly used sample is the **24-hour** specimen. In this procedure, the patient empties the bladder and discards the urine. This is usually done about 8 o'clock AM. All urine is collected for 24 hours thereafter, including the sample at 8 AM the next day. The container that is used for the 24-hour specimen should be kept in the refrigerator during the entire collection period. Various chemical preservatives may need to be added to the collection container depending on the substance to be tested. For some tests, such as creatinine and protein, refrigeration alone is sufficient. To get an accurate test result, it is important that all urine excreted during the timed period be collected. It is also important that the timing be exact. Because of the difficulty that is sometimes encountered when obtaining 24-hour collections, physicians sometimes order 12-hour or 2-hour timed specimens. However, if not properly collected, these can give misleading results.

Specimen Preservation

RATIONALE FOR ADDING PRESERVATIVES TO URINE

Ideally, the specimen for routine urinalysis should be examined while fresh (less than 1 hour). If this is not possible, then it should be refrigerated until examined. Specimens that are stored at room temperature will soon begin to decompose, mainly due to the presence of bacteria in the sample. **Urea-splitting bacteria** produce ammonia, which then combines with hydrogen ions to produce ammonium, thereby causing an increase in the pH of urine. This increase in pH will result in decomposition of any casts that may be present, because casts tend to dissolve in alkaline urine. If glucose is present, bacteria may use it as a source of energy potentially resulting in a false-negative test for glycosuria. Even if bacterial contamination is not present, some urinary components such as blood cells and casts still tend to deteriorate on in the specimen over time. However, if the pH of the sample is low and the specific gravity (concentration) is high, deterioration will take longer to occur. Table 6-1 emphasizes these potential changes in unpreserved urine specimens.

Table 6-1 Changes Occurring to Unpreserved Urine

POTENTIAL CHANGE	CHANGE OCCURRING OVER TIME
Color	Oxidation of substances
Clarity	Increased turbidity due to proliferation of bacteria or precipitation of chemical substances
Odor	Increasing strength due to proliferation of bacteria
pH	Increases as bacteria convert urea to ammonia and the loss of CO_2 from the specimen
Bilirubin	Decreasing due to photooxidation and hydrolysis
Glucose	Decreases due to metabolism by microorganisms
Ketones	Volatillization
Nitrites	Increasing due to proliferation of bacteria, but also decreasing as bacteria continue to convert nitrite to nitrogen
Urobilinogen	Decreasing due to oxidation
Crystals	Appearing due to cooling of the specimen
Cells and cases	Decreasing due to cellular degeneration
Microorganisms	Increasing due to proliferation

Preservatives

There are times when a urine specimen must be saved for a longer period of time than is recommended. This is a common occurrence when specimens are sent to commercial laboratories for analysis as well as current methods for routine urine screening. There are several chemical preservatives that can be added to specimens; however most of them interfere in some way with the testing procedure. Understanding the effects of preservatives on laboratory tests will help to ensure that tests are performed on properly preserved specimens. Preservatives that can be used to preserve urine specimens include boric acid, chloroform, chlorhexidine, formalin, thymol, toluene, and formaldehyde generating preservative tablets.

BORIC ACID

Boric acid preserves formed elements but interferes with the pH reading. Boric acid is the preservative used in tubes used to preserve urine for culture and sensitivity. The Becton Dickinson brand is a gray-stoppered evacuated tube containing boric acid and sodium formate (Fig. 6-6C). This tube should

glucose preservative. Becton Dickinson manufactures a red/yellow–stoppered conical evacuated tube that contains chlorhexidine, ethylparaben, and sodium propionate (Fig. 6-6B). Although specimens transferred to this tube for transport are stable for 72 hours, if not protected from light will yield erroneous bilirubin and urobilinogen results.

FORMALIN

Formalin (1 drop/30-mL urine) is a good preservative for urinary sediment but if used in too large a concentration it will precipitate protein, give a false-positive test for reducing substances, thus making these parameters unreportable.

PRESERVATIVE TABLETS

Commercially available **preservative tablets** (1 tablet/30-mL urine), usually act by releasing formaldehyde. At this concentration the formaldehyde will not interfere with the test for reducing substances, but higher concentrations will result in false positives. Formaldehyde increases the specific gravity by 0.005/1 tablet/30 mL.

TOLUENE

Toluene (2-mL/100-mL urine) preserves, ketones, proteins, and reducing substances, but it is not effective against bacteria already present in the urine. Because toluene floats on the surface of the urine, it may be difficult trying to separate the preservative from the specimen for testing. In addition, toluene is flammable.

THYMOL

Thymol (one small crystal) is an adequate but rarely used preservative for most urinary constituents. Thymol interferes

Match the type of urine collection with its most appropriate use:

A. Catheterization

B. Clean-catch

C. Early afternoon

D. First morning

E. Postprandial

F. Random

G. Suprapubic aspiration

H. Three-glass

I. Timed

J. 24-hour

1. ______ diabetic screening

2. ______ diagnosis of prostate infections

3. ______ insertion of a needle directly into the bladder

4. ______ insertion of tubing through the urethra into the bladder

5. ______ most concentrated specimen

6. ______ routine analysis

7. ______ screening for infection

8. ______ substance quantitation

9. ______ substances showing diurnal variation

10. ______ urobilinogen quantitation

Match urine preservatives with their description:

A. Boric acid

B. Chloroform

C. Chlorhexidine

D. Formalin

E. Refrigeration

F. Thymol

11. ______ rarely used preservative

12. ______ may precipitate protein

13. ______ inhibits bacterial growth

14. ______ culture and sensitivity

15. ______ routine analysis within 72 hours

16. ______ adequate for a short time

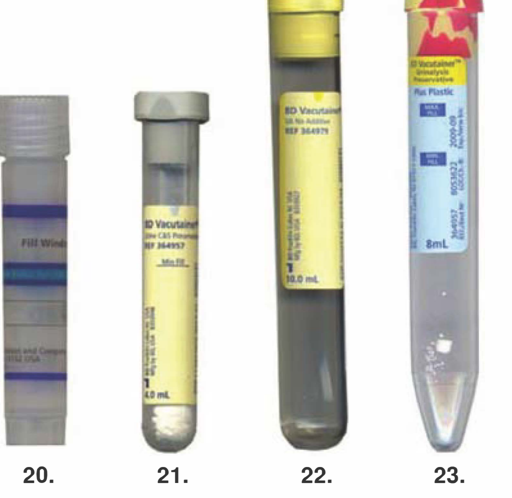
Select the best answer:

17. What method of collection is best used for urine collection from infants?
- **a.** Catheterization
- **b.** Clean-catch into a sterile urine cup
- **c.** Collection into bedpan
- **d.** Collection into a U-bag

18. What is the best method for testing urine that is delivered in a sterile cup?
- **a.** Perform urine screening tests directly in the sterile container.
- **b.** Pour off the urine into a urine preservative or testing tube.
- **c.** Use a pipet to transfer urine into a preservative or testing tube.
- **d.** Use a sterile aspiration device or cap port to transfer urine into a urine preservative tube.

19. Which specimen is the best to use for diabetes screening?
- **a.** Random
- **b.** First morning
- **c.** Postprandial
- **d.** 24-hour collection

Explain the use of the urine testing tubes in this image.



20. ________________________

21. ________________________

22. ________________________

23. ________________________

CASE STUDIES

Case 6-1 A young female patient is having difficulty following the clean-catch procedure for urine collection. There are contaminating bacteria present in the specimen which are making it difficult to diagnose urinary tract infection. Suggest how a specimen suitable for culture can be collected.

Case 6-2 A day-old urine is received in the laboratory that has not been transferred to a urine preservative tube. Comment about the suitability of this specimen for routine urine screening tests.

Case 6-3 A urine specimen that was collected by clean-catch into a sterile urine cup is received in the laboratory. How should it be transferred into appropriate urine tubes for urine culture and molecular testing for sexually transmitted disease?

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Physical Examination of Urine

Chapter 7

KEY TERMS

Alkaptonuria
Anthocyanins
Bilirubin
Biliverdin
Clarity
Colligative Property
Color
Foam
Freezing Point
Hematuria
Hemoglobinuria
Homogentisic Acid
Hyposthenuria
Hypersthenuria
Isosthenuria
Melanin
Melaninogen
Myoglobinuria
Odor
Osmolality
Phenolsulfonphthalein
Porphyrinuria
Pyridium
Refractive Index
Refractometer
Specific Gravity
Urinometer
Urobilin
Urobilinogen
Urochrome
Uroerythrin
Vapor Pressure

LEARNING OBJECTIVES

1. Explain what is included in physical examination of urine.
2. Identify normal and abnormal urine color and clarity.
3. Suggest causes for urine odor, color, and clarity.
4. Describe the methods for measuring urine concentration.
5. Judge whether a method for measurement of specific gravity needs correction for temperature and chemical effects.
6. State normal values for urine concentration.
7. Suggest causes for abnormal urine concentration.
8. Recognize and correct for sources for error when measuring urine concentration.

For centuries, physicians have used physical characteristics (urine appearance and odor) as diagnostic tools. These characteristics point to possible renal and metabolic disorders. In addition, urine appearance correlates with chemical and microscopic findings in these disorders.

Urine Color

NORMAL URINE COLOR

Normal urine has a wide range of **color** (shades of pigmentation) that is mainly determined by its concentration. This color may vary from a pale yellow to dark amber, depending on the concentration of the pigments **urochrome** and, to a lesser extent, **urobilin** and **uroerythrin**. The more pigment present, the deeper the color will be. Figure 7-1 displays the array of colors that may be exhibited by urine.

ABNORMAL URINE COLOR

Many factors and constituents that can alter the normal urine color. These include medications and diet as well as various chemicals that can be present in disease. Table 7-1 lists some of the substances that may influence the color of urine. This table should not be considered as an all-inclusive list, for there are numerous drugs that are capable of changing the color of urine. It should be noted that the pH of the urine influences the color that many chemicals produce. In addition, there may be several coloring factors present in the same urine, which may result in a different color than that expected.

Pale to Colorless Urine

Very pale or colorless urine is very dilute and can result from high fluid consumption, diuretic medication, natural diuretics such as coffee and alcohol, and in such disease states as diabetes mellitus and diabetes insipidus.

Figure 7-1. Urine specimens of varying normal colors.

Red Urine

The most common cause of red urine is the presence of **hematuria** (the presence of red blood cells). Red urine may also be due to **hemoglobinuria** (the presence of free hemoglobin), **myoglobinuria** (myoglobin in urine), or large amounts of uroerythrin that can occur in acute febrile disease. In some types of **porphyrinuria** the urine may have a red or a port-wine color; and only develop a red color if left standing. The dye **phenolsulfonphthalein**, which is used kidney function tests, can produce a red color in alkaline urine.

In addition, some individuals have an inherited metabolic sensitivity which results in the excretion of red urine after eating beets due to the presence of complex pigments called **anthocyanins**.

Brown and Black Urine

Urine that contains red cells or heme pigments can vary in shades from pink through black. The final color is determined by the amount of RBCs or pigment present, pH of the urine, and how long the pigment has been in contact with the urine. For example, an acid urine which contains hemoglobin will darken over time because of the formation of methemoglobin (oxidized hemoglobin). This reaction can occur either in vivo, as in the bladder, or in vitro, while waiting to be tested.

Another cause of dark brown to black urine is **alkaptonuria**, a rare disorder that is characterized by the excretion of **homogentisic acid** in the urine. The presence of homogentisic acid in alkaptonuria is due to the congenital lack of the enzyme homogentisic acid oxidase, which mediates an important step in the catabolism of tyrosine and phenylalanine. The urine is normal in color when freshly voided but turns dark on standing or when alkalinized (Fig. 7-2). In patients with malignant melanoma, a colorless pigment called **melanogen** occurs in the urine. On exposure to light, this chromogen is converted to **melanin**, which is black, thus darkening the urine.

Yellow-Green Urine

Patients with obstructive jaundice will excrete bile pigments such as **bilirubin**, and the urine will be yellow-brown to yellow-green, or similar to olive green in color. The green pigment is due to **biliverdin**, the oxidized product of bilirubin, and over time the green color will intensify.

Other Urine Colors

There are several medications and dyes that can impart a characteristic color to the urine. Although these colors are not clinically significant, they may interfere with color-based testing methods (explained in the chapter on chemical testing). Penazopyridine (**Pyridium**), which acts as an analgesic in the

Table 7-1 Causes for Urine Color and Clarity

APPEARANCE	PATHOLOGIC CAUSES	NONPATHOLOGIC CAUSES
White	Chyle Lipids Pyuria (many WBCs)	Phosphates Vaginal creams
Yellow to amber to orange	Bilirubin Urobilin (excessive)	Acriflavine Azo gantrisin Carrots Concentrated urine Food color Nitrofurantoin Pyridium Quinacrine Riboflavin Rhubarb Senna Serotonin Sulfasalazine Vitamin B complex
Yellow to green	Bilirubin–biliverdin	
Pink to red	Hemoglobin Myoglobin antipyrine Porphobilin Porphyrins bromosulfophthalein Red blood cells	Aminopyrine Beets (anthocyanin) Cascara Diphenylhydantoin Food color Methyldopa Phenacetin Phenolphthalein Phenolsulfonphthalein Phenothiazine Pyridium Senna
Red to purple	Porphyrins	
Red to brown	Methemoglobin Myoglobin	
Brown to black	Bilirubin Homogentisic acid Indican Melanin Methemoglobin Myoglobin Phenol p-Hydroxyphenylpyruvate Porphyrins	Chloroquine Hydroquinone Iron compounds Levodopa Methyldopa Metronidazole Nitrofurantoin Quinine Resorcinol
Blue to green	Biliverdin Indicans Pseudomonas infection	Acriflavine Amitriptyline Azure A Chlorophyll Creosote Evans blue Methylene blue Phenyl salicylate Thymol Tolonium Triamterene
Clear	Very dilute as in diabetes insipidus	Polyuria
Hazy to cloudy to turbid	Varying degrees of casts Cells Crystals and calculi Fat (lipid, chyle) Microorganisms	Varying degrees of creams, lotions, and salves Crystals Fecal contamination Microorganisms Mucus Radiographic dyes Powders Spermatozoa

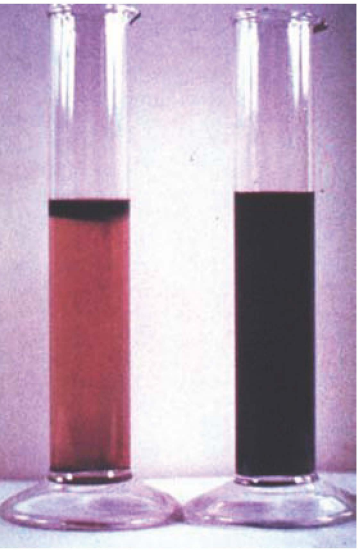

Figure 7-2. Urine specimens from a patient with alkaptonuria **(A)** near fresh specimen; **(B)** turns black over time.

Figure 7-3. Urine specimens of varying abnormal colors and clarity.

bladder, gives an orange color to the urine and to any foam that may be present. Methylene blue is also used as a urinary antiseptic and can make the urine blue or blue-green. The presence of Azure A following the Diagnex Blue test for HCl may also turn urine a blue or blue-green color for several days after the test. Multivitamins and riboflavin can contribute a bright yellow color to urine. Even food dyes such as those used in candies can be excreted in the urine, thus affecting its color.

CLARITY

Clarity reflects the degree of urine transparency, often reported as clear, hazy, or cloudy. Normal urine is usually clear but it may become cloudy due to the precipitation of amorphous crystals. Amorphous phosphates are a white precipitate which will dissolve when acid is added. Amorphous urates frequently have a pink color from urinary pigments, and they will dissolve if the specimen is heated.

Urine can be cloudy from the presence of leukocytes or epithelial cells. The presence of these cells can be confirmed by microscopic examination of the sediment. Bacteria can also cause cloudiness, especially if the specimen has been sitting at room temperature. Mucus can give the urine a hazy appearance, and RBCs can result in a smoky or turbid urine. Fat and chyle give urine a milky appearance. Figure 7-3 also includes urines of various degrees of clarity.

Miscellaneous

FOAM

Although not routinely reported, **foam** (bubble accumulation at the top of a specimen) may be a significant finding. White stable foam that is formed upon agitating the specimen can be seen in urine containing a moderate or large amount of protein (Fig. 7-4). Foam that is present after urine is shaken may appear yellow to yellow-green if sufficient amount of bilirubin

Figure 7-4. Urine containing protein may produce a foam when agitated.

is present. Other substances that alter urine color usually do not alter the color of foam that may be formed upon agitating the specimen. The observance of foam and its color should guide the technologist's interpretation of chemical tests and selection of confirmatory procedures.

ODOR

Although not routinely reported urine **odor** (a smell) may be a significant observation. Ketones smell sweet or fruity. A specimen contaminated with bacteria may have a pungent smell from the ammonia that is produced. The excretion of urine that smells like maple syrup is an indication of a congenital metabolic disorder which has been appropriately named "maple syrup urine disease." A "musty or mousy" odor of an infant's urine may indicate phenylketonuria. A "sweaty feet" odor is found in isovaleric acidemia or in individuals who have excessive amounts of butyric or hexanoic acid. Hypermethioninemia has been associated with a "rancid butter" or "fishy" odor. Prolonged presence of any strong unusual odor may be associated with inherited disorders.

Concentration

Measurements of urine concentration reflect the amount of solutes present in a urine sample. Urine concentration varies according to the amount of water and solutes eliminated by the kidneys, thereby making urine concentration useful for assessing renal functions such as the ability to conserve water through tubular reabsorption. Specific gravity and osmolality are nonspecific tests used to determine the concentration of urine. They can only indicate or support a suspected decrease in renal function. The underlying problem—whether it be renal disease, diabetes insipidus, or the effect of diuretic therapy—cannot be discerned by using these tests.

SPECIFIC GRAVITY

Specific gravity is the ratio of the weight of a volume of urine to the weight of the same volume of distilled water at a constant temperature. It is an indicator of the concentration of dissolved material in the urine; however, it is dependent not only upon the number of particles but also upon the weight of the particles in the solution. The specific gravity is used to measure the concentrating and diluting ability of the kidney in its effort to maintain homeostasis in the body. The concentrating ability of the kidney is one of the first functions to be lost as a result of tubular damage.

The normal range of specific gravity for a random specimen is 1.003 to 1.035, although in cases of excess hydration the reading may be as low as 1.001 (water is 1.000). The specific gravity value varies greatly depending on the state of hydration and the urinary volume. Usually the specific gravity rises when the fluid intake is low and falls when fluid intake is high. Because the specific gravity varies throughout the day, a single random reading may not give the physician sufficient information, so a 24-hour collection may be ordered. The range for a 24-hour specimen is 1.015 to 1.025.

The specific gravity can be useful in differentiating between diabetes insipidus and diabetes mellitus. Both diseases produce a high urinary volume, but in diabetes insipidus the specific gravity is very low because in this disease there is a deficiency of antidiuretic hormone. In diabetes mellitus, there is a deficiency of insulin and thus an excess of glucose, which exceeds the renal threshold and is excreted in the urine. Glucose molecules are very dense and, therefore, the urine will have a very high specific gravity reading by some methods.

Because the specific gravity is affected by the presence of very dense molecules such as protein and glucose, some laboratory protocols require correction for high glucose and protein concentrations. This correction involves subtracting 0.003 from the specific gravity reading (after temperature correction) for each 1 g/dL of protein and 0.004 for each 1 g/dL of glucose. There is some question whether this correction is necessary, so few laboratories correct for protein and glucose. In addition, some methods of measuring specific gravity are temperature dependent, requiring that results be corrected for temperature (explained under urinometer procedures in Appendix D).

Hyposthenuria is a term that is used to describe urine with a consistently low specific gravity (less than 1.007). The specific gravity of the glomerular filtrate is believed to be around 1.007. In hyposthenuria there is a concentration problem. Causes of decreased specific gravity include excessive fluid intake, collagen disease, pyelonephritis, hypertension, protein malnutrition, polydipsia, and diabetes insipidus. Diuretic medication as well as the natural diuretics (coffee, alcohol) will also result in specimens having low specific gravities.

The excretion of urine of unusually high specific gravity is called **hypersthenuria** and this can result from deprivation of water. **Isosthenuria** refers to a fixed specific gravity of 1.010, which indicates poor tubular reabsorption. Some of the causes of increased specific gravity include dehydration, proteinuria, glycosuria, eclampsia, heart failure, renal stenosis, syndrome of inappropriate antidiuretic hormone secretion, lipid nephrosis, and water restriction.

Specific gravity can also be falsely elevated by the presence of such high-density compounds as dextrans and the radiographic dyes used in radiographs. Depending upon how soon the urine sample is collected after the x-ray procedure, the specific gravity may be greater than 1.050. Because the kidney is limited in how high it can concentrate the urine, a specific gravity reading of greater than 1.035 should be suspected to be caused by abnormal solutes or dyes.

Osmolality

Osmolality is another measure of the concentrating ability of the kidneys. Osmolality is better than specific gravity for this determination because small quantities of high–molecular-weight solutes, such as glucose and protein, will affect specific gravity measurements but not osmolality measurements. When glucose and protein are present in urine, they indicate a disease process and not a change in kidney concentrating ability. Changes in osmolality are directly proportional to the amount of solute regardless of the type of solute.

A normal adult on a normal diet will produce urine with an osmolality of about 500 to 850 mOsm/kg water. The normal kidney should be able to produce urine as dilute as 40 to 80 mOsm/kg water during excessive hydration and as concentrated as 800 to 1,400 mOsm/kg water during dehydration. In terminal renal failure the urine osmolality may stay around 285 mOsm/kg, which is the osmolality of plasma and the glomerular filtrate, indicating that the kidney is unable to dilute or concentrate the urine.

Specific Gravity Versus Osmolality

Urine osmolality and specific gravity are both measures of total solute concentration, but they do not provide the same information. Osmolality depends on the number of particles in the solution, whereas specific gravity depends on the number and weight of the solutes. Osmolality is a better indicator of the concentrating and diluting abilities of the kidney, because it is unaffected by the density of such solutes as glucose, protein, dextrans, and radiographic dyes. Osmolality required more time, expense, and equipment than specific gravity, which is why it has not been included in the routine urinalysis procedure.

Normally the urinary osmolality and specific gravity have a fairly straight line relationship with approximately 40 mOsm (milliosmoles) being equal to each unit of specific gravity. Specific gravity values of 1.010, 1 .020, and 1.030 are roughly equivalent to 400, 800, and 1,200 mOsm/kg water. However, in renal disease and in the presence of dense substances, this relationship is altered.

Examination Methods

The following sections will discuss examination methods and tools such as urinometer and refractometer, specific gravity reagent strips, and harmonic oscillation densitometry.

URINOMETER

The urinometer was previously used to measure specific gravity at a specific temperature, usually 20°C. Its use is no longer recommended for clinical measurements per the Clinical and Laboratory Standards Institute. However, the **urinometer**, which is a hydrometer, is an instrument that truly measures specific gravity. Other methods for measuring concentration (refractive index, reagent strip), actually measure a characteristic other than specific gravity, even though the result is reported as specific gravity. An explanation of the urinometer is included in Appendix D.

REFRACTOMETER

A **refractometer** is a meter that measures total solids (TS) of a solution. The refractometer actually measures the refractive index of the solution, but some models have scales that are calibrated to give readings for specific gravity, total protein, and TS. Studies have established the relationship between the refractive index and these other measurements of concentration.

Refractive index is the ratio of the velocity of light in air to the velocity of light in solution. The path of light is deviated when it enters a solution, and the degree of deviation or refraction is proportional to the density of the solution. Refractive index varies with temperature, but the TS meter is temperature-compensated for temperatures between 60°F and 100°F and, therefore, requires no corrections in that range. The TS meter contains a liquid in a sealed chamber in the optical path, and this liquid will also have a refractive index change with temperature, thus compensating for changes in the refractive index of the sample. The chamber also contains an air bubble which allows for the expansion of the liquid, but a bubble trap prevents it from getting into the light path. Figure 7-5 shows a schematic diagram of the refractometer showing an example of the light path entering and being deviated by the solution and the internal prisms. The refractometer can be used in a flow-through method (Fig. 7-6) or manually. The manual method requires only one drop of specimen, which gives refractive index measurements an advantage over other methods.

To perform the test manually, first rinse off, then dry the surface of the cover and the prism. Close the cover plate and allow the sample to be drawn under the cover by capillary action. Hold the instrument up to a light source and read the specific gravity scale at the light-dark boundary. Figure 7-7 shows the appearance of the refractometer scale. The scale reads up to 1.035, so specimens that fall of f-scale must be diluted. The result obtained on the diluted sample must be adjusted for the dilution by multiplying the numbers after the decimal point by the dilution factor. For example, if one in two dilutions obtains a result of 1.025, the actual result is 1.050.

The calculation is $1.000 + (0.025 \times 2) = 1.050$.

The zero setting of the instrument should be checked daily with distilled water, but it should rarely, if ever, need adjustment. If the reading is not 1.000, repeat the test before adjusting the setscrew, which moves the objective lens in the light path. This type of instrument does not contain mechanically moving parts and it, therefore, retains its accuracy at any

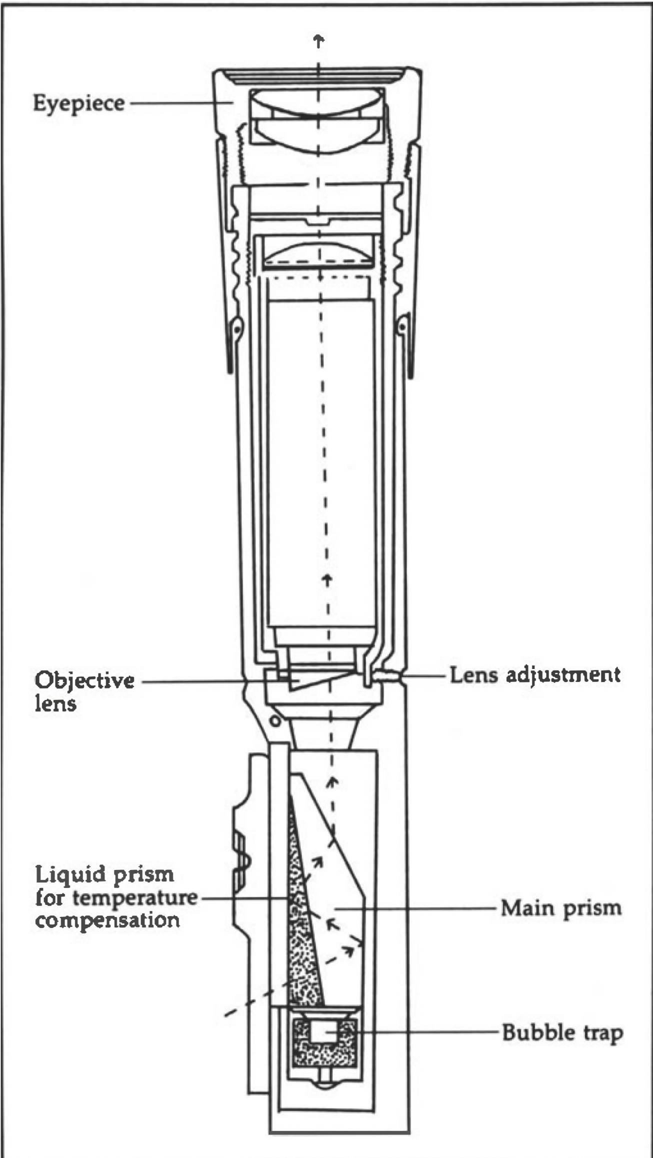

Figure 7-5. Schematic diagram of the total solids refractometer. (Courtesy of the American Optical Company, New York, NY, USA.)

point in the scale. By checking the correctness of a reading at one point against a known standard, accuracy over the entire scale is verified.

SPECIFIC GRAVITY REAGENT STRIPS

Some reagent dipsticks contain a reagent pad for measuring specific gravity. The test is based on the pK_a change of certain pretreated polyelectrolytes in relation to ionic concentration; therefore, the procedure is actually measuring the ionic concentration of the urine, which relates to the specific gravity. The polyelectrolytes in the reagent pad contain acid groups that disassociate according to the ionic concentration of the specimen. When more ions are present, more acid groups become disassociated, releasing hydrogen ions and causing the pH to change. The reagent pad contains a pH indicator (bromothymol blue) which then measures the change in pH. When urine has increased specific gravity, the reagent pad becomes more acidic. The colors of the reagent pad will range from deep blue-green in urines of low ionic concentration through green and yellow-green in urines of increasing ionic concentration. The color blocks are in increments of 0.005 for specific gravity readings between 1.000 and 1.030. Figure 7-8 shows the correlation between specific gravity ranges and color changes on the reagent strip pad.

The chemical nature of the specific gravity reagent strip may cause slightly different results from those obtained with

Figure 7-6. Total solids refractometer using flow-through setup.

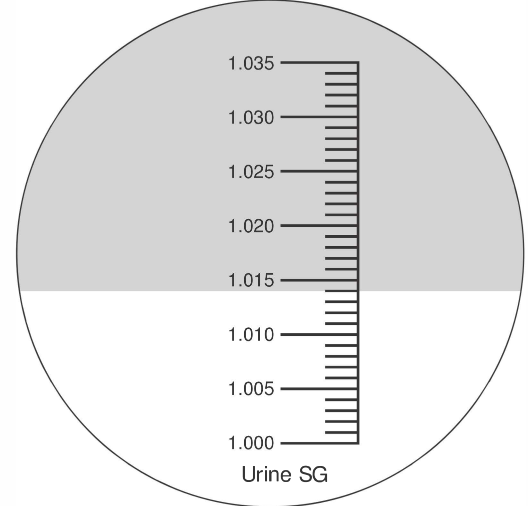

Figure 7-7. Schematic representation of the refractometer scales of measurement. This measurement represents a reading of 1.014.

Figure 7-8. Specific gravity color chart. (Modified from Tarrytown, NY: Bayer Corporation Diagnostics Division, 1996.) Note: this chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing.

other specific gravity methods when elevated amounts of certain urine constituents are present. Unlike other methods for determining specific gravity, the reagent strip method is not affected by nonionizing substances such as glucose, protein, urea, and radiographic dyes. Urines that contain glucose or urea in concentrations greater than 1% may have lower specific gravity readings than by other methods, while moderate amounts of protein (100 to 750 mg/dL) may cause elevated specific gravity readings. Urines that contain radiographic dyes will have lower readings than by other methods, because the iodine in the dye is not ionic and, therefore, it will not react with the reagent. Highly buffered alkaline urines may cause low readings, so the manufacturers often suggest that, for greater accuracy, a value of 0.005 may be added to the readings from urines with pH greater than 6.5. Confirmation of specific gravity readings may also be performed using the refractometer, especially when the reading exceeds the reagent strip maximum of 1.030.

HARMONIC OSCILLATION DENSITOMETRY

Similar to urinometry, harmonic oscillation densitometry is not commonly used in the clinical laboratory. This method uses sound waves to measure urine concentration and is discussed in further detail in Appendix B.

OSMOMETRY

Osmometry measures the concentration of solutes that contribute to the osmotic pressure of a solution. An osmole is defined as 1 g molecular weight of a substance divided by the number of parts into which it dissociates. Sample amounts vary from 0.25 to 2 mL depending upon the instrument and the type of cuvette used in the analysis. Urine osmolality is determined by comparing the **colligative property** (changes in characteristic of a solution depending on its concentration) of a sample to that of a known solution. When solutes are present, they change the sample's colligative property by altering its boiling point, freezing point, osmotic pressure, and vapor pressure. Most common methods for measuring osmolality assess a sample's freezing point or by vapor pressure.

Freezing Point Depression

Freezing point depression methods are based on the fact that a solution containing 1 osmole (or 1,000 milliosmoles) per kilogram of water lowers the freezing point 1.86°C below the freezing point of water (0°C). Osmometers that measure colligative properties using freezing point depression do so by supercooling (27°C) a measured amount of sample. The cooled sample is subjected to vibration to induce crystallization of water in the sample, which temporarily raises the temperature of the sample to its freezing point. This information is compared against the freezing point of a standard sodium chloride solution of known concentration; and the number of milliosmoles is calculated as follow:

msmol/kg H_2O = fp°C / −1.86 × 1,000
fp = measured freezing point

Vapor Pressure Depression

Vapor pressure depression (dew point) methods measure colligative properties by determining the temperature at which water vapor in a sample condenses to its liquid state. Solutes present in the sample decrease the sample's vapor pressure. A filter paper disk, to which sample has been added, is placed into a special chamber that controls evaporation of the sample to vapor. The temperature is lowered, allowing water in the sample to condense. Heat is produced, raising the temperature to the sample's dew point, which is proportional to the vapor pressure. This information is compared against vapor pressure of a standard sodium chloride solution of known concentration; and the number of milliosmoles is calculated. Care must be taken to prevent evaporation of the sample before testing with vapor pressure osmometers. Osmolality measurements by vapor pressure methods are used primarily on serum specimens and not normally performed on urine specimens.

STUDY QUESTIONS

Label the parts of this refractometer.

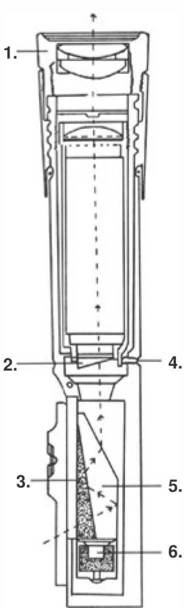

Figure 7-9. Image for questions 1 to 7.

1. ______________________________

2. ______________________________

3. ______________________________

4. ______________________________

5. ______________________________

6. ______________________________

7. What does the dashed line in Figure 7-9 represent?

____

Match urine appearance with its listed cause:

A. Amber and clear
B. Blackening with time
C. Brown and cloudy
D. Colorless and clear
E. Foamy when shaken
F. Greenish tinge
G. Orange
H. Red (wine) and clear
I. Red and cloud
J. Yellow and clear

8. __________ normal urine appearance

9. __________ alkaptonuria

10. __________ bilirubin

11. __________ concentrated urine

12. __________ medications

13. __________ red blood cells

14. __________ old specimen with RBCs

15. __________ porphyrins

16. __________ proteins

17. __________ very dilute urine

Match urine odor with their causative constituent.

A. Fishy
B. Fruity
C. Musty
D. Pungent
E. Sweaty feet
F. Syrupy

18. __________ Amino acid disorder

19. __________ Ammonia

20. __________ Bacteria

21. __________ Hypermethioninemia

22. __________ Ketones

23. __________ Leucine and isoleucine

24. __________ Phenylalanine

25. Which methods can be used to determine specific gravity? (choose all that apply)
   a. Freezing point depression
   b. Reagent strip
   c. Refractometer
   d. Vapor pressure depression

26. Which methods can be used to determine osmolality? (choose all that apply)
   a. Freezing point depression
   b. Hydrometer
   c. Refractometer
   d. Vapor pressure depression

27. Refractive index compares the velocity of light in urine to the velocity of light in:
   a. Air
   b. Oil
   c. Saline
   d. Water

28. Which principle is used in the determination of specific gravity by reagent strip methods?
   a. The pK_a of a polyelectrolyte is altered by the urine's ionic strength.
   b. Cations are chelated by a color-changing ligand.
   c. Ions catalyze the oxidation of a chromogen.
   d. Solutes release H ions to change the pK_a.

CASE STUDY

Case 7-1 A urine is received in the laboratory and is found to be yellow and cloudy. Suggest possible causes for this observation.

Case 7-2 A few hours after having eaten a meal, a person notices that his or her urine is red but clear.

1. What benign cause may be given as an explanation for this occurrence?

2. What may cause clear red urine on a more regular basis?

3. What may be indicated by a cloudy red urine?

Case 7-3 A specific gravity reading of 1.000 is obtained by reagent strip method on a urine with a pH of 8.0. What should you do before reporting this result?

Case 7-4 A specific gravity reading of 1.030 is obtained by reagent strip method on a urine sample and is confirmed by refractive index as greater than 1.035. What should you do before reporting this result?

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Chemical Analysis of Urine

8

Chapter

KEY TERMS

Acetest
Ascorbate (Ascorbic Acid)
Bilirubin
Calcium
Chromogen
Clinitest
Creatinine
Glycosuria
Hematuria
Hemoglobinuria
Ictotest
Ketones
Leukocyte Esterase
Microalbumin
Myoglobin
Nitrite
ph
Protein
Protein Error of Indicators
Proteinuria
Reagent Strip
Reducing Substance
Run-Over Effect
Tamm–Horsfall Protein
Urobilinogen

LEARNING OBJECTIVES

For Each Chemical Test Performed by Dipstick Methodology

1. Describe the principle and procedure, for each chemical test.
2. Compare and contrast the reagent strip characteristics among manufacturers.
3. Interpret the results of urine chemistry tests.
4. Define expected normal values.
5. Suggest the causes for abnormal findings.
6. Identify the sources of error.
7. Suggest appropriate confirmatory tests.
8. Correlate results of chemical tests with those of physical examination.
9. Predict findings of microscopic examination.

For the Confirmatory Urine Tests (Tablet Tests)

10. Describe the principle and procedure.
11. Interpret the results.
12. Recognize the sources of error.
13. Suggest appropriate clinical applications.

The routine urinalysis includes chemical testing for pH, protein, glucose, ketones, occult blood, bilirubin, urobilinogen, nitrite, leukocyte esterase, and strip test method for specific gravity. The urinalysis offered by laboratories depends on the type of dipstick that is used. In addition, most laboratories routinely screen for reducing substances as part of the routine urinalysis for children 2 years old and younger. These procedures are either qualitative (positive or negative) or semiquantitative (e.g., trace through 4+) measurements. Since the introduction of single- and multiple-test reagent strips, test tapes, and tablets, the chemical screening of the urine has become a sensitive and rapid procedure. Completion of urine chemistry using reagent test strips occurs in 2 minutes. Several brands of dipsticks are available worldwide. A comparison of some of the strips available from various manufacturers is made in this chapter.

A **reagent strip**, also called a dipstick, is a narrow strip of plastic with small pads attached to it. Each pad contains reagents for a different reaction, thus allowing for the simultaneous determination of several tests. The colors generated on each reagent pad vary according to the concentration of the analyte present. Colors generated by each pad are visually compared against a range of colors on brand-specific color charts. Color charts for one brand of reagent strips discussed in this text is included in Appendix C. Figure 8-1 illustrates a typical urine chemistry reagent strip (dipstick).

To obtain accurate and reliable results with the dipsticks, certain precautions must be taken to help maintain the reactivity of the reagents. The strips must not be exposed to moisture, direct sunlight, heat, or volatile substances; and they should be stored in their original containers. The container should not be kept in the refrigerator nor exposed to temperatures over 30°C. Each vial or bottle contains a desiccant, but the strips should still not be exposed to moisture. Remove only the number of strips needed at the time of testing and then tightly close the container. If the color blocks on the strip do not resemble the negative blocks on the color chart or if the expiration date on the container has past, discard the strips. Figure 8-2 shows the appearance of an improperly stored reagent strip.

The manual method for using a reagent strip to test urine calls for dipping the entire strip into the specimen and withdrawing it in one continuous motion while removing excess urine by dragging across the edge of the specimen container (Fig. 8-3). A critical requirement is that the reactions be read at the prescribed time after dipping and then compared closely with the color chart provided by the manufacturer as shown

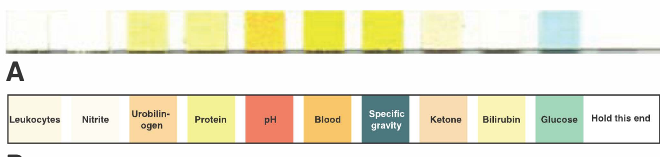


Figure 8-1. **A:** Multistix 10 SG. **B:** Illustration of Multistix 10 SG with pad identification labels. (Modified from Siemens [formerly Bayer Corporation, Tarrytown NY]; 1996.)

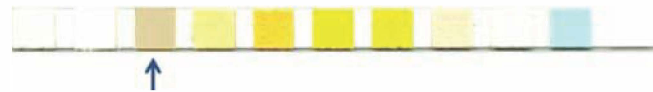


Figure 8-2. Notice the discoloration of reagent pad (*arrow*) on this test strip that was exposed to moisture during storage (compare with Fig. 8-1A).

in Figure 8-4. Urine should be tested at room temperature. If the urine specimen has been refrigerated, it should be brought room temperature before testing. The procedure for using the dipstick is outlined in Box 8-1.

Several brands of urine chemistry dipsticks are compared in this text (Fig. 8-5). The reagents used for these dipsticks vary according to manufacturer.

The reagents for each parameter measured by these manufacturers along with their sensitivities are listed in tables that appear with the discussion of each parameter. Although examples of each parameter's color reactions are also included, they portray the results obtained by only one manufacturer. Chemical reaction colors vary slightly as do the timing of the reactions. Color charts for one brand of reagent strips discussed in this text is included in Appendix C. Always review and follow the manufacturers' latest directions, as improvements to the reagent strips may have been made for more recently manufactured lot numbers of strips.

Even with the widespread use of the rapid and convenient screening procedures, it is still necessary to understand the basic principles of the tests as well as the correct technique to be used. This chapter includes a clinical explanation of the chemical constituents most often tested in urine, the principles behind the tests, some causes for abnormal results, and use of confirmatory procedures.

Urinary pH

One of the functions of the kidney is to help maintain acid–base balance in the body. To maintain a constant **pH** (hydrogen ion concentration) in the blood (about 7.40), the kidney must vary the pH of the urine to compensate for diet and

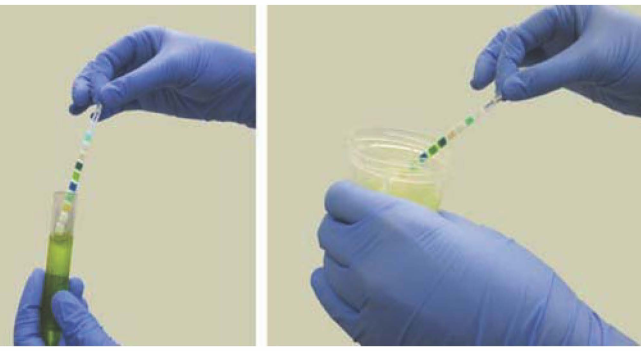


Figure 8-3. The reagent strip is dipped into a specimen and removed quickly, dragging the edge of the strip along the lip of the container to remove excess urine.

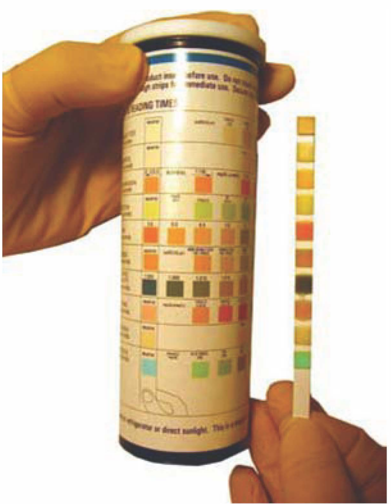


Figure 8-4. Follow the manufacturer's required times for reading of reagent strip test results.

products of metabolism. This regulation occurs in the distal portion of the nephron with the secretion of both hydrogen and ammonia ions into the filtrate, and the reabsorption of bicarbonate. If sufficient hydrogen ions (H^+) are secreted into the tubule, all of the bicarbonate present will be reabsorbed, but if fewer H^+ are secreted or if an excess of bicarbonate is present, some of the bicarbonate will be excreted in the urine. The continued secretion of H^+ after all bicarbonate has been reabsorbed will drop the pH of the filtrate and result in an acidic urine. The secretion of H^+ in the tubule is regulated by the amount present in the body. If there is an excess of acid in the body (acidosis), more H^+ will be excreted and the urine will be acid. When there is an excess of base in the body (alkalosis), less H^+ will be excreted and the urine will be alkaline. The hydrogen ions in the urine are excreted as either free H^+, in association with a buffer such as phosphate, or bound to ammonia as ammonium ions. The pH of the urine is determined by the concentration of the free H^+.

BOX 8-1 Manual Urine Chemistry Screening Procedure

1. Ensure that the specimen is at room temperature.
2. Ensure that the specimen label contains two patient identifiers.
3. Prepare test result documentation for this specimen.
4. Completely dip the test areas of the strip in fresh, well-mixed, uncentrifuged urine and remove immediately. Care should be taken not to touch the test areas.
5. Remove the excess urine from the stick by dragging the edge of the strip along the top of the urine container. Follow the manufacturer's requirement for maintaining the reagent strip in either a horizontal or vertical position.
6. At the correct times, compare the test areas with the corresponding color charts on the container. The strip should be read in good lighting for accurate color comparison.
7. Record results as prescribed by your laboratory's protocol.

Figure 8-5. Many companies exist worldwide who manufacture urine chemistry reagent strips.

Because pH is the reciprocal of the hydrogen ion concentration, as the H^+ concentration increases, the pH decreases or becomes more acidic. As the H^+ concentration decreases, the pH increases or becomes more alkaline. The pH of the urine may range from 4.6 to 8.0 but averages around 6.0, so it is usually slightly acidic. There is no abnormal range as such, since the urine can normally vary from acid to alkaline. For this reason, it is important for the physician to correlate the urine pH with other information to determine whether there is a problem. Metabolic and renal disorders that affect urine pH are discussed in other chapters.

REAGENT TEST STRIPS

All brands of dipsticks discussed in this chapter use the same two indicators, methyl red and bromothymol blue, and measure a range of pH from 5.0 to 8.5. The results may be reported in whole units or interpolated to half units. If a more precise reading is needed, measurement may be made using a pH meter with a glass electrode. Some laboratories report the reaction as "acid," "neutral," or "alkaline," instead of giving numerical values. Figure 8-6 shows the color reactions that correspond to pH values from 5.0 to 8.5.

Most manufacturers recommended that the pH be read immediately as this will prevent misreadings due to the phenomenon of "**run-over effect**" (Fig. 8-7). This term is used to describe what happens when excess urine is left on the stick after dipping, and so the acid buffer from the reagent in the protein area runs onto the pH area. This type of contamination can cause a false lowering of the pH reading, especially in the case of alkaline or neutral urine.

Run-over can sometimes be recognized by the technologist, because the edge nearest the protein area will usually change color first. However, if the strip is not observed constantly after dipping, this occurrence can be overlooked.

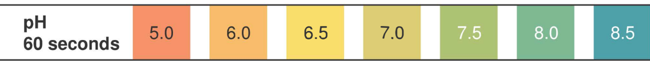


Figure 8-6. pH color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

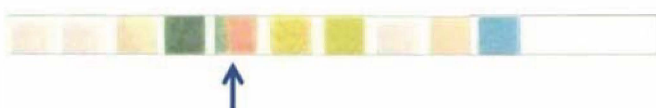


Figure 8-7. The discoloration on the pH pad, due to improper removal of excess urine from the reagent test strip, causes a run-over effect of color change in the pH pad.

Advances have been made to prevent "run-over." Multistix has a hydrophobic inter pad surface which causes the urine to bead up on it and thereby reduces "run-over." The design of the Chemstrip is such that a nylon mesh holds the test pads and underlying absorbent papers in place on the plastic strip. The mesh allows for even diffusion of the urine on the test pads, and the underlying paper absorbs excess urine to prevent "run-over." If pH is the only test needed to be done on a urine specimen, litmus paper or Nitrazine paper can also be used to obtain an approximate reading.

Protein

Proteinuria, the presence of increased amounts of protein in urine, can be an important indicator of renal disease. It may be the first sign of a serious problem and may appear long before other clinical symptoms. There are, however, physiologic conditions such as exercise and fever that can lead to increased protein excretion in the urine in the absence of renal disease. There are also some renal disorders in which proteinuria is absent.

In the normal kidney, only a small amount of low–molecular-weight protein is filtered at the glomerulus. The structure of the glomerular membrane prevents the passage of high–molecular-weight proteins including albumin (mol wt 69,000). After filtration, most of the protein is reabsorbed in the tubules with less than 150 mg/24 h (or 20 mg/dL) being excreted. In a child, the normal excretion is less than 100 mg/m^2/24 h. The protein that is normally excreted includes a mucoprotein called **Tamm–Horsfall protein**, which is not contained in the plasma but is secreted by the renal tubules. This protein forms the matrix of most urinary casts (see chapter on urinary sediment). Various renal disorders that cause proteinuria are described in the Chapter 2.

SCREENING TESTS

The screening tests for proteinuria are based either on the "**protein error of indicators**" principle or on the ability of protein to be precipitated by acid or heat. Sensitivity differs among these tests. The dipsticks are more sensitive to albumin than to other proteins, whereas the heat and acid tests are sensitive to all proteins. In addition, some substances that interfere with the precipitation tests do not interfere with the reaction on the dipstick.

Contamination of the urine with vaginal discharge, semen, heavy mucus, pus, and blood can result in a false-positive reaction with any method that is used. Very dilute urine can give a false-negative reaction because the concentration of protein fluctuates with the urine flow. Therefore, it is important to interpret the protein result by correlating it with the specific gravity. A trace of protein in dilute urine indicates a greater loss of protein than does a trace amount in a concentrated specimen. If protein is present in large quantities, the surface tension of the urine will be altered. Agitation of the urine will cause white foam to develop on the surface of the urine.

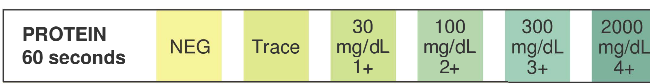
REAGENT TEST STRIPS

This colorimetric method used in dipsticks is based on the concept known as the "protein error of indicators," a phenomenon which means that the point of color change of some pH indicators is different in the presence of protein from that observed in the absence of protein, because proteins act as hydrogen ion acceptors at a constant pH. Usually, the indicator changes from yellow to blue (or green) between pH 3 and pH 4, but in the presence of protein, this color change will occur between pH 2 and pH 3. Therefore, in the presence of protein an "error" occurs in the behavior of the indicator. Indicators used on the various reagent strips vary by manufacturer and are outlined in Table 8-1. Sensitivities

Table 8-1 Protein Indicators and Sensitivities by Reagent Strip

BRAND AND SENSITIVITY	INDICATOR
AimStick (15 mg/dL)	Tetrabromophenol blue
Chemistrip (6 mg/dL)	3,3,5,5-Tetrachlorophenol-3,4,5,6-Tetrabromsulfophthalein
Combi-Screen PLUS (15 mg/dL)	Tetrabromophenol blue
DiaScreen (5 mg/dL)	Tetrabromophenol blue Citric acid
Dirui H-Series (0.15–0.3 g/L)	Tetrabromophenol blue
Mission (18–30 mg/dL)	Tetrabromophenol blue
Multistix (15 mg/dL)	Tetrabromophenol blue
Self-Stik (5–10 mg/dL)	Tetrabromophenol blue Citric acid Sodium citrate
URiSCAN (10 mg/dL albumin)	Tetrabromophenol blue
Uritest 13G (0.1–0.3 g/L albumin)	Tetrabromophenol blue
Uro-dip 10C (not given)	Tetrabromophenol blue
URS (15 mg/dL)	Tetrabromophenol blue

Note: sensitivities are for albumin.



PROTEIN 60 seconds	NEG	Trace	30 mg/dL 1+	100 mg/dL 2+	300 mg/dL 3+	2000 mg/dL 4+

Figure 8-8. Protein color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

for protein are also listed. Be aware that reagent strips detect primarily albumin and are less sensitive to globulins.

An acid buffer is added to the reagent area to maintain a constant pH of 3, which in the absence of urine protein produces a yellow color. The development of any green to blue color indicates the presence of protein. The intensity of the color is proportional to the amount of protein that is present. The protein area is read at 60 seconds for most brands of dipsticks (follow the manufacturer's latest directions). The color of the reagent area should be carefully compared with the color chart supplied by the manufacturer. The results display a range of colors from yellow to blue and are usually reported as negative, 1+, 2+, 3+, or 4+. Figure 8-8 displays the color chart for protein values.

The dipstick procedure is very sensitive to albumin, the protein that is primarily excreted as the result of glomerular damage or disease. Other urine proteins such as gamma globulin, glycoprotein, ribonuclease, lysozyme, hemoglobin, Tamm–Horsfall mucoprotein, and Bence-Jones protein are much less readily detected than albumin. Therefore, a negative urinary dipstick result does not necessarily rule out the presence of these proteins.

False-Positive Results

False-positive results may occur in highly buffered alkaline urine, which may result from alkaline medication or stale urine. The alkaline pH can overcome the acid buffer in the reagent and the area may change color in the absence of protein. If the dipstick is left in the urine for too long, the buffer will be washed out of the reagent, the pH will increase and the strip will turn blue or green, even if protein is not present.

Quaternary ammonium compounds that may be used to clean the urine containers will alter the pH and result in a false-positive reaction. False positives may occur on some dipsticks during treatment with phenazopyridine and after the infusion of polyvinylpyrrolidone as a plasma expander. Chlorhexidine gluconate, found in skin cleansers, may produce false-positive results. Specimens containing blood may cause a false-positive protein reaction; and high amounts of bilirubin may obscure color reactions.

False-Negative Results

False-negative results can occur in dilute urines and when proteins other than albumin are present in slightly elevated concentrations. The various acid precipitation tests that also screen for urinary proteins are not routinely performed in most clinical laboratories. The principles and procedures for these tests are included in Appendix B as reference material.

REAGENT STRIP GLUCOSE OXIDASE TEST

Reagent strips that are impregnated with the enzyme glucose oxidase detect only glucose. These strips use the following double sequential enzyme reaction:

Reaction A:

$$\text{Glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

Reaction B:

$$H_2O_2 + \text{chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen}$$

Glucose and Other Reducing Substances

The presence of significant amounts of glucose in the urine is called **glycosuria** (or glucosuria). The quantity of glucose that appears in the urine is dependent upon the blood glucose level, the rate of glomerular filtration, and the degree of tubular reabsorption. Usually, glucose will not be present in the urine until the blood level exceeds 160 to 180 mg/dL, which is the normal renal threshold for glucose. When the blood glucose exceeds the renal threshold, the tubules cannot reabsorb all of the filtered glucose, and so glycosuria occurs. Normally, this level is not exceeded even after ingestion of a large quantity of carbohydrate. A small amount of glucose may be present in normal urine, but fasting levels in an adult is only about 2 to 20 mg of glucose per 100 mL of urine.

SCREENING TESTS

There are two basic types of tests that are used to screen for or monitor glycosuria. The procedures that use the enzyme glucose oxidase are specific for glucose, while the copper reduction test will detect any **reducing substance** (a chemical that loses an electron to a different chemical during an oxidation–reduction reaction). As with all screening procedures, a positive test result should be correlated with other findings. The interpretation of a positive glucose test should be based on other screening tests including specific gravity, ketones, and albumin. But more importantly, a correlation must be made with the blood glucose level as well as the case history, family history, and clinical picture.

A previously undiagnosed glycosuria should be followed up by such studies as a glucose tolerance test, 2-hour postprandial glucose, and fasting blood sugar. A positive reducing substance other than glucose can best be differentiated by either thin-layer or paper chromatography.

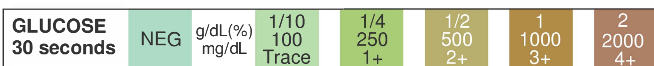


Figure 8-9. Glucose color chart Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

Chromogens (precursors to biochemical pigments) are subject to change by the manufacturer, so always consult manufacturer package inserts. Indicators used on the various reagent strips are outlined in Table 8-2. Glucose results are read at 30 or 60 seconds, depending on the manufacturer. The color changes displayed by these values range from blue to brown (Fig. 8-9). Results are reported as semiquantitative values ranging from negative to 4+ (negative to 2,000 mg/dL). The color reaction is kinetic and will continue to react after the prescribed time. A reading taken after this time will be falsely elevated. Glucose oxidase methods are more sensitive to solutions of aqueous glucose than to glucose in urine; therefore, they are more sensitive to dilute urine than concentrated urine. Urines which have been refrigerated must be first brought to room temperature before accurate testing can be performed, because these methods are enzymatic and are effected by temperature.

False-Positive Results

No known constituent of urine will give a false-positive enzyme test, but if the urine specimen is contaminated with strong oxidizing cleaning agents peroxide or hypochlorite, a false-positive reaction may occur. In urines positive for glucose, a falsely elevated glucose may result in the presence of elevated urobilinogen when using automated methods for some brands of reagent strips.

False-Negative Results

Sensitivity for glucose may be affected by temperature, specific gravity, and pH. Reactivity for glucose can vary with temperature because of the effect temperature can have on enzymatic reactions. An elevated specific gravity may decrease the sensitivity of glucose oxidase. Alkaline pH may decrease sensitivity to glucose. The combination of high specific gravity and alkaline pH may result in false negatives at low concentrations of glucose.

High urinary concentrations of **ascorbate** (**ascorbic acid** or vitamin C) can inhibit the enzymatic reaction which will result in a reduced or false-negative reading. Ascorbic acid will be oxidized by the hydrogen peroxide in the second part of the enzyme reaction, and will, therefore, compete with the oxidation of the chromogen, resulting in the inhibition of the color formation. The ingestion of normal amounts of vitamin C usually presents no problem. Large concentrations of urinary ascorbic acid can also occur with the parenteral administration of vitamin C or antibiotics that contain ascorbic acid as a stabilizing agent (e.g., tetracycline). If vitamin C interference is suspected, a repeat test should be performed at least 24 hours after the last intake of ascorbic acid.

Moderately high ketone levels (40 mg/dL) may reduce the sensitivity and may cause false negatives with glucose levels of 100 mg/dL. However, such a high level of ketones in a patient with diabetes with only a small amount of glucose is unusual. For some reagent strips, ketones as high as 250 mg/dL have been shown not to interfere with the glucose test.

SCREENING FOR REDUCING SUBSTANCES

In addition to glucose, other sugars that may be found in urine, such as galactose, lactose, fructose, and maltose, are reducing substances. Procedures, which are based on the ability of glucose to reduce copper, will also detect these sugars if they are present. Any other reducing substances that can occasionally be found in the urine will also give positive reduction tests. Sucrose (table sugar) is not a reducing substance and will give a negative result.

Clinitest, a copper reduction test (similar to Benedict's test), can be used to test for glucose but is usually used to screen for other reducing substance which may be present. This test is based on the fact that in strongly alkaline solutions and in the presence of heat, reducing sugars will reduce cupric ions to cuprous oxide. The reaction produces a color change of blue through green to orange depending upon the amount of reducing substances present in the urine.

A test for reducing substances should be included in the routine urinalysis of all pediatric patients. This will provide for the early detection of those metabolic defects that are characterized by the excretion of reducing sugars such as galactose, which is present in the urine in patients with galactosemia.

CLINITEST PROCEDURE

Clinitest is a self-heating method for the semiquantitative determination of reducing substances in the urine. The tablet contains the following reagents: copper sulfate, citric acid, sodium hydroxide, and sodium carbonate. When placed in a mixture of water and urine, the tablet is rapidly dissolved by the action of sodium carbonate and citric acid which act as an effervescent. The sodium hydroxide provides the alkaline medium necessary for the reaction, and the heat required is provided by the reaction of sodium hydroxide with water and citric acid. The reducing substances in the urine then react with the copper sulfate to reduce the cupric ions to cuprous oxide. Box 8-2 outlines the steps involved in the Clinitest procedure, including reporting information. Colors produced by this reaction are shown in Figure 8-10.

During the reaction, if the color should rapidly "pass-through" bright orange to a dark brown or greenish-brown, report the result as being greater than 2%. Clinitest is a very accurate procedure if the manufacturer's directions are carefully followed. Failure to observe the reaction as it takes place

BOX 8-2 Clinitest Procedure

1. Ensure that the specimen is at room temperature.
2. Ensure that the specimen label contains two patient identifiers.
3. Place five drops of urine into a glass test tube (or use 0.3 mL).
4. Add 10 drops of water (or 0.6 mL) and mix by shaking.
5. Drop one Clinitest tablet into the tube and observe the complete reaction. DO NOT shake the tube during the reaction or for 15 seconds after the boiling has stopped (Fig. 8-11).

   Warning: The bottom of the tube will become very hot! Plastic test tubes may expand because of the heat and become difficult to remove from test tube racks.

6. At the end of the 15-second waiting period, shake the tube gently and then compare with the color chart that is provided.
7. Record results according to your laboratory's policies.

Clinitest Interpretation

The test is reported as negative, 1/4% (or trace), 1/2% (1+), 3/4% (2+), 1% (3+), or 2% (4+).

will result in a falsely low reading. The "pass-through" phenomenon can occur so rapidly that it can be missed if not observed closely. If measurement beyond 2% is medically desirable, an alternate two-drop method is available. This method involves adding only 2 drops of urine to 10 drops of water, but a special color chart must be used. The two-drop method will allow for quantitation up to 5% but the "pass-through" phenomenon may still occur when very large concentrations of sugar are present.

To determine whether a positive copper reduction test is due to the presence of glucose or another reducing substance, both the glucose oxidase test and the reduction test must be performed and a correlation made of results. Table 8-3 lists possible results along with the interpretation. A positive enzyme test but negative reducing test can occur when only a small amount of glucose is present because the enzyme test can measure as little as 0.1%, but the Clinitest reducing test can detect only 0.25% or higher.

False-Positive Results

Nalidixic acid, cephalosporins, probenecid, and the urinary preservatives such as formalin and formaldehyde if present in large quantities may cause false-positive results. High concentrations of ascorbic acid have been considered to give false-positive results. The sensitivity of Clinitest (1/4%) is such that a number of other substances that react positively with Benedict's solution (sensitivity is around 0.05%) will, in most cases, not be present in sufficient quantities to react with Clinitest, for example, salicylates and penicillin. Dextrins, homogentisic acid, and glucuronates will also give positive reduction tests. Urines having a low specific gravity and containing glucose may produce slightly elevated results.

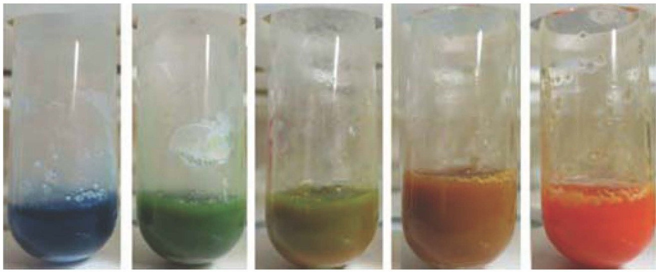


Figure 8-10. Clinitest reactions: Negative, 1+, 2+, 3+, and 4+.

Table 8-2 Glucose Chromogens and Sensitivities by Reagent Strip

BRAND AND SENSITIVITY	CHROMOGEN
AimStick (50 mg/dL)	Potassium iodide
Chemistrip (40 mg/dL)	Tetramethylbenzine
Combi-Screen PLUS (40 mg/dL)	Glucose oxidase Peroxidase O-Tolidine-hydrochloride
DiaScreen (50 mg/dL)	Potassium iodide
Dirui H-Series (2.8–5.5 mmol/L)	Glucose oxidase Peroxidase Potassium iodide
Mission (25–50 mg/dL)	Glucose oxidase Peroxidase O-Tolidine
Multistix (75 mg/dL)	Potassium iodide
Self-Stik (50–100 mg/dL)	Glucose oxidase Peroxidase Potassium iodide
URiSCAN (50 mg/dL)	Glucose oxidase Peroxidase Potassium iodide
Uritest 13G (2.2–2.8 mmol/L)	Glucose oxidase Peroxidase 4-Aminoantipyrine
Uro-dip 10C (100–150 mg/dL)	Glucose oxidase Peroxidase Potassium iodide
URS (100 mg/dL)	Glucose oxidase Peroxidase Potassium iodide

False-Negative Results

Urines containing a high protein concentration may need extra time before interpreting the Clinitest, because the elevated levels of protein increase the boiling time of the reaction. The presence of Hypaque (x-ray contrast media) may produce a false-negative result.

Table 8-3 Interpretation of Glucose Oxidase and Copper Reduction Tests

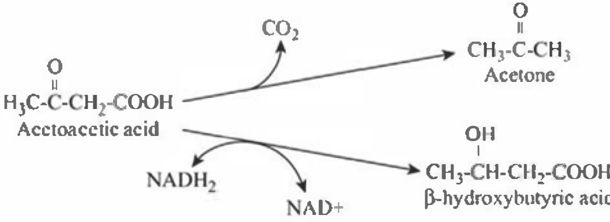
GLUCOSE OXIDASE	COPPER REDUCTION	INTERPRETATION
+	+	Glucose or glucose plus other reducing substances
−	+	Non-glucose reducing substance or interference from ascorbic acid
+	−	Small quantity of glucose

Ketones

Ketones or ketone bodies are formed during the catabolism of fatty acids. One of the intermediate products of fatty acid breakdown is acetyl CoA. Acetyl CoA enters the citric acid cycle (Krebs cycle) in the body if fat and carbohydrate degradation are appropriately balanced.

The first step in the Krebs cycle is the reaction of acetyl CoA with oxaloacetate to yield citrate. When carbohydrate is not available or is not being properly utilized, all available oxaloacetate will be used to form glucose, and so there will be none available for condensation with acetyl CoA. CoA cannot enter the Krebs cycle; therefore, it is diverted to the formation of ketone bodies.

The ketone bodies are acetoacetic acid (diacetic acid), β-hydroxybutyric acid, and acetone. Acetoacetic acid is the first ketone that is formed from acetyl CoA, and the other ketones are formed from acetoacetic acid as shown in the following reaction:



β-Hydroxybutyric acid is formed by reversible reduction, and acetone is formed by a slow spontaneous decarboxylation. Acetoacetic acid and β-hydroxybutyric acid are normal fuels of respiration and are important sources of energy. In fact, the heart muscle and the renal cortex prefer to use acetoacetate instead of glucose. But glucose is the major fuel of the brain in well-nourished individuals, even though the brain can adapt to utilize acetoacetate in the absence of glucose. The odor of acetone may be detected in the breath of an individual who has a high level of ketones in the blood because acetone is eliminated via the lungs.

Normally small amounts of ketones are present in the blood, 2 to 4 mg/dL. The relative proportion of each is approximately 20% acetoacetic acid, 2% acetone, and 78% β-hydroxybutyric acid. There may, however, be considerable proportional variation among individuals. Acetone is lost into the air if a sample is left standing at room temperature. Therefore, urines should be tested immediately or refrigerated in a closed container until testing.

Reagent Test Strips

Laboratory tests that screen for ketones include reagent test-strip methods and tablet-based tests such as **Acetest**.

Multistix contains the reagents sodium nitroprusside and an alkaline buffer, which react with diacetic acid in urine to form a maroon color, as in the following reaction:

Nitroprusside reaction:

Acetoacetic acid + Na nitroprusside +

$$\text{Glycine} \xrightarrow[\text{pH}]{\text{alkaline}} \text{violet–purple color}$$

Sodium nitroprusside is used by each manufacturer as listed in Table 8-4. However, sensitivities do vary. Some brands of reagent strips are sensitive only to acetoacetic acid (diacetic acid) whereas others also detect acetone. None of these reagent strips detects β-hydroxybutyric acid.

Multistix and DiaScreen dipsticks do not react with acetone or β-hydroxybutyric acid but will detect as little as 5 to 10 mg/dL of diacetic acid. In addition to diacetic acid, Chemistrip and AimStick dipsticks detect high levels of acetone but neither detects β-hydroxybutyric acid. Ketone results are read at 40 or 60 seconds, depending on the manufacturer. Color change is from buff-pink to maroon and the reaction is reported as either negative, trace, moderate, or large or negative to 160 mg/dL. Figure 8-11 displays a ketone color chart.

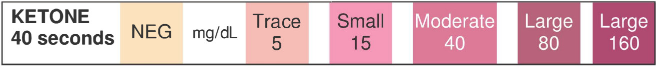


Figure 8-11 Ketone color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

Table 8-4 Ketone Reagents and Sensitivities by Reagent Strip

BRAND AND SENSITIVITY	REAGENT
AimStick (5 mg/dL diacetic acid; 48 mg/dL acetone)	Sodium nitroprusside
Chemistrip (9 mg/dL diacetic acid; 70 mg/dL acetone)	Sodium nitroprusside
Combi-Screen PLUS (5 mg/dL aceto-acetic acid; 50 mg/dL acetone)	Sodium nitroprusside
DiaScreen (5 mg/dL diacetic acid)	Sodium nitroprusside
Dirui H-Series (0.5–1.0 mmol/L)	Sodium nitroprusside
Mission (2.5–5 mg/dL)	Sodium nitroprusside
Multistix (5 mg/dL diacetic acid)	Sodium nitroprusside
Self-Stik (5 mg acetoacetic acid per 100 mL of urine)	Sodium nitroprusside Magnesium sulfate
URiSCAN (5 mg/dL acetoacetic acid; 70 mg/dL acetone)	Sodium nitroprusside
Uritest 13G (0.5–1.0 mmol/L acetoacetic acid)	Sodium nitroprusside
Uro-dip 10C (5 mg acetoacetic acid per 100 mL of urine)	Sodium nitroprusside
URS (5–10 mg/dL acetoacetic acid)	Sodium nitroprusside

False-Positive Results

False-positive results may occur when the urine specimen is highly pigmented or when it contains large amounts of levodopa metabolites. Some specimens that have both a high specific gravity and a low pH may produce false-positive reactions. Compounds that contain sulfhydryl groups may cause a false-positive or atypical color reactions.

Phenylketones may cause a red-orange coloration. Phthalein compounds used in liver and kidney function tests produce a reddish coloration due to the alkalinity of the test zone. These colors, however, are easily distinguishable from the colors obtained with ketone bodies. Some laboratories chose to confirm positive and questionable result with a tablet test.

False-Negative Results

Because of the specificity of Multistix and DiaScreen for diacetic acid, these brands of dipstick will not give a positive ketone result with controls that contain acetone.

ACETEST TABLETS

The Acetest tablet contains sodium nitroprusside, glycine, a strong alkaline buffer (disodium phosphate), and lactose. Acetest can be used to test urine, serum, plasma, or whole blood. Diacetic acid and acetone react with sodium nitroprusside and glycine in an alkaline medium to form a purple color (Fig. 8-12). Lactose in the tablet helps enhance the color. Refer to the Acetest procedure outlined in Box 8-3.

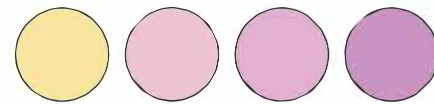


Figure 8-12. Acetest color chart. Note: This chart is for color demonstration only and should not be used for interpreting reactions for diagnostic testing. (Modified from Siemens [formerly Elkhart, IN: Bayer HealthCare LLC]; 2006.)

Acetest is about 10 times more sensitive to diacetic acid than to acetone. However, Acetest will not react with β-hydroxybutyric acid. In urine it will detect as little as 5 to 10 mg/dL of diacetic acid and 20 to 25 mg/dL of acetone.

Those substances that interfere with the dipsticks will also interfere with the Acetest tablet because the same reaction is involved. Other screening tests for ketones that are no longer routinely performed are included in Appendix B.

Occult Blood

The term "occult" means "hidden," and the methods used to test for blood in the urine are capable of detecting even minute amounts not visualized macroscopically. Another reason for this title is that these procedures actually detect the free hemoglobin from lysed red blood cells (RBCs). Recent improvements in the dipsticks now allow for the detection of intact RBCs by causing them to lyse while on the test pad. Formerly, some intact RBCs could not be detected. In cases in which all of the red cells stayed intact, it was possible to get a negative test for blood even though the microscopic examination revealed the presence of RBCs. The chemical methods used in

BOX 8-3 Acetest Procedure

1. Place the tablet on a piece of clean, dry white paper.
2. Put one drop of urine, serum, plasma, or whole blood directly on top of the tablet.
3. Compare reaction color with color chart.
   a. For urine, compare at 30 seconds.
   b. For serum or plasma, compare after 2 minutes.
   c. For whole blood, remove the clotted blood from the tablet after 10 minutes and compare.

Acetest Interpretation:
Results are reported as "small, moderate, or large."
For urine, the small color block corresponds to approximately 5–10 mg/dL of diacetic acid, the moderate block is 30–40 mg/dL, and the large block is about 80–100 mg/dL.
For serum, plasma, and whole blood, the lowest limit of detection is 10 mg of diacetic acid per 100 mL.

the routine urinalysis for detecting blood (hematuria) will also detect free hemoglobin (hemoglobinuria) and myoglobin (myoglobinuria). The urine is normally free of all of these substances; therefore, a positive test for occult blood should be followed by determination of the exact cause and origin of this abnormal finding. A correlation must also be made with the microscopic examination, and this may be done by asking the following questions: Are there red cells present? Does the number of red cells agree with the intensity of the chemical test? Are there red cell casts or hemoglobin casts? Are there empty red cell membranes (ghost cells)? Are there numerous squamous epithelial cells present (possible menstrual contamination)? It should be noted that hematuria, hemoglobinuria, and myoglobinuria can occur either individually or together.

HEMATURIA

Hematuria is the presence of blood or intact RBCs in the urine. Urine that is highly alkaline or has very low specific gravity (1.007) can cause the red cells to lyse, thus releasing their hemoglobin into the urine. The presence of this type of hemoglobin is still considered to be hematuria as far as the origin is concerned, but it is very difficult to distinguish from true hemoglobinuria. When lysing occurs, the microscopic examination may show the empty red cell membranes which are often referred to as "ghost" cells. In microhematuria there is such a small amount of blood in the urine that the color of the specimen is unaffected and the hematuria can only be detected chemically or microscopically. Gross hematuria alters the color of the urine and is easily visible macroscopically.

HEMOGLOBINURIA

Hemoglobinuria is the presence of free hemoglobin in the urine as a result of intra vascular hemolysis. The hemolysis that occurs in the urine while in the urinary tract or after voiding because of a low specific gravity or highly alkaline pH may be considered to be hemoglobinuria, but it does not bear the same significance as true hemoglobinuria. Hemoglobinuria without hematuria occurs as a result of hemoglobinemia and, therefore, it has primarily nothing to do with the kidneys even though it may secondarily result in kidney damage.

MYOGLOBINURIA

Myoglobin is the heme protein of striated muscle. It serves as a reserve supply of oxygen and also facilitates the movement of oxygen within muscle. Injury to cardiac or skeletal muscle results in the release of myoglobin into the circulation. Even just subtle injury to the muscle cells can bring about the release of myoglobin. Myoglobin has a molecular weight of approximately 17,000 and so it is easily filtered through the glomerulus and excreted in the urine.

Because myoglobin is cleared so rapidly from the circulation, the plasma is left uncolored even though the urine may be red to brown to black, depending on the degree of myoglobinuria.

SCREENING TESTS

Those tests which screen for occult blood will detect hematuria, hemoglobinuria, and myoglobinuria. As previously mentioned, these states can coexist. If the correlation of the microscopic and chemical results does not imply hematuria, then further evaluation and studies may be done to differentiate between hemoglobinuria and myoglobinuria. The definitive diagnostic test for differentiating these two states is electrophoresis. Other methods that can be used are immunodiffusion, hemagglutination inhibition, or immunoelectrophoresis. Hemoglobinuria and myoglobinuria can be rapidly differentiated by the following screening criteria: red plasma plus red urine equals hemoglobin; clear plasma plus red urine equals myoglobin. Another screening procedure is the ammonium sulfate test described in Appendix B.

Testing for blood by using benzidine has long been the standard procedure for the detection of occult blood. However, benzidine is carcinogenic and the routine use of it has been discouraged. Therefore, benzidine tests procedures are not included in this text.

Reagent Test Strips

The dipstick procedure is based on the peroxidase-like activity of hemoglobin and myoglobin which catalyzes the oxidation of a chromogen by organic peroxide as in the following reaction:

Hemoglobin reaction:

$$H_2O_2 + \text{chromogen} \xrightarrow[\text{peroxide}]{\text{hemoglobin}} \text{oxidized chromogen} + H_2O_2$$

The indicators used by the most common reagent strips are listed in Table 8-5 along with their sensitivities.

Most dipsticks are capable of detecting intact erythrocytes as well as free hemoglobin and myoglobin. Intact RBCs in the urine will hemolyze on the test pad. The freed hemoglobin will react with the reagent and will result in green spots on a yellow or orange back ground. Thus, the presence of intact red cells will produce a spotted green reaction, whereas free hemoglobin and myoglobin will show a uniform green or green to dark blue color.

Blood is usually read at 60 seconds, and the color change is from orange to green to dark blue. There are two separate color scales for erythrocytes and hemoglobin. Intact RBCs may display a speckle-pattern reaction in the absence of free hemoglobin. The results are reported as trace or moderate numbers of intact RBCs or trace through 3 (large) amount of hemoglobin. Figure 8-13 displays a color chart of blood reactions.

Table 8-5 Hemoglobin Chromogens and Sensitivities by agent Strip

BRAND AND SENSITIVITY	OXIDANT; CHROMOGEN
AimStick (5 RBCs; 0.3 mg/dL Hb)	Diisopropylbenzene Dihydroperoxide; tetramethylbenzidine
Chemistrip (5 RBCs; Hb 10 RBCs)	2,5-Dimethylhexane-2,5-dihydroperoxide; tetramethylbenzidine
Combi-Screen PLUS (5 Ery/uL)	Tetramethylbenzidine-dihydrochloride Isopropylbenzol-hydroperoxide
DiaScreen (5 RBCs; 0.02 mg/dL Hb)	2,5-Dimethylhexane-2,5-dihydroperoxide; tetramethylbenzidine
Dirui H-Series (5–15 Ery/uL)	Diisopropylbenzene Dihydroperoxide; tetramethylbenzidine
Mission (0.018–0.060 mg/dL)	Diisopropylbenzene Dihydroperoxide; tetramethylbenzidine
Multistix (5 RBCs; 0.015 mg/dL Hb)	Diisopropylbenzene Dihydroperoxide; tetramethylbenzidine
Self-Stik (5–10 RBCs/mL urine)	Cumene hydroperoxide O-Tolidine
URiSCAN (5 RBC/uL or 3–5 RBC/HPF; 0.015 mg/dL hemoglobin)	Cumene hydroperoxide Tetramethylbenzidine
Uritest 13G (0.3–0.6 mg/L hemoglobin)	Cumene hydroperoxide 3,3,5,5-Tetramethylbenzidine
Uro-dip 10C (0.05 mg/dL hemoglobin)	Cumene hydroperoxide Tetramethylbenzidine
URS (0.015 mg/dL Hb or 5–10 intact RBCs/uL)	Cumene hydroperoxide Tetramethylbenzidine

False-Positive Results

Most dipsticks will give false-positive results in the presence of certain oxidizing contaminants such as hypochlorites which may be used to clean urine-collection containers. Other strong oxidizing agents such as bromides and iodides will also produce false positive results. When the urine is contaminated with a high bacterial content, a false-positive reaction may occur because of bacterial peroxidases.

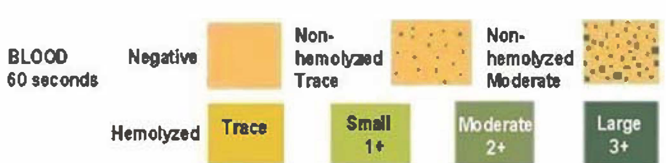


Figure 8-13. Blood color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

False positives will result if the urine is contaminated with menstrual blood. False-positive reactions may occur if the urine or test strip is contaminated with povidone-iodine (Betadine).

False-Negative Results

The test is slightly more sensitive to free hemoglobin and myoglobin than to intact RBCs. If the urine sample is not mixed well before testing, a false-negative result can occur because the red cells tend to settle in the bottom of the container.

Some dipsticks give lower or false-negative readings in the presence of high levels of ascorbic acid. If necessary, the test should be repeated at least 24 hours after the last dose of vitamin C. Captopril (Capoten) may reduce the reagent pad's sensitivity. Sensitivity is less in urines with high specific gravity, nitrites, or protein. In addition, specimens preserved using formalin will yield a false-negative result.

Bilirubin and Urobilinogen

Bilirubin is formed from the breakdown of hemoglobin in the reticuloendothelial system. It is then bound to albumin and transported through the blood to the liver. This free or unconjugated bilirubin is insoluble in water and cannot be filtered through the glomerulus. In the liver, bilirubin is removed by the parenchymal cells and is conjugated with glucuronic acid to form bilirubin diglucuronide. This conjugated bilirubin, which is also called direct bilirubin, is water soluble and is excreted by the liver through the bile duct and into the duodenum.

Normally, very small amounts of conjugated bilirubin regurgitate back from the bile duct and into the blood system. Therefore, very small amounts of conjugated bilirubin can be found in the plasma, but not in concentrations higher than 0.2 to 0.4 mg/dL. Because conjugated bilirubin is not bound to protein, it is easily filtered through the glomerulus and excreted in the urine whenever the plasma level is increased. Normally, no detectable amount of bilirubin (sometimes ref erred to as "bile") can be found in the urine.

In the intestines, bacterial enzymes convert bilirubin, through a group of intermediate compounds, to several related compounds which are collectively referred to as **urobilinogen**. Most of the urobilinogen (a colorless pigment), and its oxidized variant, urobilin (a brown pigment), are lost in the feces. About 10% to 15% of the urobilinogen is reabsorbed into the bloodstream, returns to the liver, and is reexcreted into the intestines. A small amount of this urobilinogen is also excreted by the kidneys into the urine, with a normal level of about 1 to 4 mg/24 h or less than 1.0 Ehrlich unit/2 h. Figure 8-14A illustrates the normal pathway of bilirubin and urobilinogen metabolism. (The anatomical parts have been rearranged for the purpose of illustration.)

The normal level of total bilirubin in the serum is about 1.0 mg/dL or less. This consists mainly of indirect or unconjugated bilirubin, but there is also a very small amount of direct or conjugated bilirubin present. When the level of total bilirubin exceeds approximately 2.5 mg/dL, tissues of the body take on the yellow color of bilirubin, and this is called jaundice. If the jaundice is due to an increase in unconjugated bilirubin, no bilirubin will be excreted in the urine because unconjugated bilirubin cannot be filtered at the glomerulus. But if jaundice is due to an increase in the water-soluble conjugated bilirubin, then bilirubin will be present in the urine.

There are three major types of jaundice: hepatic, obstructive, and hemolytic. Since these types differ in the substances excreted in the urine, they can be differentiated by testing for the presence of bilirubin and urobilinogen.

The first type of jaundice to be discussed is that which results from liver damage. The clinical picture varies according to the type and degree of hepatic injury. There may be

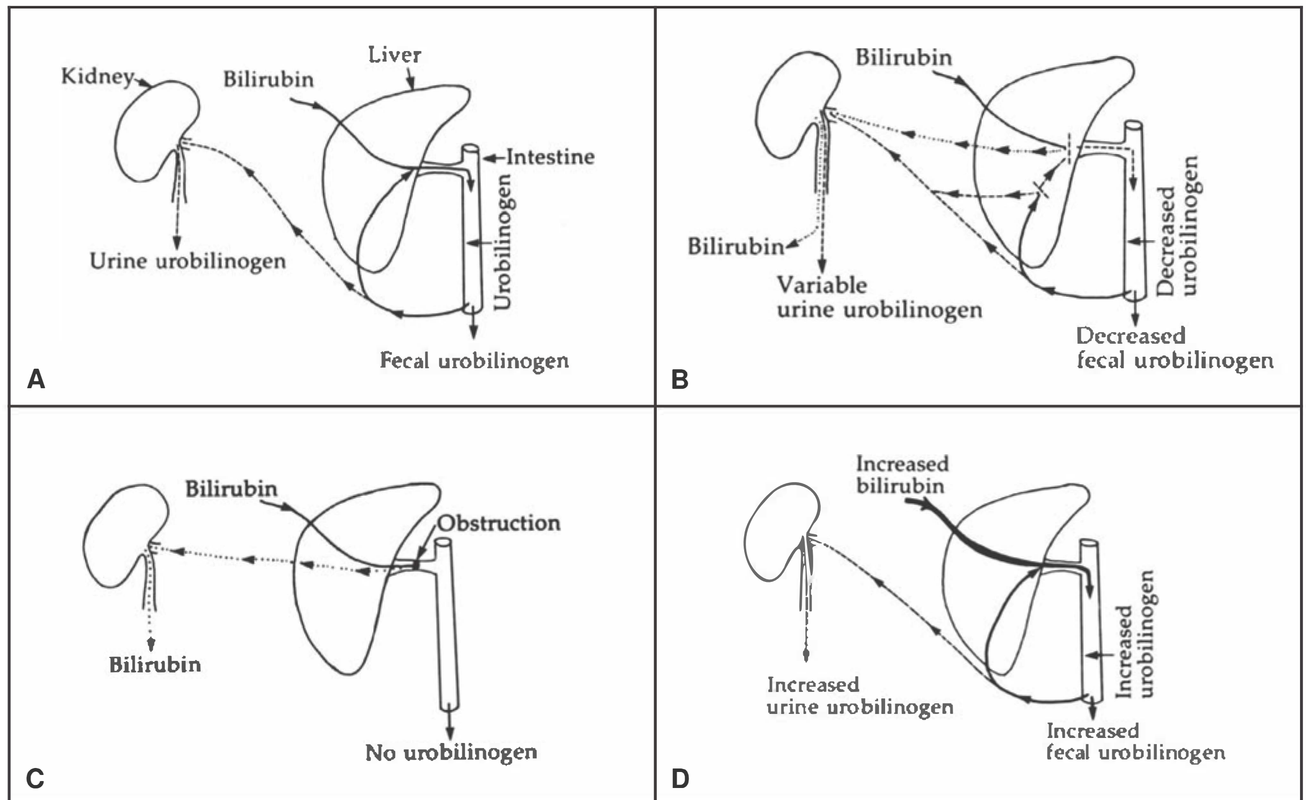


Figure 8-14. **A:** Normal pathway of bilirubin and urobilinogen metabolism; **(B)** pathway in hepatic jaundice; **(C)** pathway in obstructive jaundice; **(D)** pathway in hemolytic jaundice.

injury to the parenchymal cells caused by viral hepatitis or cirrhosis. Intrahepatic disease as a result of chemical intoxication or drug reactions can also cause hepatic jaundice. Figure 8-14B demonstrates the possible pathway in hepatic jaundice. The flow of conjugated bilirubin into the duodenum is inhibited, so the bilirubin backs up into the blood and jaundice may be present, depending on the degree of inhibition. In some types of liver damage, the liver may also not be able to conjugate the normal amount of bilirubin and so the resulting jaundice will be due to both conjugated and unconjugated bilirubin. Although there is partial obstruction to the flow of bilirubin into the duodenum, some is still able to pass into the intestines where urobilinogen is formed. The feces will be lighter in color due to the decrease in urobilin, which is the oxidized form of urobilinogen. When part of the urobilinogen is reabsorbed, the liver may not be able to reabsorb or reexcrete the circulating urobilinogen, thus causing more to appear in the urine. The clinical picture in hepatic jaundice may be: positive urine bilirubin; decreased fecal urobilinogen; and, depending on the type of liver damage, either normal, decreased, or increased levels of urine urobilinogen.

In some kinds of liver cell damage, the normal pathway of bilirubin conjugation and excretion is not affected, but the liver cells are unable to remove the circulating urobilinogen. This can sometimes be seen in cirrhosis of the liver, metastatic carcinoma, and congestive heart failure. The resulting picture will then be: negative urine bilirubin, normal fecal urobilinogen, and increased urine urobilinogen.

The second major type of jaundice is obstructive jaundice, which may be due to an obstruction in the common bile duct caused by gallstones, carcinoma, pancreatitis, diseased lymph nodes surrounding the duct, or by carcinoma of the head of the pancreas. There may also be obstruction from intrahepatic blockage of small biliary ducts by tumors. Severe drug toxicity can also cause a type of intrahepatic obstruction. Figure 8-14C gives a good illustration of the path of bilirubin in the presence of total obstruction. The obstruction prevents the entry of bilirubin into the duodenum. The bilirubin backs up into the blood, which causes jaundice due to conjugated bilirubin, and then bilirubin is excreted in the urine. Since bilirubin is unable to get into the intestines, urobilinogen cannot be formed; thus, the feces will have a characteristic "clay-colored" or gray-white appearance. The clinical picture in total obstruction is: positive urine bilirubin, negative urine urobilinogen, and negative or only very trace amounts of fecal urobilinogen. If the obstruction is only partial, then the clinical picture will resemble that which is seen in Figure 8-14B.

Hemolytic jaundice is that type of jaundice which is the result of the excessive production of bilirubin. The increased breakdown of RBCs produces bilirubin at a rate that exceeds the ability of the liver to conjugate and excrete it. The jaundice is, therefore, due to unconjugated bilirubin. As shown in Figure 8-14D, the liver is able to excrete all of the conjugated bilirubin as it is formed, but the increased excretion of conjugated bilirubin results in an increased amount of fecal urobilinogen. This usually leads to more urobilinogen being reabsorbed from the intestines and, therefore, the urine will also contain increased levels. So the clinical picture in hemolytic jaundice is: negative urine bilirubin, increased urine urobilinogen, and increased fecal urobilinogen. In some cases there may also be a positive test for occult blood due to the presence of free hemoglobin in the urine. Some of the possible causes of hemolytic jaundice include: intravascular hemolysis; anemia, especially hemolytic anemia; and thalassemia.

SCREENING TESTS FOR BILIRUBIN (BILE)

Bilirubin can be detected in the urine before other clinical symptoms are present or recognizable. The detection of small quantities is very important in the early diagnosis of obstructive and hepatic jaundice. This test is also useful in the differential diagnosis of obstructive (positive) and hemolytic (negative) jaundice. Bilirubin is light sensitive and so the urine should be protected from the light and examined as quickly as possible. On standing and especially when exposed to light, bilirubin, which is a yellow-brown color, will be oxidized to biliverdin, which is a green color. Many of the procedures used to detect bilirubin will not react with biliverdin, so false-negative results may occur if the urine is not tested when fresh.

Detectable amounts of bilirubin are not normally present in the urine, so the results of some methods are just reported as positive or negative. When liver disease is suspected, confirmatory methods provide more accurate results because of the sensitivity of these tests.

REAGENT TEST STRIPS

Most dipsticks are based on the coupling reaction of a diazonium salt with bilirubin in an acid medium as show by this reaction:

Bilirubin reaction:

$$\text{Bilirubin} + \text{diazide} \xrightarrow{\text{acid}} \text{azobilirubin}$$

Some dipsticks differ, however, in the diazonium salt that is used and the color that develops. The indicators used by the most common manufacturers are listed in Table 8-6 along with their sensitivities. Bilirubin results are read from 30 to 60 seconds, depending on the manufacturer and display a range of colors from buff through various shades of tan or tannish-purple. These colors correspond to levels of bilirubin from negative to large (3+). Figure 8-15 displays a bilirubin color chart.

False-Positive Results

If the bilirubin pad is observed after the prescribed amount of time, it may develop other colors that may interfere with the reading of bilirubin reactions. Several compounds may produce atypical color reactions on the bilirubin pad. Indican and metabolites of etodolac (Lodine) can produce an interfering color reaction. Patients receiving large doses of chlorpromazine (Thorazine) may have false-positive results.

Table 8-6 Bilirubin Indicators and Sensitivities by Reagent Strip

BRAND AND SENSITIVITY	INDICATOR
AimStick (0.2 mg/dL)	2,4-Dichlorobenzene amine diazonium salt
Chemistrip (0.5 mg/dL)	2,6-Dichlorobenzene-diazoniuim-tetrafluoroborate
Combi-Screen PLUS (0.5–1 mg/dL)	Diazonium salt
DiaScreen (0.5 mg/dL)	2,4-Dichlorobenzene diazonium salt Sulfosalicylic acid
Dirui H-Series (8.6–17 umol/L)	2,4-Dichloroaniline diazonium salt
Mission (0.4–1.0 mg/dL)	2,4-Dichloroaniline diazonium salt
Multistix (0.4 mg/dL)	2,4-Dichloroaniline diazonium salt
Self-Stik (not given)	2,4-Dichlorobenzene diazonium Na Oxalic acid
URiSCAN (0.5 mg/dL)	Sodium nitrite
Uritest 13G (8.6–17 *u*mol/L)	2,4-Dichloroaniline diazonium
Uro-dip 10C (not given)	2,4-Dichlorobenzene diazonium
URS (0.4–0.8 mg/dL)	2,4-Dichloroaniline diazonium salt

Metabolites of drugs such as phenazopyridine produce a red color at an acid pH and cause misinterpretation of results that could lead to false-positive reports. A confirmatory test should be used to confirm bilirubin results on urines that generate a positive or atypical color reaction.

False-Negative Results

Large amounts of ascorbic acid decrease the sensitivity of this test. Repeating the test at least 10 hours after the last dose of vitamin C will produce more accurate results. Elevated levels of nitrite will lower the bilirubin result. A false-negative result will be obtained if the bilirubin has been oxidized to biliverdin, as occurs when specimens are exposed to room temperature and light.

CONFIRMATORY TESTS

Ictotest

Ictotest is a tablet test that is based on the same diazo reaction as the dipsticks. However, Ictotest is much more sensitive than the dipsticks, being able to detect as little as 0.05 mg/dL. Because of this sensitivity, Ictotest is the recommended procedure when a test for just bilirubin is ordered. It also serves as a good confirmatory test for a positive dipstick result.

The tablet contains 2,6-dichlorobenzene-diazoniuim-tetrafluoroborate, sulfosalicylic acid, and sodium bicarbonate. The mats that are used in the procedure are made of an asbestos–cellulose mixture. When the urine is placed on the mat, the absorbent qualities of the mat cause the bilirubin to remain on the outer surface. The sulfosalicylic acid provides the acid environment for the reaction. It also acts with the sodium bicarbonate to provide an effervescence that helps partially dissolve the tablet. The diazonium salt then couples with the bilirubin on the mat, giving a blue or purple reaction product. Refer to the Ictotest procedure outlined in Box 8-4.

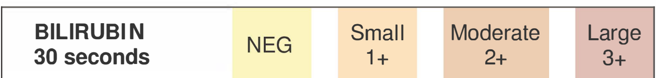


Figure 8-15. Bilirubin color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

BOX 8-4 Ictotest Procedure

1. Place five drops of urine on one square of the special test mat supplied with Ictotest.
2. Place a tablet in the center of the moistened area.
3. Flow two drops of water onto the tablet so that the water runs off of the tablet and onto the mat.
4. Observe the color of the mat around the tablet at the end of 30 seconds. If a blue or purple color develops, the test is positive.

All other colors including pink or red are negative. Figure 8-16 shows the examples of positive and negative Ictotest reactions.

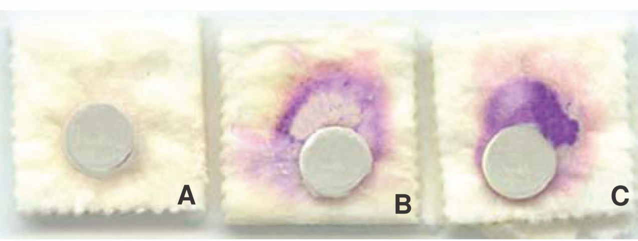


Figure 8-16. Ictotest color reactions. **A:** negative, **(B)** moderate, and **(C)** large.

False-Positive Results

Urine from patients receiving large doses of chlorpromazine may give false-positive reactions. If the urine is suspected of containing a large amount of chlorpromazine, the wash-through technique can be used. Prepare duplicate mats with five drops of urine on each. To one mat add 10 drops of water to wash through the drug metabolites. Add a tablet to each mat and perform the Ictotest procedure. If the color is about the same on both mats, bilirubin is present, because it stays adsorbed on the mat surface. If the wash-through mat is either much lighter or if no color is present, then the reaction is probably due to the drug metabolites.

Ictero-Check

Similar to Ictotest, the Ictero-Check (Fig. 8-17) test is used to qualitatively detect bilirubin in urine or confirm positive reagent strip results. The Ictero-Check test is based on the diazo coupling reaction between bilirubin that adsorbs onto a test pad and stabilized diazonium salt of nitrobenzene in the reagent disc. The reaction results in varying shades of purple to blue-purple color in proportion to the amount of bilirubin present in the specimen.

Bilirubin is labile (breaking down rapidly), therefore fresh specimens are best used for this test. If testing must be delayed, urine may be stored refrigerated for up to six hours. Normal levels of bilirubin (less than 0.02 mg/dL of urine) are not and give a negative result. Ictero-Check can detect abnormal levels of urinary bilirubin as low as 0.1mg/dL of urine.

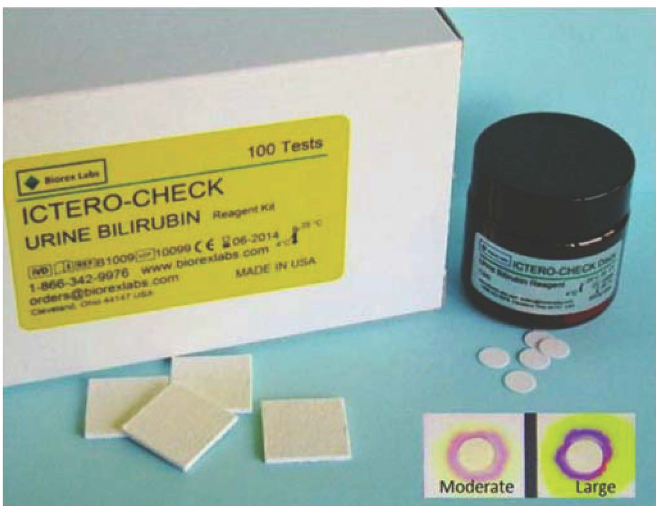


Figure 8-17. ICTERO-CHECK test for urine bilirubin. (Courtesy of Biorex Labs LLC Cleveland, OH.)

False-Positive Results

Certain drugs may interfere with the performance of the Ictero-Check test. Chlorpromazine and etodolac (NSAID) metabolites in urine may cause unusual or false-positive results. Metabolites of phenazopyridine produce bright orange color urine and mask the color produced by small amounts of bilirubin present in the urine.

FOAM TEST

If the urine is a yellowish-brown or greenish-yellow color and bilirubin is suspected, shake the urine. If yellow or greenish-yellow foam develops, then bilirubin is most likely present. Bilirubin alters the surface tension of urine and foam will develop after shaking. The yellow color is from the bilirubin pigment. A false-positive foam test occurs when the urine contains phenazopyridine. The foam test must be followed up by another more accurate procedure. It can, however, be a good clue that bilirubin is present, and the technologist should then test out the possibility of bilirubinuria. Other screening tests for bilirubin that are not regularly performed are included in Appendix B.

SCREENING TESTS FOR UROBILINOGEN

Screening for urobilinogen is useful in the diagnosis of liver function disorders. There are two other factors other than liver disease which must be taken into account when interpreting urobilinogen results. Patients receiving broad-spectrum antibiotics and other substances which will alter the normal bacterial flora in the intestines will excrete little or no urobilinogen in their urine because urobilinogen cannot be formed in the intestines. In addition, in cases of intestinal obstruction, significant quantities of urobilinogen may be absorbed from the intestine and thus the urine levels will increase.

Unlike bilirubin, urobilinogen is normally present in the urine but in concentrations of 1 Ehrlich unit or less per 100 mL of urine. Some procedures will detect only amounts in excess of this, but dipsticks are capable of detecting normal amounts.

Decreased or absent levels of urobilinogen cannot be detected by any of these screening procedures. One of the important problems in measuring urobilinogen is its instability. The urobilinogen is converted to urobilin on standing in the presence of oxygen and on exposure to air. For this reason, the test should be performed on a fresh specimen. Urobilinogen excretion reaches peak levels between 2 and 4 PM. Therefore, when screening for liver damage it is advisable to do a collection during these hours.

Table 8-7 Urobilinogen Reagents and Sensitivities by Reagent Strip

BRAND AND SENSITIVITY	REAGENT
AimStick (0.2 mg/dL)	*p*-Diethylaminobenzaldehyde
Chemistrip (0.4 mg/dL)	4-Methloxybenzene-diazonium-tetrafluoroborate
Combi-Screen PLUS (not given)	Diazonium salt
DiaScreen (0.4 mg/dL)	4-Methloxybenzene-diazonium-tetrafluoroborate
Dirui H-Series (3.3–0.6 mmol/L)	Fast B blue
Mission (0.2–1.0 mg/dL)	*p*-Dimethylaminobenzaldehyde
Multistix (0.2 mg/dL)	*p*-Dimethylaminobenzaldehyde
Self-Stik (not given)	4-Methloxybenzenediazonium salt
URiSCAN (Trace-1EU/dL)	*p*-Diethylaminobenzaldehyde
Uritest 13G (3.3–16 umol/L)	Fast blue B salt
Uro-dip 10C (not given)	*p*-Diethylaminobenzaldehyde
URS (0.2 EU/dL)	*p*-Dimethylaminobenzaldehyde

REAGENT TEST STRIPS

Screening tests for urobilinogen are based on the Ehrlich Aldehyde Reaction:

p-Dimethylaminobenzaldehyde + urobilinogen = red-colored azo dye

This is a simple color development reaction in which aldehyde or diazonium compounds react with urobilinogen to produce a pink to red color in an acid environment. The reagents used for this reaction varies by manufacturer but have similar sensitivities as seen in Table 8-7.

Urobilinogen results are read at 30 or 60 seconds, depending on the manufacturer and display a range of colors in the pink spectrum from light to dark. Most brands of dipsticks show two blocks on the color chart for normal levels of urobilinogen of 0.1 and 1 mg/dL. The other color blocks range from 2 to 8 or 12, depending on the manufacturer. Figure 8-18 displays a color chart for urobilinogen.

False-Positive Results

Several interfering substances may react with the urobilinogen test pad to produce atypical colors. These interfering substances include high levels of bilirubin, *p*-aminosalicylic acid, sulfonamides, and *p*-amino-benzoic acid. Reagent strips using *p*-dimethylaminobenzaldehyde may react with porphobilinogen, although this is not a reliable method for detecting porphobilinogen. Urine from patients receiving phenazopyridine may show a false-positive reaction.

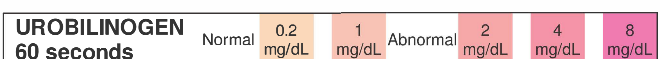


Figure 8-18. Urobilinogen color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

False-Negative Results

A true absence of urobilinogen is not detectable. Several substances may decrease the color reaction of this test. Urines containing nitrite or those preserved with formalin may produce false-negative results. False negatives may also occur in improperly stored samples allowing the oxidation of urobilinogen to urobilin.

Other qualitative methods are available for the detection of urobilinogen. The Watson-Schwartz test can be used to differentiate between urobilinogen and porphobilinogen. This and other tests are outlined in Appendix D.

Nitrite

The **nitrite** (reduced form of nitrate) test is a rapid, indirect method for the early detection of significant and asymptomatic bacteriuria. Common organisms that can cause urinary tract infections, such as *Escherichia coli, Enterobacter, Citrobacter, Klebsiella,* and *Proteus* species, produce enzymes that reduce urinary nitrate to nitrite. For this to occur, the urine must have incubated in the bladder for a minimum of 4 hours, making the first morning urine the specimen of choice.

Table 8-8 Nitrite Reagents and Sensitivities by Reagent Strip

BRAND AND SENSITIVITY	REAGENT
AimStick (0.09 mg/dL)	*p*-Arsanilic acid *N*-ethylenediamine Tetrahydroquinoline
Chemistrip (0.05 mg/dL)	Sulfanilamide 3-Hydroxy-1,2,3,4-tetrahydro-benzo (h) quinoline
Combi-Screen PLUS (0.05–0.1 mg/dL)	Tetrahydrobenzoquinoline Sulfanilic acid
DiaScreen (0.05 mg/dL)	*p*-Arsanilic acid Hydroxy(3)-1,2,3,4-tetrahydro-benzo (h) quinoline
Dirui H-Series (13–22 *u*mol/L)	*p*-Arsanilic acid-*N*-1-(naphthol)-ethylenediamine Tetrahydroquinoline
Mission (0.05–0.1 mg/dL)	*p*-Arsanilic acid-*N*-1-(naphthol)-ethylenediamine
Multistix (0.06 mg/dL)	*p*-Arsanilic acid 1,2,3,4-Tetrahydro-benzo (h) quinoline-3-ol
Self-Stik (not given)	*p*-Arsanilic acid *N*-(1-naphthyl) ethylenediamine 2HCl
URiSCAN (0.05 mg/dL nitrite ion)	*p*-Arsanilic acid
Uritest 13G (18–26 *u*mol/L)	Sulfanilamide *N*-(naphthyl) ethylenediammonium dihydrochloride
Uro-dip 10C (0.05 mg/dL)	Sulfanilamide *N*-(naphthyl) ethylenediammonium Dihydrochloride
URS (0.075 mg/dL)	*p*-Arsanilic acid

REAGENT TEST STRIPS

Reagent strips for the detection of nitrite in the urine commonly use *p*-arsanilic acid and a quinoline compound. Nitrite reacts with *p*-arsanilic acid to form a diazonium compound. This compound then couples with the quinoline compound to produce a pink color as in the following reaction:

Reaction A:

Nitrite + *p*-arsanilic acid ⟶ diazonium compound

Reaction B:

3-Hydroxyl-1,2,3,4 tetrahydrobenz-(h)-quinoline + diazonium compound = pink color

The reagents used for this reaction vary slightly by manufacturer but have similar sensitivities as seen in Table 8-8.

Nitrite results are read at 30 or 60 seconds, depending on the manufacturer. Any degree of uniform pink color should be interpreted as a positive nitrite test suggesting the presence of 10^5 or more organisms per milliliter.

The color development is not proportional to the number of bacteria present. Pink spots or pink edges should not be considered a positive result. If the uniform pink color is very light, it may best be seen by placing the strip against a white background. The test is reported as positive or negative. Figure 8-19 displays a nitrite color chart.

False-Positive Results

The urine should be tested shortly after being voided, because if the urine is allowed to stand at room temperature for several hours, organisms may grow in the specimen and generate nitrite. Results may be misinterpreted as positive in urines that appear red or contain phenazopyridine and other substances that turn red in acid.

False-Negative Results

The sensitivity of the test is reduced in urine with a high specific gravity or elevated level of ascorbic acid. A negative test

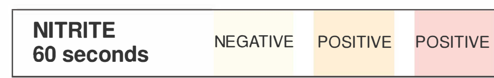


Figure 8-19. Nitrite color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

should never be interpreted as indicating the absence of bacterial infection. There are several reasons for this:

1. There may be pathogens present in the urine that do not form nitrite.
2. The urine may not have remained in the bladder long enough for the nitrate to be converted to nitrite.
3. There are cases in which the urine does not contain any nitrate, so bacteria may be present but the dipstick will be negative.
4. Under certain circumstances, the bacterial enzymes may have reduced nitrate to nitrite and then converted nitrite to nitrogen, which will give a negative nitrite result.
5. False-negative nitrite determinations or negative interferences can be the result of abnormally high levels of urobilinogen.
6. The presence of ascorbic acid levels as low as 5 mg/dL, or acidic urine (pH is 6.0 or less) may inhibit the reaction.

The nitrite test is not meant to take the place of other routine bacteriology studies such as cultures and smears. The dipstick procedure is just used as a screening test which is capable of detecting bacteriuria even when not clinically suspected. If there are clinical symptoms, then regular bacteriology tests should be performed, even if the nitrite test is negative.

Leukocyte Esterase

White blood cells can be present in any body fluid depending on a cause for their presence. The most common white blood cell seen in a urine sample is the neutrophil, which is normally present in low numbers. Increased numbers of neutrophils usually indicate the presence of a urinary tract infection; and their presence is indicated by a positive **leukocyte esterase** test. Screening for urinary tract infections also includes evaluation of pH, protein, and nitrite. Most accurate results are obtained on fresh, uncentrifuged, well-mixed specimens at room temperature.

REAGENT TEST STRIPS

Neutrophils contain enzymes known as esterases. These esterases can be detected by reagent strips that contain an appropriate substrate such as indoxylcarbonic acid ester and are based on the following reaction:

Reaction A:

$$\text{Indoxyl or pyrrole carbonic acid ester} \xrightarrow[\text{esterase}]{\text{granulocytic}} \text{Indoxyl or pyrrole}$$

Reaction B:

$$\text{Indoxyl or pyrrole} + \text{diazonium salt} = \text{purple}$$

The reagents used for this reaction vary by manufacturer and seem to affect test sensitivities as seen in Table 8-9.

Table 8-9 Leukocyte Esterase Reagents and Sensitivities by Reagent Strip

BRAND AND SENSITIVITY	REAGENT
AimStick (5 WBCs/uL)	Pyrrole amino acid ester Diazonium salt
Chemistrip(20 WBCs/uL)	Indoxylcarbonic acid ester Diazonium salt
Combi-Screen PLUS	Carboxylic acid ester
(10–20 leukocytes/uL)	Diazonium salt
DiaScreen (20 WBCs/uL)	Indoxylcarbonic acid ester Diazonium salt
Dirui H-Series (5–15 leukocytes/uL)	Pyrrole amino acid ester Diazonium salt
Mission (9–15 WBCs leukocytes/uL)	Pyrrole amino acid ester Diazonium salt
Multistix (5 WBCs/uL)	Pyrrole amino acid ester Diazonium salt
Self-Stik (not given)	Phenylthiazole amino acid ester Diazonium salt
URiSCAN (10 WBC/uL or 2–5 WBC/HPF)	Naphthol AS-D chloroacetate 2-Chloro-4-benzamide-5 Methylbenzenediazonium chloride
Uritest 13G (15–40 cells/uL granulocyte)	Indoxyl ester Diazonium salt
Uro-dip 10C (not given)	Indoxyl ester Diazonium salt
URS(10–15 WBCs/uL)	Indoxyl ester Diazonium salt

Leukocyte esterase results are read at 2 minutes. A positive reaction produces lavender to purple color reactions with a reporting range of values from trace to large. Values reflecting cell numbers from negative to 500 may be reported. These results may not correlate with the numbers of neutrophils seen during microscopic examination. Figure 8-20 displays a color chart for leukocyte esterase.

False-Positive Results

Strong oxidizing agents cause a false-positive leukocyte esterase result. This occurs when strong detergents used to clean the collection container remain present. False-positive results may also be obtained on females due to contamination of the urine with vaginal discharge. Some preservatives such as formalin will cause a false-positive result.

Nitrofurantoin contributes a color to urine that may cause misinterpretation of this test. False-positive results may be caused by drugs that contain imipenem, meropenem, and clavulanic acid.

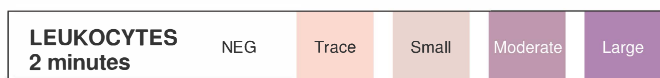


Figure 8-20. Leukocyte esterase color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Siemens Healthcare Diagnostics Inc. Tarrytown, NY; 2010).

False-Negative Results

False-negative results may occur with high specific gravity and in urines containing glucose and protein. Significantly high levels of protein or glucose can contribute to increased specific gravity. In such an environment, white blood cells will crenate and be unable to release esterase.

Various drugs and chemicals interfere with this test. Check the packaging insert of the reagent strip manufacturer for specifics concerning interfering substances. Some drugs and chemicals that may cause false-negative results include ascorbic acid, oxalic acid, cephalexin, cephalothin, gentamicin, and tetracycline.

Additional Parameters Available on Urine Reagent Strips

Some manufacturers offer additional tests that may be performed using reagent strip methods including calcium, creatinine, and microalbumin. In addition, some brands include a test pad for ascorbic acid to provide a way to access potential interference by this substance.

ASCORBIC ACID

Vitamin C is essential for many metabolic functions of the body. Yet humans cannot produce their own vitamin C and must consume vitamin C rich foods or take supplements. Recommended daily doses of vitamin C are 75 mg for females and 90 mg for males.

Because vitamin C is water soluble, excess amounts will be excreted in the urine (provided normal renal function). Increased rate of vitamin C excretion can occur from stress caused by heat or cold exposure. In addition, some drugs may increase vitamin C excretion. Some researches stress the importance of measuring urine vitamin C in patients with consistently low levels. In addition, screening for increased levels of urine vitamin C can help physicians monitor patients who are prone to oxalate kidney stones.

Excess ascorbic acid (vitamin C) can interfere with the chemical reactions for bilirubin, blood, glucose, and leukocytes; and may result in false low or negative results in these parameters. Detecting the presence of ascorbic acid may be helpful in correlating negative results with other findings.

Figure 8-21. Ascorbic acid color chart. (Modified from Combi-Screen PLUS® [color chart]. Analyticon Biotechnologies AG, Lichtenfels, Germany; 2008.)

Reagent strips that test for ascorbic acid use Tillman's reagent, 2,6-dichlorphenol-indophenole-sodium, which is reduced by ascorbic acid producing a change in color on the reagent pad from blue to orange (Figure 8-21).

CALCIUM

The most common mineral in the human body, **calcium**, is needed for many metabolic functions including bone, heart, muscle, and nerve health. Calcium is primary stored in bones, which release calcium if blood calcium levels fall too low. If blood calcium levels become elevated, calcium is either stored or excreted in urine or stool. Calcium levels are dependent upon consumption of calcium-rich foods, vitamin D levels, intestinal absorption ability of calcium and vitamin D, phosphate levels, and hormones (calcitonin, estrogen, parathyroid hormone). Urine calcium screening can detect the need for further investigation into the disorders of these various factors. Screening for increased levels of urine calcium can also help physicians monitor patients who are prone to calcium-containing kidney stones.

Reagent strip tests for urine calcium are based on the formation of a complex between calcium ions and glyoxal-bis-2-hydroxyanil in the presence of hydrogen peroxide. This reaction forms a compound that provides the color reactions shown in Figure 8-22.

CREATININE

Creatinine is waste product generated from muscle metabolism of the amino acid creatine. Most of the creatinine is removed by the kidneys, making it a good substance to use when testing renal filtration function as described in the Chapter 1. In the reagent strip reaction for creatinine, creatinine reacts with a creatinine indicator (copper sulfate and benzidine) at an alkaline pH to form a purplish-brown color. Figure 8-23 displays a creatinine color chart. The

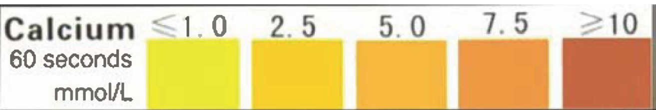


Figure 8-22. Calcium color chart. (Modified from UriTest 13G® Urine Reagent Strips [color chart]. Uritest Medical Electronic Co., Ltd. GuiLin, PR China; 2006.)

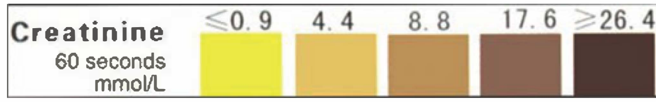


Figure 8-23. Creatinine color chart. (Modified from UriTest 13G® Urine Reagent Strips [color chart]. Uritest Medical Electronic Co., Ltd. GuiLin, P.R China; 2006.)

reference range for creatinine is 10 to 300 mg/dL (0.9 to 26.5 mmol/L).

Testing creatinine allows examiners to determine if a specimen is altered, as may be the case in attempts to foil urine drug screening tests. In these attempts both creatinine and specific gravity are much lower than typically found in urine. In addition, the absence of creatinine may indicate the specimen is not human urine.

False Results

Because, this reagent strip must be read manually, heavily colored urines due to the presence of large amount of hemoglobin or bilirubin can cause misinterpretation of results. In addition, substances that add color to urine (pyridium, nitrofurantoin, riboflavin) may affect test results as well.

MICROALBUMIN

Testing for **microalbumin** (small amounts of albumin) is used to screen people with conditions that have an associated increased risk for developing kidney disease. This is explained further in the chapter Urinary System Anatomy and Physiology and Urine Formation.

In the reagent strip reaction, albumin binds sulfonephthalein dye at a constant pH to develop a blue-green color. The resulting color ranges are shown in Figure 8-24. Less than 20mg/L is normal, whereas 20 to 200 mg/L indicates early kidney disease. Greater than 200 mg/L albumin is present in advanced renal disorders.

False Results

Because, this reagent strip must be read manually, heavily colored urines due to the presence of large amount of hemoglobin or bilirubin can cause misinterpretation of results. In addition, substances that add color to urine (pyridium, nitrofurantoin, riboflavin) may affect test results as well. Microalbumin testing should not be performed during urinary tract infection as this condition causes an increase in urinary albumin and may also cause urinary tract bleeding.

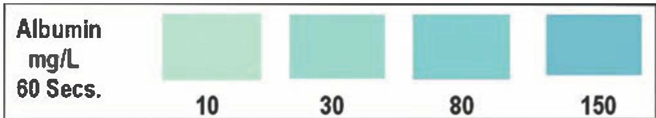


Figure 8-24. Microalbumin color chart. (Modified from URS-3ACC® Reagent Strips for Urinalysis [color chart]. Jilin Shenhua Economic Trade Co., Ltd. 1188 Jinxin Street, Changchun, China.)

STUDY QUESTIONS

Match the reagents listed below to the test in which they are used.

a. Bilirubin
b. Blood
c. Glucose
d. Ketone
e. Leukocytes
f. Nitrite
g. pH
h. Protein
i. Urobilinogen

1. __arsanilic acid
2. __bromothymol blue
3. __cumene hydroperoxide
4. __dichloroaniline
5. __dimethylaminobenzaldehyde
6. __indoxylcarbonic acid ester
7. __methyl red
8. __potassium iodide
9. __sodium nitroprusside
10. __tetrabromophenol blue
11. __tetrachlorophenol-tetrabromosulfophthalein
12. __tetrahydroquinoline
13. __tetramethylbenzine
14. Specimens for urine chemistry analysis must be well mixed to ensure an accurate reading of:
    a. pH and specific gravity
    b. Blood and leukocytes
    c. Glucose and ketones
    d. Bilirubin and urobilinogen
15. Timing of reagent strip readings is especially critical for:
    a. Diazo compound formation
    b. Dye-binding reactions
    c. Enzymatic reactions
    d. Protein error of indicators

16. Testing specimens that contain high levels of ascorbate may affect the reading of all of these EXCEPT:
- **a.** Bilirubin
- **b.** Glucose
- **c.** Nitrite
- **d.** Urobilinogen

17. A high specific gravity will affect all of the following reactions EXCEPT:
- **a.** Glucose
- **b.** Leukocytes
- **c.** Nitrite
- **d.** Protein

18. Which of the following tests does not have a negative reading on reagent strip color charts?
- **a.** Blood
- **b.** Glucose
- **c.** Ketone
- **d.** Urobilinogen

19. Purple colors are observed in the positive reactions for:
- **a.** Blood and glucose
- **b.** Ketone and leukocytes
- **c.** Bilirubin and urobilinogen
- **d.** Protein and nitrite

20. The ketone most detectable by all reagent strips is:
- **a.** Acetoacetic acid
- **b.** Acetone
- **c.** β-hydroxybutyric acid
- **d.** Phenylketone

21. A false-positive protein may be produced by:
- **a.** Albumin
- **b.** Alkaline pH
- **c.** Ascorbic acid
- **d.** Run-over effect

22. Positive bilirubin reactions should be confirmed by:
- **a.** Acetest
- **b.** Clinitest
- **c.** Foam Test
- **d.** Ictotest

23. The principle of "protein error of indicators" is based on:
- **a.** Protein changing the pH of the specimen.
- **b.** Protein changing the pK_a of the specimen.
- **c.** Protein accepting hydrogen from the indicator.
- **d.** Protein giving up hydrogen to the indicator.

24. Early detection of renal disease indicates testing for:
- **a.** Calcium
- **b.** Creatinine
- **c.** Macroalbumin
- **d.** Microalbumin

25. Non-routine urine reagent strip tests that may be useful to screen for substances associated with kidney stones include (*choose all that apply*).
- **a.** Ascorbate
- **b.** Calcium
- **c.** Creatinine
- **d.** Microalbumin

CASE STUDY

Case 8-1 When performing routine urinalysis quality control you observe when you remove the dipsticks from the bottle that the urobilinogen pad is of brown color. What is your course of action?

Case 8-2 When performing routine urinalysis you observe a pink color on the bilirubin pad. How should you proceed?

Case 8-3 When performing a routine urinalysis you observe a 1+ leukocyte esterase. No cells are seen upon microscopic examination. What can account for these results?

Case 8-4 A urine test on a 1-month-old baby shows a positive copper reduction test with a negative oxidase test. How should these results be reported and what is their significance?

Case 8-5 A physician questions the results of a urinalysis which was reported to show a negative nitrite, yet contained 2+ bacteria. Suggest a course of action and explanation why these findings are consistent.

Case 8-6 A urine chemical screening test results in all negative results and includes a specific gravity of 1.000. What other test can be performed to determine whether the fluid is actually urine?

Case 8-7 The brand of urine reagent strips you use at your facility includes a pad for ascorbic acid (ascorbate). The pad gives a reading of 2+.

1. What are the possible effects on other laboratory tests?

2. What does this result suggest about the nutritional status of the patient?

3. What could this result suggest about renal disorders in the patient?

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Microscopic Examination of Urinary Sediment

KEY TERMS

Ammonium Biurate
Amorphous Phosphates
Amorphous Urates
Artifacts
Bacteria
Calcium Carbonate
Calcium Oxalate
Calcium Phosphate
Calcium Sulfate
Cholesterol
Cloth Fibers
Cylindroids
Cystine
Epithelial Cell Casts
Erythrocytes
Fatty Casts
Glitter Cells
Granular Casts
Hippuric Acid
Hyaline Casts
Leucine
Leukocytes
Kova System
Mucous Threads
Oval Fat Bodies
Parasites
Radiographic Dyes
Red Blood Cell Casts
Renal Tubular Epithelial Cells
Sodium Urates
Spermatozoa
Squamous Epithelial Cells
Starch Crystals
Sternheimer–Malbin
Sulfonamide Drugs
Tamm–Horsfall Mucoprotein
Transitional Epithelial Cells
Triple Phosphate
Tyrosine
Uric Acid
Waxy Casts
White Blood Cell Casts
Yeast

LEARNING OBJECTIVES

1. Compare methods for specimen preparation.
2. Describe the procedure for proper microscopic examination and enumeration of urinary sediment.
3. Identify causes for altered appearance and distribution of urinary sediment on the slide.
4. Identify urinary sediment and artifacts.
5. Describe characteristics of urine sediment.
6. Recognize sources of error in identification of urine sediment.
7. Suggest methods to confirm the identification of urine sediment (staining, alternate microscopy, solubility tests).
8. Describe the appearance of urine sediment using confirmatory methods of identification.
9. Distinguish among cells seen in urine.
10. Distinguish among crystals seen in urine.
11. Distinguish among urine casts.
12. Explain the formation of casts and their sequence of degeneration.
13. Suggest the clinical significance of urine sediment.
14. Correlate urine sediment with chemical analysis.

The microscopic examination is a vital part of the routine urinalysis. It is a valuable diagnostic tool for the detection and evaluation of renal and urinary tract disorders as well as other systemic diseases. The value of the microscopic examination is dependent on two main factors: the examination of a suitable specimen and the knowledge of the person performing the examination.

The best specimen for the routine urinalysis is the first morning specimen. Casts and red blood cells (RBCs) tend to dissolve or lyse in specimens with a low specific gravity or alkaline pH. The first morning specimen usually provides the concentrated and acidic environment needed to maintain these structures. The sediment should be examined as soon as possible after collection, but it may be refrigerated for a few hours if the examination cannot be per formed immediately.

There have been some advances made in an effort to aid the technologist with the microscopic examination. These include the use of stains, the development of the phase and interference contrast microscopy techniques, and automated computerized imaging.

The most common stain for urinary sediments is the **Sternheimer–Malbin** supravital stain. Sternheimer–Malbin contains crystal violet and safranin stains and can be used as a general stain for most urinary structures. Some of the other staining techniques that can be used to differentiate certain urinary components include Sudan III, Sudan IV, and Oil Red O, which are used to stain fat a pink to red color; eosin, which stains RBCs and helps distinguish them from yeast cells which will not pick up the stain; and iodine, which can be used to stain starch granules and vegetable fibers a dark brown.

Sediment Preparation

COMMON METHOD

The microscopic examination should be performed on a centrifuged sample. If the volume of the specimen is too small to be centrifuged, then examine the sample directly, but note in the report that the results are from an uncentrifuged urine. Mix the specimen and then place approximately 10 to 15 mL of urine into a centrifuge tube and centrifuge at 2,000 rpm for about 5 minutes. In an attempt to standardize the microscopic examination, the laboratory should adopt a regulated volume, and speed and time for the centrifugation of the urine specimens. Pour off the supernatant fluid and resuspend the sediment in the urine that drains back down from the sides of the tube. Some laboratories leave exactly 1 mL of sediment/supernatant mixture in the tube. Flick the bottom of the tube to mix the sediment and place a drop of sediment on a clean slide or in a counting chamber. Cover with a coverslip and examine immediately.

KOVA SYSTEM

Some laboratories use the **KOVA system** in an effort to standardize the procedure for microscopic examination of urinary sediment. The KOVA system (Fig. 9-1) consists of centrifuge tubes and caps, special pipets, and plastic slides with 10 standardized chambers (with or without 3-mm × 3-mm grids). Plain slides are most commonly used for semiquantitative analysis of urine sediment, whereas slides with grids are used for quantitative cell counts on other body fluids (explained in the Chapter 11). KOVA stain, a modified Sternheimer–Malbin stain, may be used with this system. Well-mixed urine is transferred from a collection cup to the conical centrifuge tube and centrifuged for 5 minutes at 1,500 revolutions per minute (rpm). The Kova Petter is inserted into the tube and the supernatant is poured off and discarded, leaving 1 mL of sediment/supernatant mixture. The KOVA Petter is removed and one drop of KOVA stain may be added and gently resuspended using the Kova Petter. A small sample of the stained sediment is aspirated into the KOVA Petter and dispensed onto the

A

B

Figure 9-1. Kova system: **(A)** Kova Petter is inserted into a tube of centrifuged urine; and is used to decant, resuspend, and deliver sediment into one of the counting chambers; **(B)** Kova slide. Some models of Kova slides have 3-mm × 3-mm grids (as indicated by the call out).

KOVA slide by touching a drop of the sediment to the notch of one of the chambers. The slide is scanned under low- and high-power magnifications and sediment is enumerated as described in the next section.

Microscopic Observation and Enumeration

The first rule for examining unstained urinary sediment with the bright field microscope is that subdued light must be used to provide adequate contrast. This is obtained by partially closing the iris diaphragm and then adjusting the condenser downward until optimum contrast is achieved. If there is too much light, some of the structures will be missed. For example, hyaline casts, which are gelled protein, have a very low refractive index and will be overlooked if the light is too bright or if there is not enough contrast.

The second important rule is that the fine adjustment should be continuously adjusted up and down to enable the viewer to see the depth of the object as well as other structures that may be on a different focal plane. Proper adjustment of the microscope is explained in the Chapter 5. Figure 9-2A is an example of why the focus should be constantly adjusted. The field seems to contain only amorphous phosphates; but when the fine adjustment knob is moved slightly, a hyaline cylindroid appears (Fig. 9-2B). Sediment should be viewed first under low-power magnification (100×). Scan the slide and observe for casts, crystals, and elements that are present in only a few fields. Enumerate the number of casts. Switch to high dry power (400×) when necessary to delineate the structures that are seen.

Casts have a tendency to move toward the edge of the coverslip, so the entire periphery of the coverslip should be scanned. Casts are reported as the average number that is present in 10 to 15 fields under low-power magnification (100×). For example, if the number of hyaline casts in 10 different fields is 1, 3, 2, 1, 1, 2, 2, 3, 1, and 3, then the report would be 1–3 hyaline casts/low-power field (LPF). Some laboratories use ranges for reporting casts: 0–2, 2–5, 5–10. Other laboratories may report casts as rare, few, moderate, or many.

Cells are enumerated using high dry power (400×) and are reported in ranges (0–2, 2–5, 5–10, 10–20, 20–50, <50) or as rare, few, moderate, many, and packed. Crystals, bacteria, parasites, and other rare sediments may be reported as being present, or may be reported as rare, occasional, moderate, and many.

Cells

Cells that can be present in the urine include **erythrocytes** (RBCs), **leukocytes** (white blood cells or WBCs), and epithelial cells from anywhere in the urinary tract from the tubules to the urethra or as contaminants from the vagina or vulva. Microscopic evaluation of urine is important for detection of these cells not only for confirmation of chemical findings but also for detection of RBCs and WBCs in specimens that may contain interfering substances for these cells.

ERYTHROCYTES

Red blood cells in the urine may have originated in any part of the urinary tract from the glomerulus to the urethral meatus, and in the female they may be the result of menstrual contamination. They can appear in a variety of forms depending upon the environment of the urine (Fig. 9-3). When the urine specimen is fresh, the red cells have a normal, pale, or yellowish appearance and are smooth, biconcave disks approximately 7 microns in diameter and 2 microns thick. They contain no nuclei and, when viewed from the side, they have an hourglass appearance.

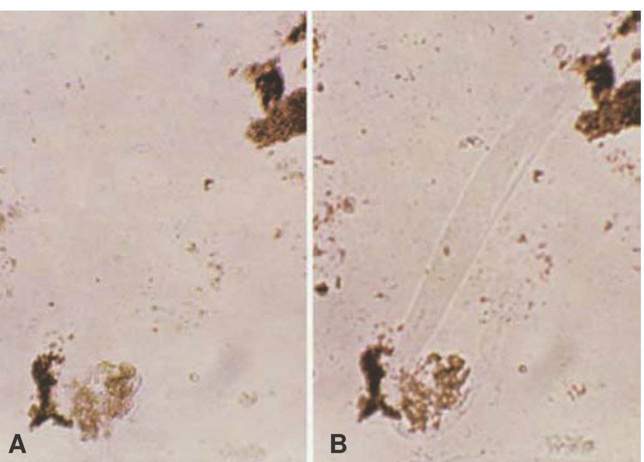

Figure 9-2. Amorphous phosphates and hyaline cylindroid. The cylindroid is not visible in **A** but appears in **B** when the focus is adjusted (200×).

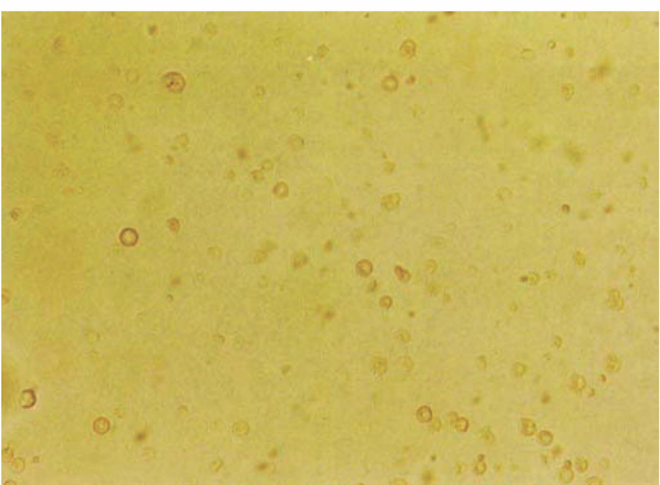

Figure 9-3. Red blood cells. The field also contains a white cell and several "ghost" cells (400×).

In dilute or hypotonic urine, the red cells swell up and can lyse, thus releasing their hemoglobin into the urine. Lysed cells, which are referred to as "ghost" or "shadow" cells, are faint, colorless circles and are actually the empty red cell membranes. Lysing will also occur in alkaline urine. Red blood cells will crenate in hypertonic urine and sometimes the crenations may resemble granules.

There are some structures that can be confused with RBCs in the microscopic examination. Swollen or crenated RBCs can sometimes be mistaken for WBCs, even though they are larger and contain nuclei. This is especially true if there is only one type of cell present in the sediment not allowing for comparisons to be made among cells. The presence of a positive test for occult blood is often helpful in making a decision.

Simple adjustment of the microscope can aid in the differentiation of cells. In Figure 9-4A, which shows a field with both red cells and white cells, there should not be any problem differentiating the two cell types. Red cells in this figure resemble those that are seen on a blood smear. By turning the fine adjustment up and down, the result is that the red cells "pop out" at the viewer as black circles, as is seen in Figure 9-4B. This occurs because RBCs are very refractile and are thicker on the edges than in the center. This phenomenon will not occur if the red cells are grossly distorted by a hypotonic or hypertonic urine environment.

The best way to differentiate red cells is by the addition of a few drops of 2% acetic acid. The red cells will lyse in dilute acetic acid, but white cells will not. The addition of the acid will also emphasize the nuclei of the WBCs. Because the acid will lyse the red cells, it is important to count the cells that are present before adding the acid. Scan the entire slide before the acid is added, otherwise, structures such as red cell casts will also dissolve, or new crystals will precipitate out.

Yeast cells can be mistaken for RBCs. Yeast cells are ovoid, rather than round, and they frequently contain buds which are smaller than themselves in size. The doubly refractile border of the yeast cell tends to resemble the doughnut appearance of the red cell. Yeast cells will not dissolve in 2% acetic acid, nor will they stain with eosin.

Normally, RBCs do not appear in the urine, although the presence of 1 to 2 RBC/HPF is usually not considered abnormal. The mechanism whereby red cells enter the urine is not entirely clear. Unlike white cells, red cells do not possess amoeboid characteristics and, therefore, they must stay within the blood vessels. Injury or rupture of the blood vessels of the kidney or urinary tract releases RBCs into the urine, but this does not account for the acceptance of the normal presence of a few RBCs in the urine. Hematuria is the presence of an increased number of RBCs in the urine and the blood reagent pad will reflect the presence of RBCs or free hemoglobin. In addition, the protein test will be positive if large amounts of blood are present. As always, a correlation should be made between the chemical tests and the results of microscopic examination.

LEUKOCYTES

White blood cells can enter the urinary tract anywhere from the glomerulus to the urethra. On average, normal urine can contain up to 2 WBCs/HPF. White blood cells are approximately 10 to 12 microns in diameter and are larger than RBCs but smaller than renal epithelial cells. White blood cells are usually spherical and can appear dull gray or greenish-yellow in color (Fig. 9-5). WBCs may appear singly or in clumps (Fig. 9-6). The WBCs that are seen in urine are mostly neutrophils, which can be identified by their characteristic granules and nuclear lobulations. The leukocyte esterase pad on reagent strips indicates the presence of neutrophils. Lymphocytes do not possess leukocyte esterase and are detected by urine sediment observation. Figure 9-7 shows a field of packed WBCs.

The addition of 2% acetic acid can be used to accentuate the nuclei. Leukocytes shrink in hypertonic urine and swell or are lysed in hypotonic or alkaline urine. The number of WBCs in an alkaline and hypotonic urine decreases by 50% within 1 hour after collection if the specimen is kept at room temperature. When WBCs expand in dilute or hypotonic urine, their granules may demonstrate Brownian movement.

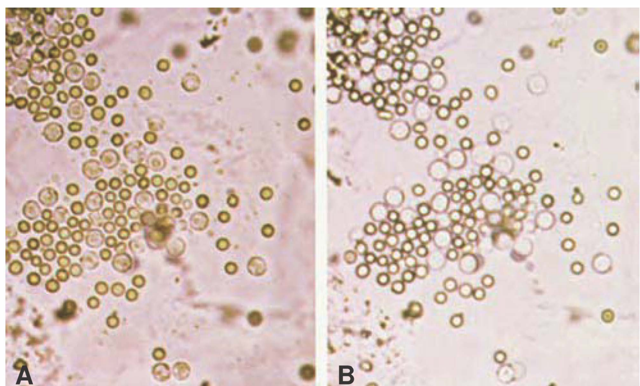

Figure 9.4 **A:** RBCs and WBCs. **B:** Changing the focus causes the red cells to appear as black circles (400×).

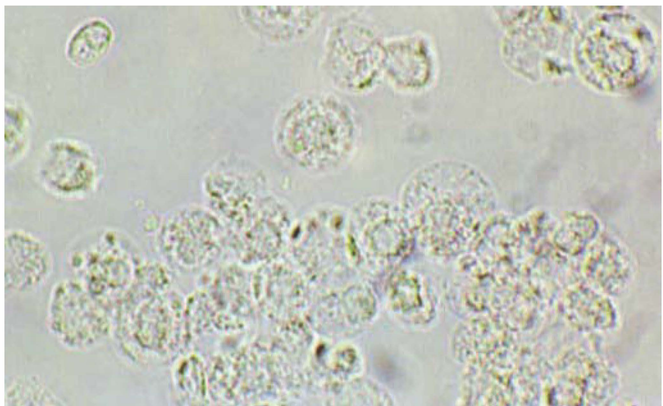

Figure 9-5. White blood cells in a hypotonic urine. The nuclei and granules are easily recognized (800×).

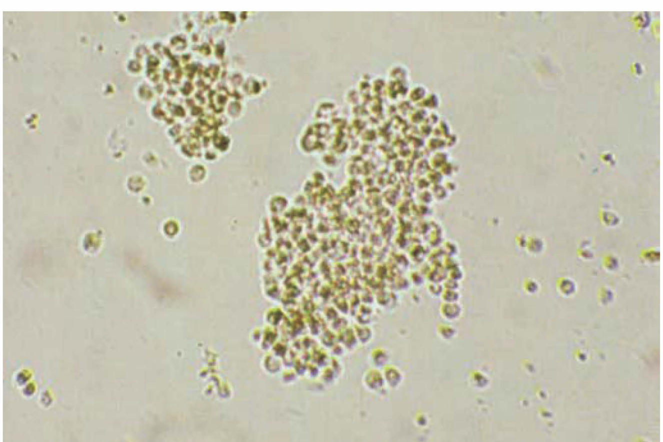

Figure 9-6. White cell clumps (200×).

Cells that develop this characteristic are called "**glitter cells**." Glitter cells were previously considered to be specific for pyelonephritis, but they can occur in a variety of conditions if the cells are exposed to a hypotonic environment.

An increase of WBCs in urine is associated with an inflammatory process in or adjacent to the urinary tract. Leukocytes are attracted to any area of inflammation and, because of their amoeboid properties, can penetrate the areas adjacent to the inflammatory site. Sometimes pyuria (pus in the urine) is seen in conditions such as appendicitis and pancreatitis. Pyuria is also found in noninfectious conditions such as acute glomerulonephritis, lupus nephritis, renal tubular acidosis, dehydration, stress, fever, and in noninfectious irritation to the ureter, bladder, or urethra. The presence of many white cells in the urine, especially when they are in clumps, is strongly suggestive of acute infection such as pyelonephritis, cystitis, or urethritis.

White blood cell casts are evidence that the WBCs originated in the kidney. White blood cell clumps are also strongly suggestive of renal origin, but they are not conclusive evidence. Because of the importance of WBC clumps, their presence should be reported. A few leukocytes can normally be found in secretions from the male and female genital tracts, so the possibility of a contaminated urine should be considered.

EPITHELIAL CELLS

The epithelial cells in the urine may originate from any site in the genitourinary tract from the proximal convoluted tubule to the urethra, or from the vagina. Normally, a few cells from these sites can be found in the urine as a result of the normal sloughing off of old epithelial cells. A marked increase indicates inflammation of that portion of the urinary tract from which the cells are derived. Making a distinction between the epithelial cells that arise in the various portions of the urinary tract is difficult. For this reason, many a laboratory reports the presence of epithelial cells without attempting to differentiate them. When distinction is possible, three main types of epithelial cells may be recognized: renal tubular, transitional, and squamous. Reagent strips do not indicate the presence of these cells.

Renal Tubular Epithelial Cells

Renal tubular epithelial cells are slightly larger than leukocytes and contain a large round nucleus. They may be flat, cuboidal, or columnar. Figure 9-8 shows a field containing WBCs, renal tubular epithelial cells, and a transitional cell. Note the variation in the sizes of these cells as well as the relative size of their nuclei. Increased numbers of tubular epithelial cells suggest tubular damage. This damage can occur in pyelonephritis, acute tubular necrosis, salicylate intoxication, and kidney transplant rejection.

Transitional Epithelial Cells

Transitional epithelial cells are two to four times as large as white cells. They may be round, pear-shaped, or may have tail-like projections. Occasionally, these cells may contain

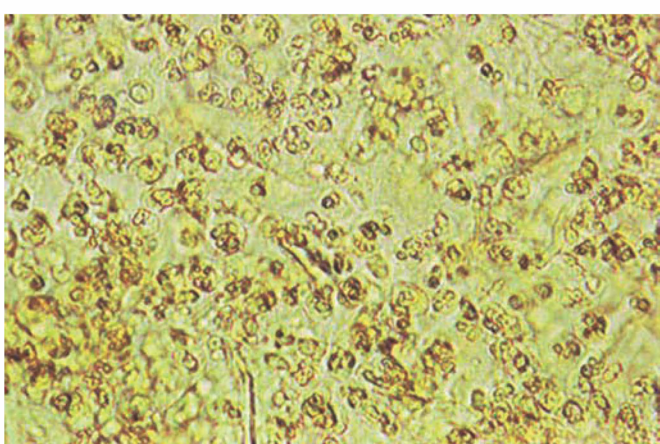

Figure 9-7. Numerous white cells. Acetic acid (2%) was added to accentuate the nuclei (400×).

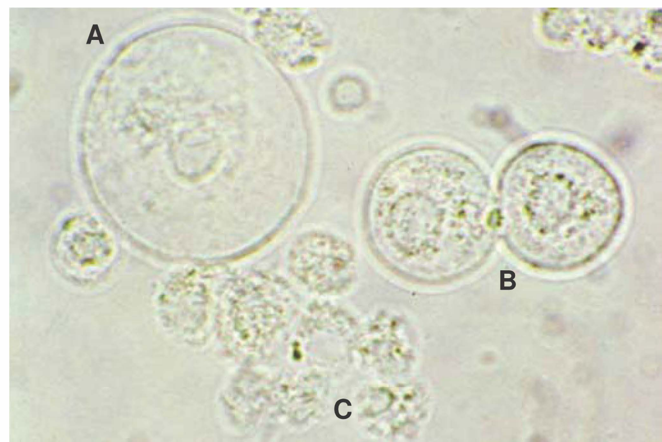

Figure 9.8 **A:** Transitional cell. **B:** Renal epithelial cells. **C:** WBCs (800×).

Figure 9-9. Transitional epithelial cells (500×).

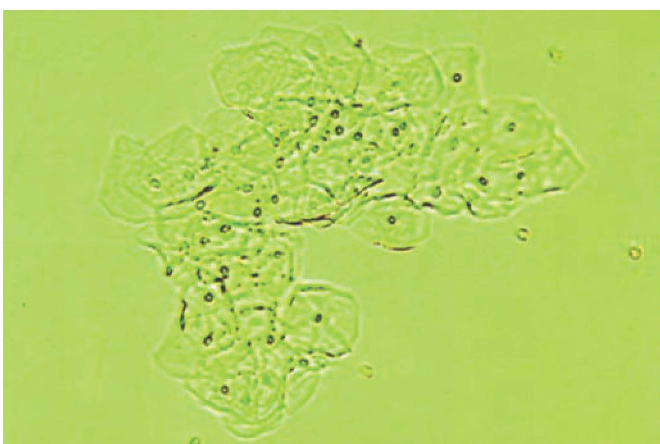

Figure 9-11. Squamous epithelial cells (160×).

two nuclei. Transitional cells line the urinary tract from the pelvis of the kidney to the upper portion of the urethra. Figure 9-9 shows pear-shaped transitional cells, and Figure 9-10 demonstrates the size of a transitional cell in proportion to the size of WBCs.

Squamous Epithelial Cells

Squamous epithelial cells are easily recognized as large, flat, irregularly shaped cells. They contain small central nuclei and abundant cytoplasm (Fig. 9-11). The cell edge is often folded over and the cell may be rolled up into a cylinder. Squamous epithelial cells occur principally in the urethra and vagina. Many of the squamous cells present in the female urine are the result of contamination from the vagina or vulva, and as such, they have little diagnostic significance.

Spermatozoa

Spermatozoa may be present in the urine of men after epileptic convulsions, nocturnal emissions, diseases of the genital organ, and in spermatorrhea. Sperm may also be found in the urine of both sexes after coitus. Spermatozoa have oval bodies and long, thin, delicate tails (Fig. 9-12).

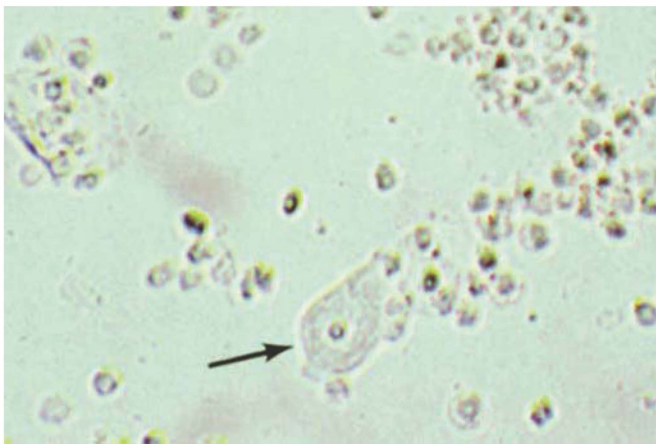

Figure 9-10. Transitional epithelial cell (*large arrow*), several squamous epithelial cells, and white cells (200×)

MICROORGANISMS

Bacteria

The urine is normally free of **bacteria** while in the kidney and bladder, but contamination may occur from bacteria present in the urethra or vagina, or from other external sources. When a properly collected, freshly voided specimen contains large numbers of bacteria, especially when accompanied by many white cells, it is usually indicative of a urinary tract infection. Bacteria are reported according to the number that is present (few, moderate, etc.), but no attempt is made, in the routine urinalysis laboratory, to identify the exact organism. The presence of bacteria is easily recognized when the sediment is viewed under high-power magnification (Fig. 9-13).

Some bacteria reduce nitrate to nitrite, allowing for the detection of bacteria by chemical methods. However, not all pathogenic bacteria are nitrate reducers. In addition,

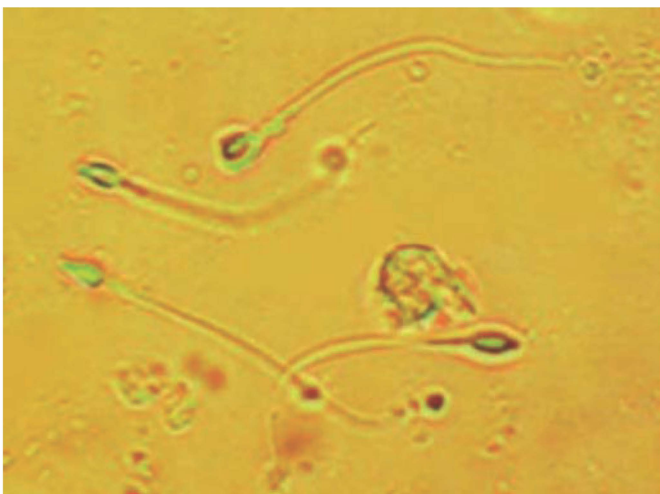

Figure 9-12. Spermatozoa (500×).

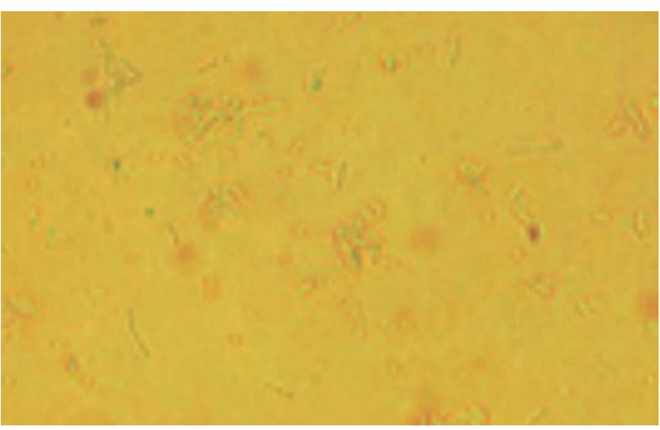

Figure 9-13. Bacteria (rods, cocci, and chains) (500×).

conditions exist which influence the presence of nitrites. The presence of leukocytes may provide more accurate correlation with bacterial infection than does nitrite.

Yeast

Yeast cells are smooth, colorless, usually ovoid cells with doubly refractile walls. They can vary in size and often show budding (Fig. 9-14). They may sometimes be mistaken for red cells, but unlike RBCs, they are insoluble in acid and alkali, and they will not stain with eosin. Yeast may be found in urinary tract infections, especially in patients with diabetes. Yeast may also be present in the urine as a result of skin or vaginal contamination. *Candida albicans* is the most common yeast to appear in the urine. Reagent strips do not detect the presence of yeast.

Parasites

Parasites may occasionally be found in the urine, either because they are indigenous to the urinary tract or as the result of vaginal or fecal contamination. Chemical analysis

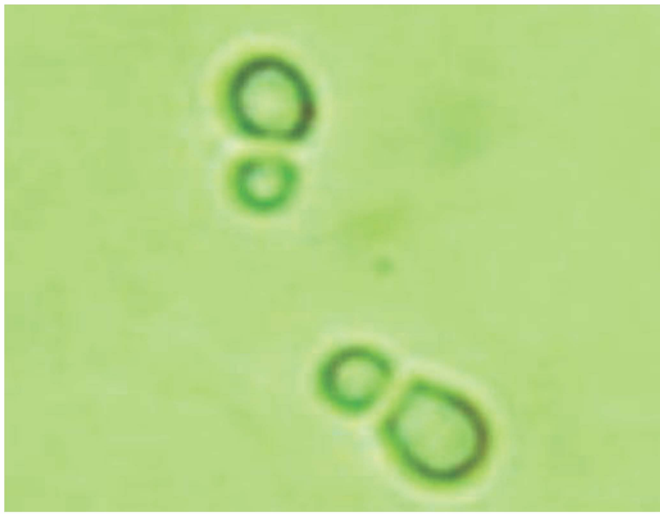

Figure 9-14. Yeast. Note the budding and doubly refractile walls (1,000×)

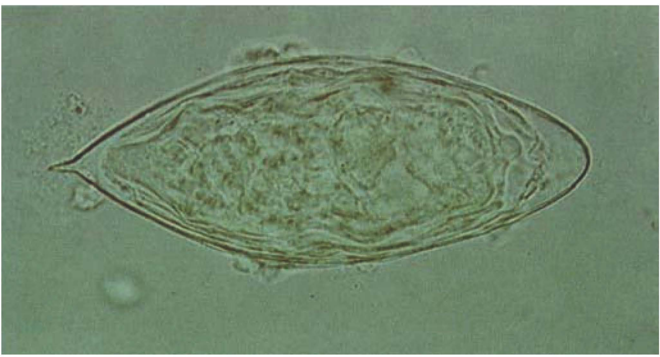

Figure 9-15. Schistosoma haematobium ovum. (Courtesy of Dr. Kenneth A. Borchardt, San Francisco State University, San Francisco, CA.)

does not detect parasites in urine. Microscopic evaluation of urinary sediment is important if parasitic infections are suspected. Chemical analysis may reveal the presence of leukocytes, if present during these infections. Reagent strips do not detect the presence of parasites.

Schistosoma haematobium

Schistosoma haematobium is a blood fluke that inhabits the veins in the wall of the urinary bladder. The adult deposits eggs in the capillaries of the mucosa. Abscesses develop around the eggs, and the eggs can be found in the urine accompanied by RBCs and WBCs. This type of schistosomiasis is endemic in Africa, especially around the Nile Valley, in the Middle East, and around the Mediterranean. The *S. haematobium* ovum has a characteristic terminal spine and measures about 50 microns by 150 microns (Fig. 9-15).

Trichomonas vaginalis

Trichomonas vaginalis is the most frequently occurring parasite in the urine. It is a flagellate organism that is about the same size as a large white cell (Fig. 9-16). In the unstained

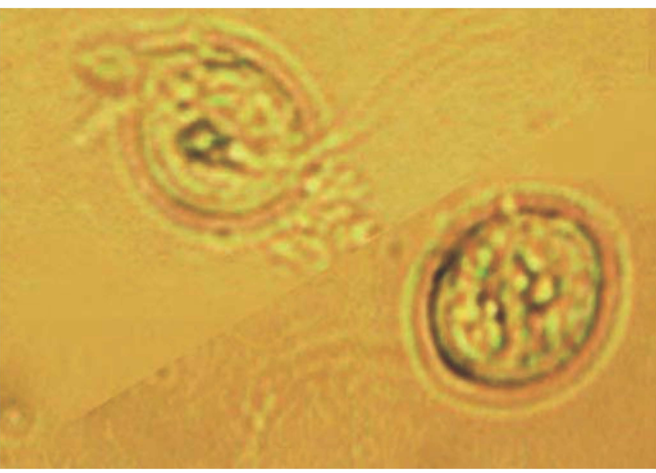

Figure 9-16. Trichomonas vaginalis. Note the four flagella (1,000×).

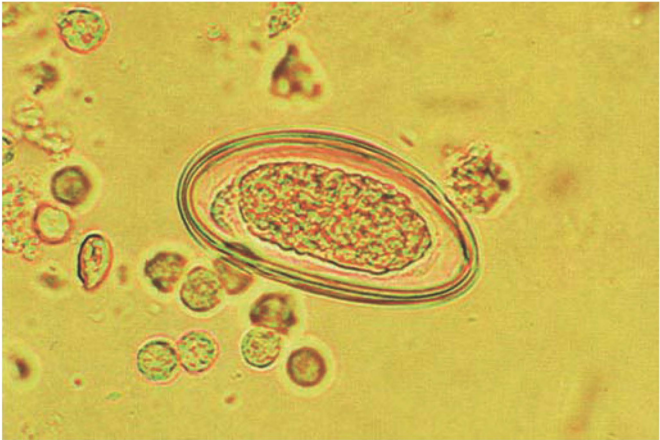

Figure 9-17. Enterobius vermicularis ovum and WBCs (500×).

wet mount, the organism should not be reported unless it is motile. Sometimes when bacteria are next to a white cell, the cell may be mistaken for Trichomonas, which is why motility is the diagnostic feature. This organism may be found in males, although it is more common in females. *T. vaginalis* is frequently accompanied by WBCs and epithelial cells.

Enterobius Vermicularis

Enterobius vermicularis (pinworm) ova and occasionally also the female adult may be found in the urine, perhaps even more frequently than was once believed. The ova are very characteristic in shape, having one flat and one rounded side (Fig. 9-17). The developing larva can usually be observed through the transparent shell of the egg. If the urine is found to contain many ova, examination of the original urine container may reveal the adult worm (Fig. 9-18).

Figure 9-18. Head of Enterobius vermicularis adult female worm (1,000×)

Crystals

Crystals are usually not found in freshly voided urine but appear after the urine stands for a while. When the urine is supersaturated with a particular crystalline compound, or when the solubility properties of that compound are altered, the result is crystal formation. In some cases, this precipitation occurs in the kidney or urinary tract and can result in the formation of urinary calculi (stones).

Many of the crystals that are found in the urine have little clinical significance, except in cases of metabolic disorders, calculus formation, and the regulation of medication. The most important crystals that may be present are cystine, tyrosine, leucine, cholesterol, and sulfa. Crystals can be identified by their appearance, pH dependency, and if necessary, by their solubility characteristics (refer to Table 9-1). Microscopic evaluation of urine is important for detection of crystals, because no chemical test detects the presence of crystals.

ACIDIC URINE

Those crystals which are frequently found in acidic urine are uric acid, calcium oxalate, and amorphous urates. Figure 9-19

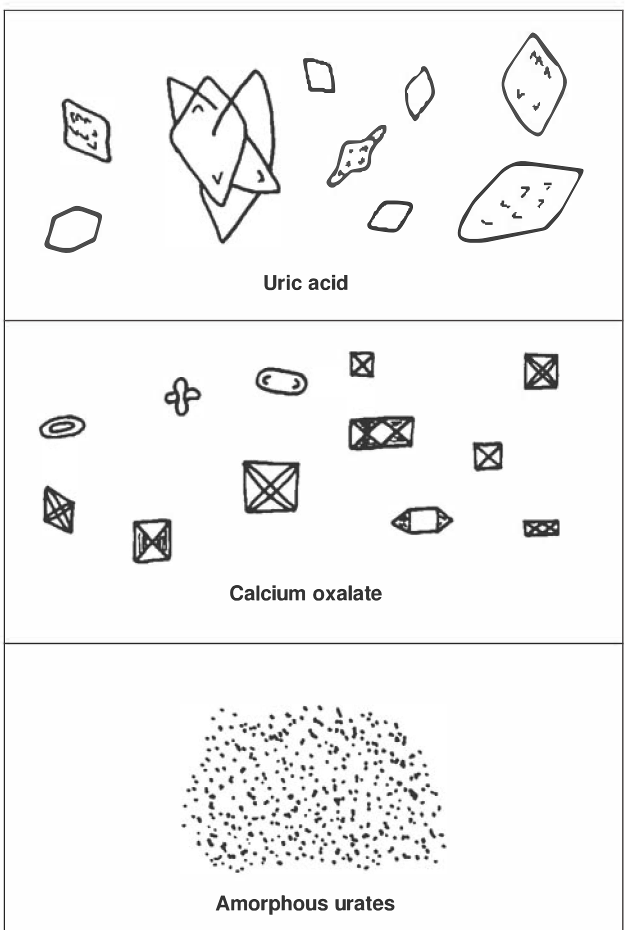

Figure 9-19. Crystals frequently found in acidic urine.

Table 9-1 Properties of Crystalline Compounds

	PH		
	ACID	ALKALINE	SOLUBILITY PROPERTIES
Amorphous urates	+	–	S-alkali, 60° C I-acetic acid
Bilirubin	+	–	S-chloroform, acid, alkali, acetone I-alcohol, ether
Calcium oxalates	+	±	S-HCl I-acetic acid
Calcium sulfate	+	–	S-acetic acid
Cholesterol	+	–	S-chloroform, ether, hot alcohol I-alcohol
Cystine	+	–	S-HCl, alkali, especially ammonia I-boiling, H_2O, acetic acid, alcohol, ether
Hippuric acid	+	±	S-hot H_2O, alkali I-acetic acid
Leucine	+	–	S-hot acetic acid, hot alcohol, alkali I-HCl
Sodium urate	+	–	S-60° C Slightly. S-acetic acid
Sulfonamides	+	–	S-acetone
Tyrosine	+	–	S-NH_4OH, HCl, dilute mineral oil I-acetic acid, alcohol, ether
Uric acid	+	–	S-alkali I-alcohol, HCl, acetic acid
X-ray dye	+	–	S–10% NaOH
Ammonium biurates	±	+	S-60° C, acetic acid, strong alkali NaOH (ammonia liberated)
Amorphous phosphates	–	+	S-acetic acid
Calcium carbonate	–	+	S-acetic acid (effervescence)
Calcium phosphates	–	+	S-dilute acetic acid
Triple phosphates	–	+	S-dilute acetic acid

shows drawings of these common forms of acid crystals. Crystals which occur less frequently include calcium sulfate, sodium urates, hippuric acid, cystine, leucine, tyrosine, and cholesterol. Drug crystals can be present in acid urine from patients taking certain medications. For example sulfonamide (sulfa) crystals. Figure 9-20 shows drawings of these lesser seen crystals.

Calcium Oxalate Crystals

Calcium oxalate crystals are colorless octahedral (double pyramid) crystals and appear to look like an "envelope"—small squares crossed by intersecting diagonal lines suggest the possibility of oxalate calculi (Fig. 9-21). When focusing on the typical calcium oxalate crystal, the "X" may appear to "pop out" of the field (Fig. 9-22). Rarely, calcium oxalates may also appear as oval spheres or biconcave disks which have a dumbbell shape when viewed from the side; and these can form clusters or rosettes (Fig. 9-23). These crystals can vary in size so that at times they are only barely discernible under high-power magnification.

Calcium oxalate crystals are frequently found in acidic and neutral urine, and occasionally they are also found in alkaline urine. They are soluble in hydrochloric acid but insoluble in acetic acid. Calcium oxalate crystals can be present normally in the urine especially after the ingestion of various oxalate-rich food such as tomatoes, spinach, rhubarb, garlic, oranges, and asparagus. Increased amounts of calcium oxalates, particularly if they are present in freshly voided urine, suggest the possibility of oxalate calculi. Other pathologic conditions in which

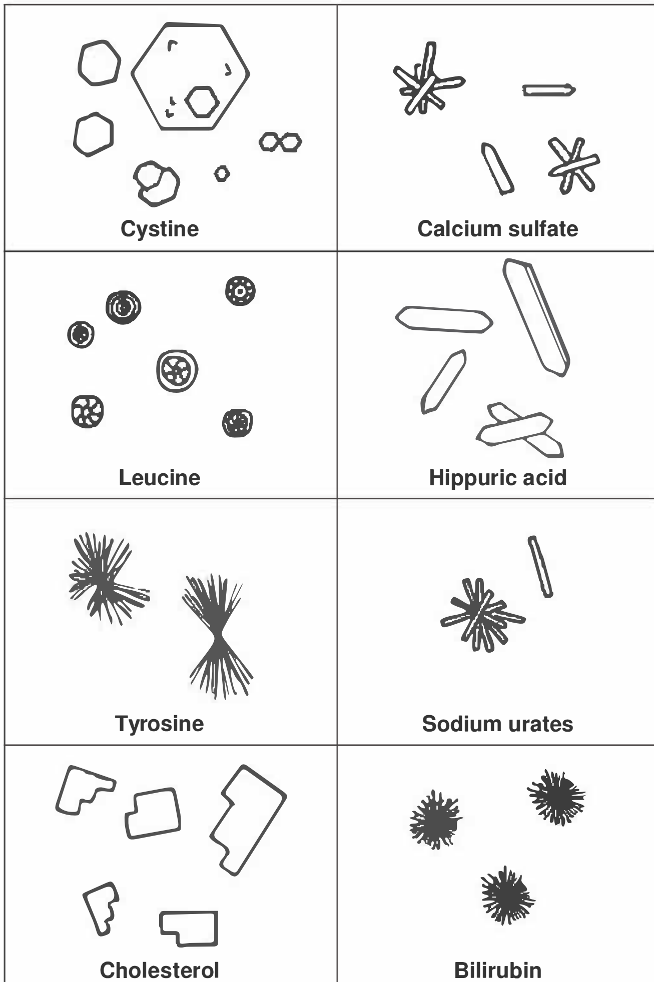

Figure 9-20. Crystals less frequently found in acidic urine.

calcium oxalates can be present in increased numbers include ethylene glycol poisoning, diabetes mellitus, liver disease, and severe chronic renal disease.

Calcium oxalate crystals may be present in the urine following the intake of large doses of vitamin C. Oxalic acid is

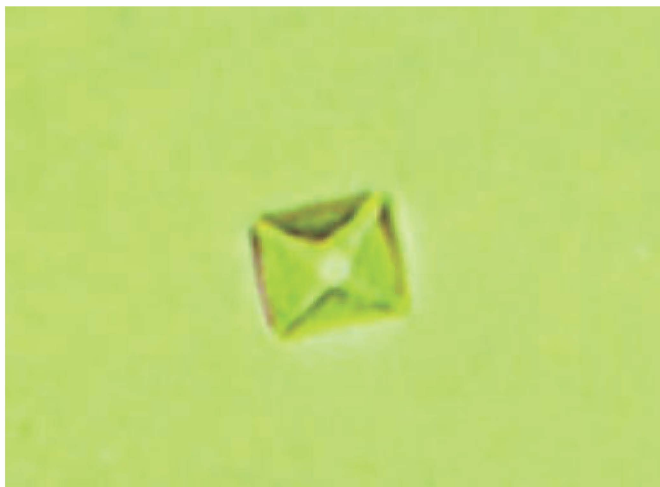

Figure 9-21. Calcium oxalate crystal (400×).

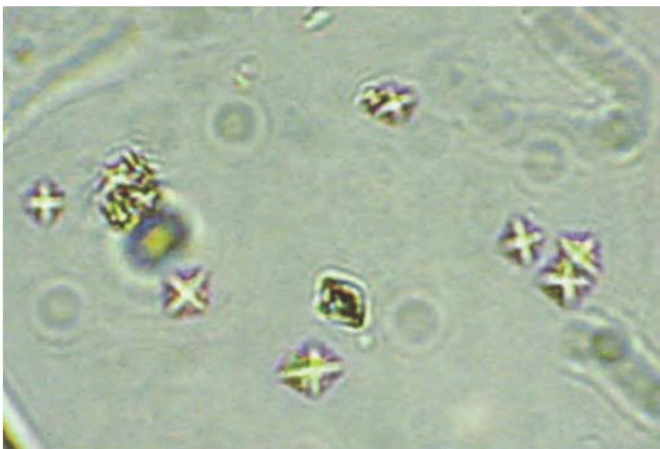

Figure 9-22. Calcium oxalate crystals. The "X" of each crystal is very prominent (500×).

one of the breakdown products of ascorbic acid, and oxalic acid precipitates ionized calcium. This precipitation may also result in a decrease in the level of serum calcium.

Uric Acid Crystals

Uric acid crystals can occur in many different shapes, but the most characteristic forms are the diamond or rhombic prism (Fig. 9-24), and the rosette (Fig. 9-25), which consists of many crystals clustered together. Uric acid crystals may occasionally have six sides (Fig. 9-26), which sometimes causes this form to be erroneously identified as cystine. However, cystine crystals are colorless. Uric acid crystals are usually stained with urinary pigments and are, therefore yellow, or red-brown in color. The color is frequently dependent upon the thickness of the crystal, so very thin crystals may be colorless.

Under polarized light, uric acid crystals may take on a variety of colors. The polarized crystal in Figure 9-27 also demonstrates the layered effect that many uric acid crystals

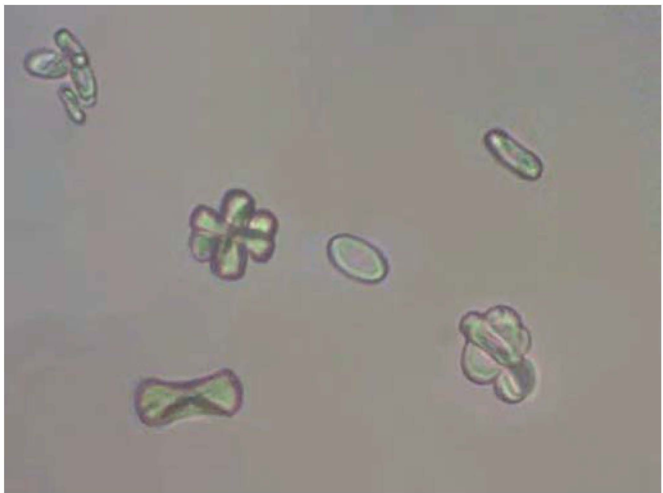

Figure 9-23. Calcium oxalate crystals can sometimes appear as oval, dumbbells, and rosettes.

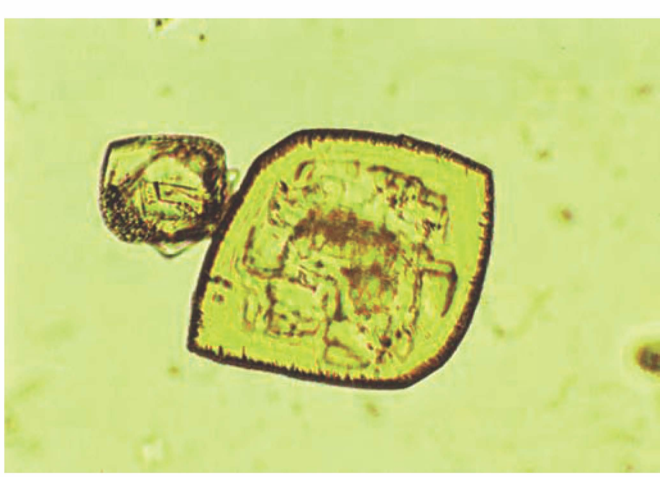

Figure 9-24. Uric acid: diamond or rhombic forms may also be referred to as "lemon-shaped" (500×).

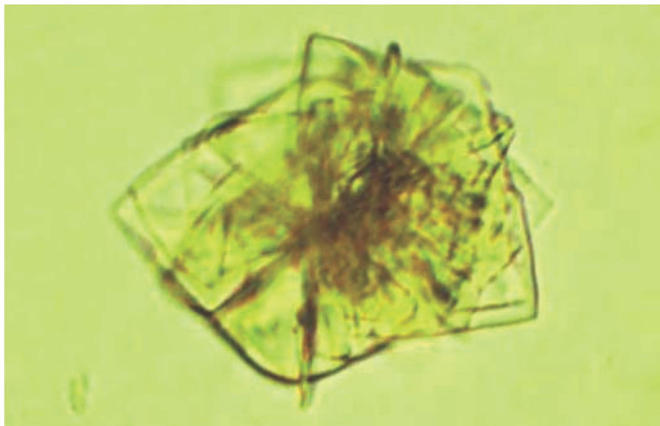

Figure 9-25. Uric acid crystals in rosette formation (500×).

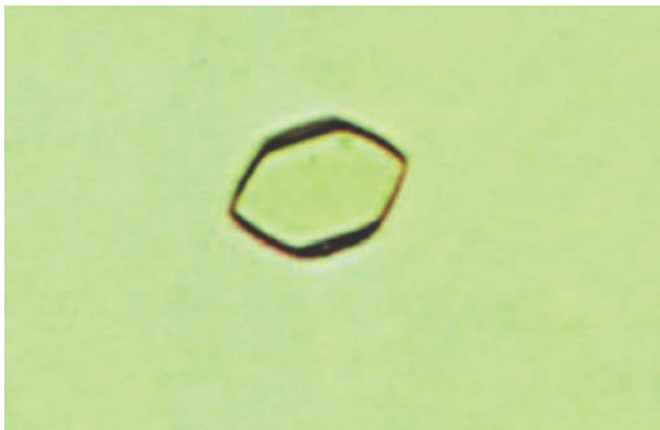

Figure 9-26. Uric acid: six-sided crystal may be confused with cystine (400×).

manifest. These crystals are soluble in sodium hydroxide and insoluble in alcohol, hydrochloric acid, and acetic acid.

The presence of uric acid crystals in the urine can be a normal occurrence. It does not necessarily indicate a pathologic condition, nor does it mean that the uric acid content of the urine is definitely increased. Pathologic conditions in which uric acid crystals are found in the urine include gout, high purine metabolism, acute febrile conditions, chronic nephritis, and Lesch–Nyhan syndrome.

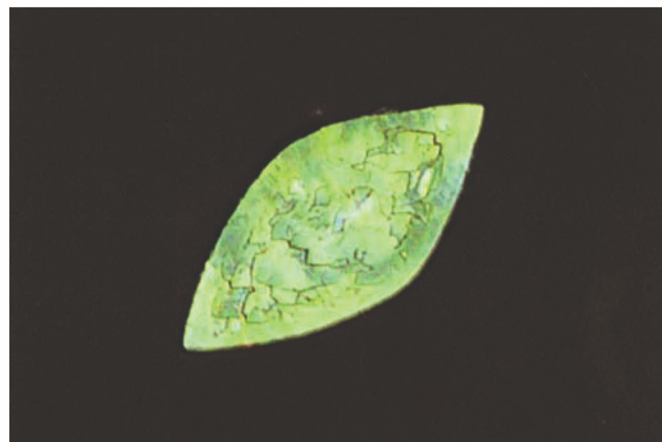

Figure 9-27. Uric acid: may display many colors under polarized light. Note the layered or laminated surface (400×).

Amorphous Urates

Amorphous urates are urate salts of sodium, potassium, magnesium, and calcium that are frequently present in the urine in a noncrystalline, amorphous form. These amorphous urates have a yellow-red granular appearance (Fig. 9-28) and are soluble in alkali and at 60°C. The presence of a granular pink-colored sediment may indicate the presence of amorphous urates. Amorphous urates have no clinical significance.

Hippuric Acid Crystals

Hippuric acid crystals are yellow-brown or colorless elongated prisms or plates (Fig. 9-29). They may be so thin as to resemble needles, and they often cluster together. Hippuric acid

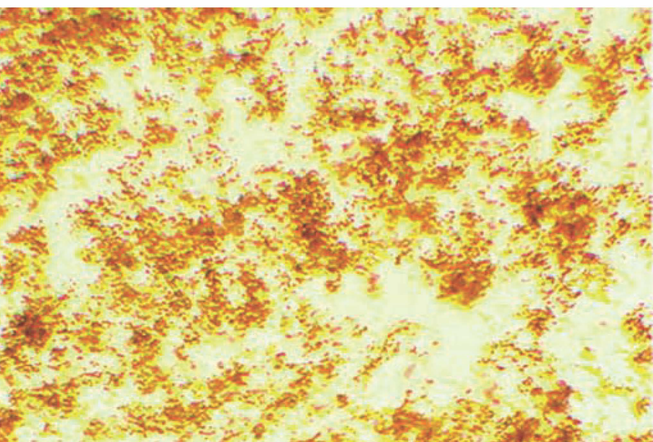

Figure 9-28. Amorphous urates (200×).

Figure 9-29. Hippuric acid crystal (400×).

crystals are more soluble in water and ether than are uric acid crystals. Hippuric acid crystals are rarely seen in the urine and have practically no clinical significance.

Sodium Urates

Sodium urates may be present as amorphous or crystalline forms (Fig. 9-30). Sodium urate crystals are colorless or yellowish needles or slender prisms occurring in sheaves or clusters. They are soluble at 60°C and only slightly soluble in acetic acid. Sodium urates have no clinical significance.

Calcium Sulfate Crystals

Calcium sulfate crystals are long, thin, colorless needles or prisms that are identical in appearance to calcium phosphates. The pH of the urine helps differentiate these two crystals, because calcium sulfate is found in acidic urine, whereas calcium phosphate is usually found in alkaline urine. Calcium sulfate is also extremely soluble in acetic acid. Calcium

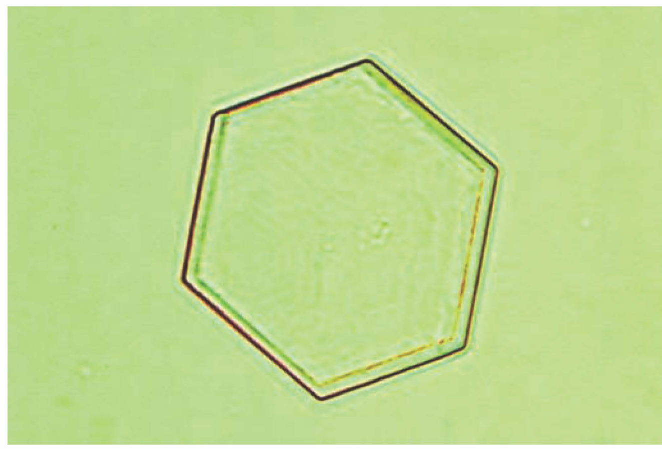

Figure 9-31. Cystine crystal (1,000×).

sulfate crystals are rarely seen in the urine and they have no clinical significance.

Cystine Crystals

Cystine crystals are colorless, refractile, hexagonal plates with equal or unequal sides (Fig. 9-31). They may appear singly, on top of each other (resembling a spiral staircase), or in clusters. Cystine crystals frequently have a layered or laminated appearance (Fig. 9-32).

Cystine crystals are insoluble in acetic acid, alcohol, acetone, ether, and boiling water. They are soluble in hydrochloric acid and alkali, especially ammonia. Solubility in ammonia helps differentiate cystine from colorless, six-sided uric acid crystals. Cystine can be detected chemically with the sodium cyanide–sodium nitroprusside test (Appendix B).

The presence of cystine crystals in the urine is always important. They occur in patients with either congenital cystinosis or congenital cystinuria. Cystine crystals can form calculi. Disorders of cysteine metabolism are discussed in the Chapter 3.

Figure 9-30. Sodium urate crystals. These needle-like crystals are not pointed at the ends (400×).

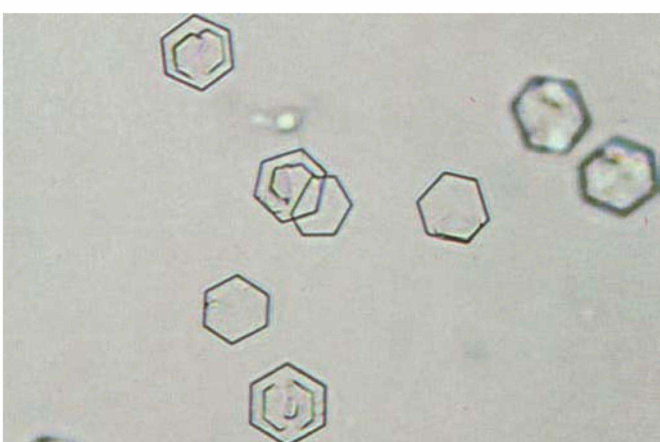

Figure 9-32. Cystine crystals. Several have laminated surfaces (160×).

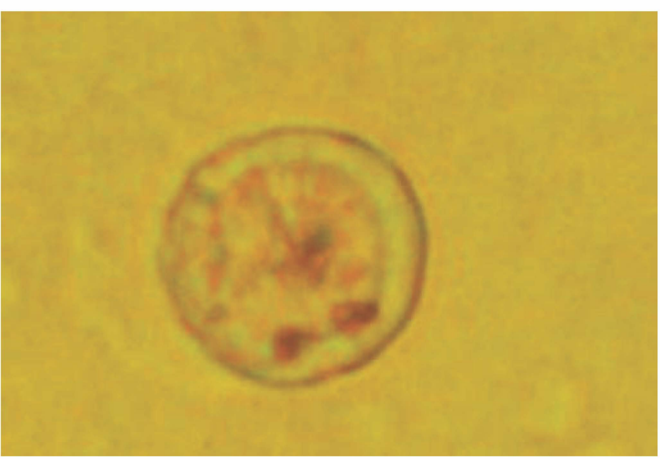

Figure 9-33. Leucine spheroid. Note the radiating and concentric striations as well as the brown coloring typical of these crystals.

Leucine

Leucine crystals are oily, highly retractile, yellow or brown spheroids with radial and concentric striations, which often resemble the sections of a grapefruit surrounded by a thick rind (Fig. 9-33). These spheroids are probably not pure leucine, because pure leucine crystallizes out in the form of plates.

Leucine is soluble in hot acetic acid, hot alcohol, and in alkali, but insoluble in hydrochloric acid. Under polarized light leucine spheroids produce a Maltese-cross pattern with the outer rim appearing as crushed diamonds (Fig. 9-34).

Leucine crystals are clinically very significant. They are found in the urine of patients with maple syrup urine disease, oasthouse urine disease, and in serious liver disease such as terminal cirrhosis of the liver, severe viral hepatitis, and acute yellow atrophy of the liver. Leucine and tyrosine crystals are frequently present together in the urine of patients with liver disease.

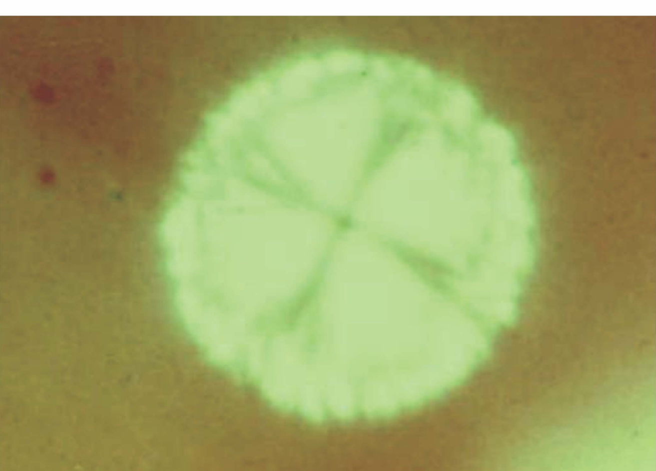

Figure 9-34. Leucine spheroid under polarized light. Notice the Maltese cross. (Modified from The Roche Historical Collection and Archive.)

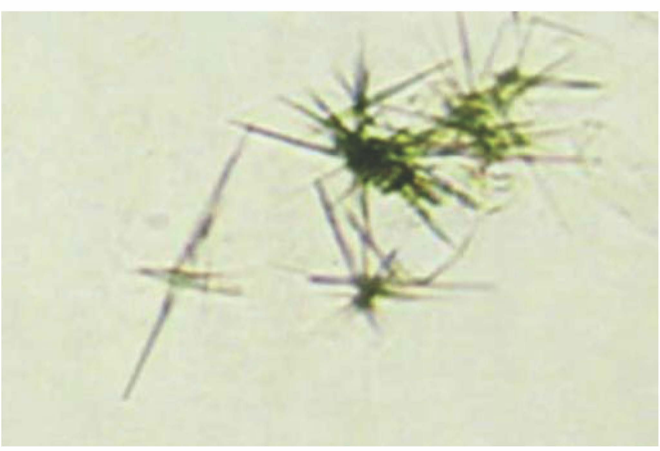

Figure 9-35. Tyrosine crystals (160×).

Tyrosine

Tyrosine crystals are very fine, highly refractile needles occurring in sheaves or clusters (Figs. 9-35). The needle clusters of tyrosine often appear to be black, especially in the center, but they may take on a yellow color in the presence of bilirubin. Tyrosine crystals polarize light (Fig. 9-36) and are soluble in ammonium hydroxide and hydrochloric acid but insoluble in acetic acid. Tyrosine crystals can be seen in tyrosinosis and oasthouse urine disease. Disorders of tyrosine metabolism are discussed in the Chapter 3.

Cholesterol

Cholesterol crystals are large, flat, transparent plates with notched corners (Figs. 9-37 and 9-38). Under polarized light they exhibit a variety of colors. They are soluble in chloroform, ether, and hot alcohol. At times, cholesterol crystals, as well as globules of esterified cholesterol, may be found as a

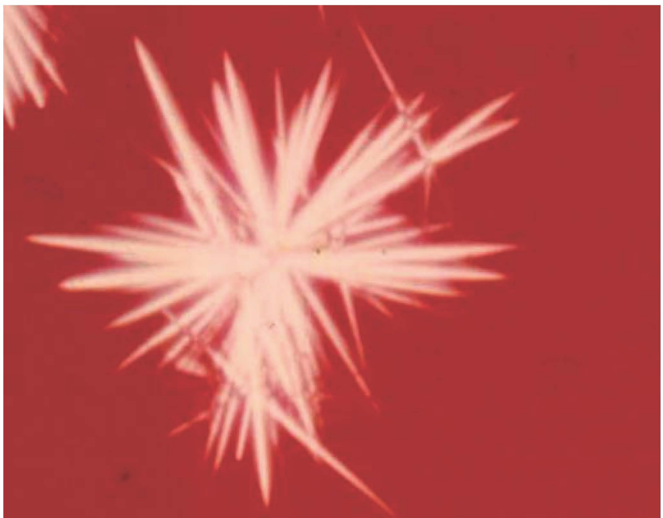

Figure 9-36. Tyrosine crystals under polarized light. (Modified from The Roche Historical Collection and Archive.)

Figure 9-37. Cholesterol crystal with typical notched edges (250×).

film on the surface of the urine instead of in the sediment. Esterified cholesterol will polarize light into a Maltese-cross formation (Fig. 9-39).

The presence of cholesterol plates in the urine indicates excessive tissue breakdown, and these crystals are seen in nephritis and nephritic conditions. They may also be present in chyluria, which is the result of either thoracic or abdominal obstruction to lymph drainage, thereby causing rupture of the lymphatic vessels into the renal pelvis or urinary tract. Some of the causes of obstruction to the lymphatic flow include tumors, gross enlargement of the abdominal lymph nodes, and filariasis.

Bilirubin Crystals

In some cases of bilirubinuria, the bilirubin may crystallize out in acidic urine as red or reddish-brown needles or granules (Fig. 9-40). Bilirubin crystals are readily soluble in chloroform, acetone, acid, and alkali but are insoluble in alcohol and ether. These crystals are of no more significance than the fact that bilirubin is present in the urine.

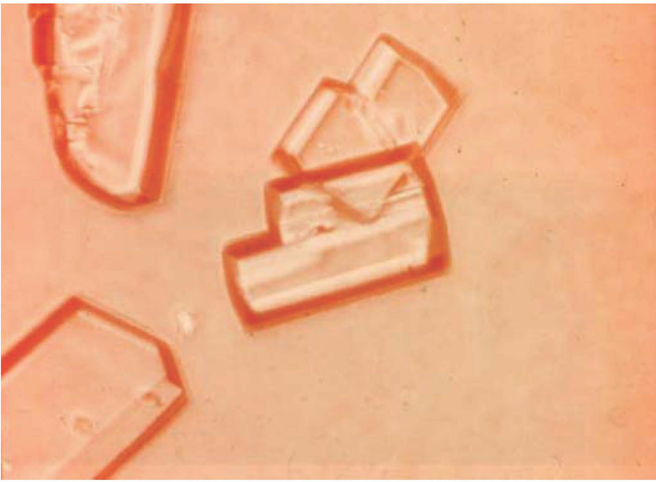

Figure 9-38. Cholesterol crystal DIT microscopy. (Modified from The Roche Historical Collection and Archive.)

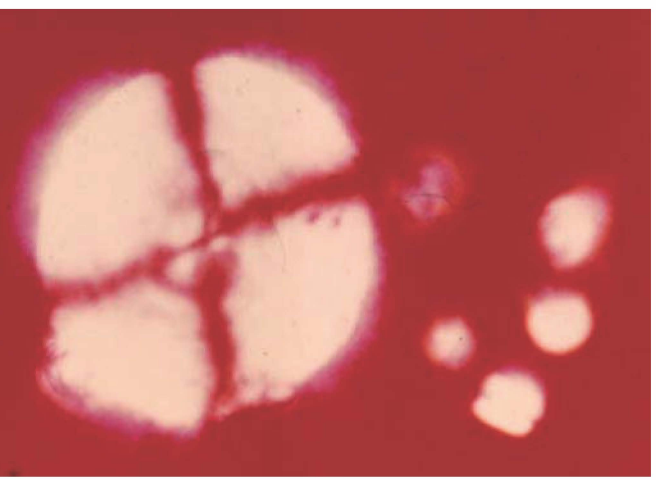

Figure 9-39. Esterified cholesterol polarizes light into a Maltese cross. Polarized light. (Modified from The Roche Historical Collection and Archive.)

Acidic Urine Crystals of Iatrogenic Origin

The administration of drugs can occasionally result in the formation of crystals that can be found in acid urine, if administered in very large doses. These include antibiotics, anti-inflammatory drugs and other drugs such as sulfonamides. Intravenous administration of radiographic dyes may also result in urine crystal formation. Ingestion of extremely high amounts of supplements such as Vitamin C or caffeine can occasionally result in urine crystal formation.

Sulfonamide Drug Crystals

When **sulfonamide drugs** were first introduced, there were many problems with renal damage resulting from the precipitation of the drug. The newer sulfa drugs are much more soluble, even in an acid environment, and so now they rarely form crystals in the urine.

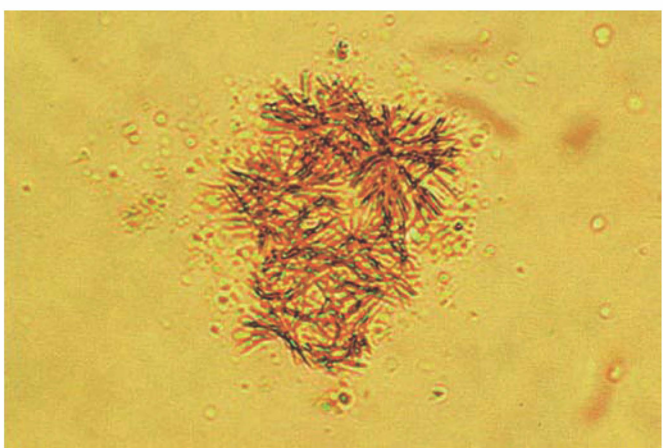

Figure 9-40. Bilirubin crystals (500×).

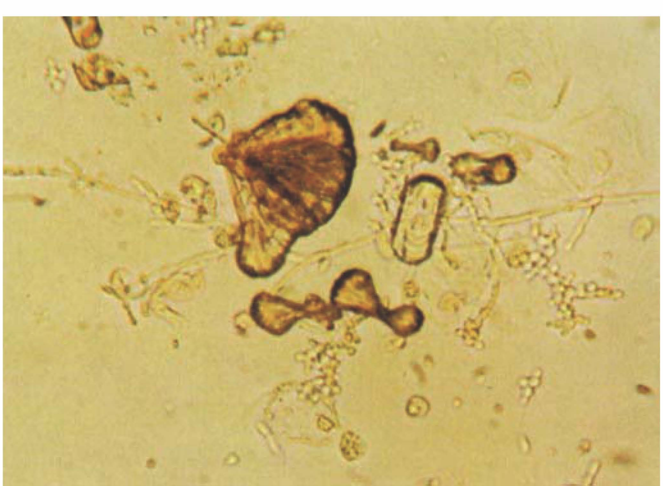

Figure 9-41. Sulfonamide crystals, yeast, and WBCs. This photograph demonstrates two typical formations of sulfa crystals: the fan or sheaf formation and sheaves with eccentric bindings (400×).

Most of the sulfonamide drugs precipitate out as sheaves of needles, usually with eccentric binding, and they may be clear or brown in color (Fig. 9-41). Two steps should be followed to confirm the presence of sulfa crystals. First, if possible, contact the nursing station (if the urine is from an inpatient) to verify that the patient is taking sulfa medication. An older test to help identify sulfonamides is the lignin test (Appendix B). Sulfonamide crystals are soluble in acetone.

Radiographic Dye Crystals

Radiographic dyes include Hypaque (Fig. 9-42) and Renografin (Fig. 9-43). Both dyes are diatrizoate meglumine plus diatrizoate sodium and can crystallize out in acidic urine following intravenous injection of the drug for x-ray studies. Both of these dyes crystalize out as pleomorphic needles that can occur singly or in sheaves. These needles may be quite large, are often seen with brown spheres. Radiographic dye crystals and will polarize light (Fig. 9-44).

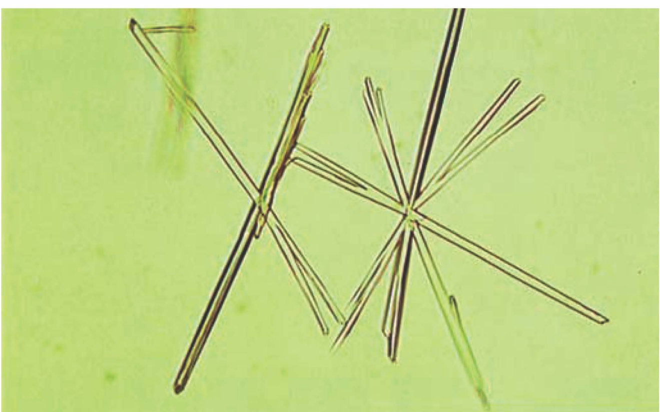

Figure 9-43. X-ray dye crystals: Renografin (400×).

Radiographic dyes are very dense, and, when present in the urine, will result in elevated specific gravity. The presence of needle crystals in a urine with a grossly elevated specific gravity (often >1.050) is usually indicative of x-ray dye. Radiographic dyes may be present in the urine for up to 3 days following injection.

Other Substances

Most substances are not ingested at a high enough dose to produce urinary crystals. However, occasionally they are and can form interesting crystals that may not be identifiable. In these cases, laboratory personnel may need to report them as unidentified crystals seen. Examples of these rare finds include aspirin (Figs. 9-45 and 9-46), acetophenetidin (Fig. 9-47), ascorbic acid (Fig. 9-48), and caffeine (Fig. 9-49).

ALKALINE URINE

Those crystals which can be found in alkaline urine include triple phosphates (ammonium magnesium phosphates), amorphous phosphates, calcium carbonate, calcium phosphate, and ammonium biurates, also called ammonium urates (Fig. 9-50).

Figure 9-42. X-ray dye crystals: Hypaque (160×).

Figure 9-44. X-ray dye crystals may polarized light (160×).

Figure 9-45. Aspirin crystals—prismatic form. (Modified from The Roche Historical Collection and Archive.)

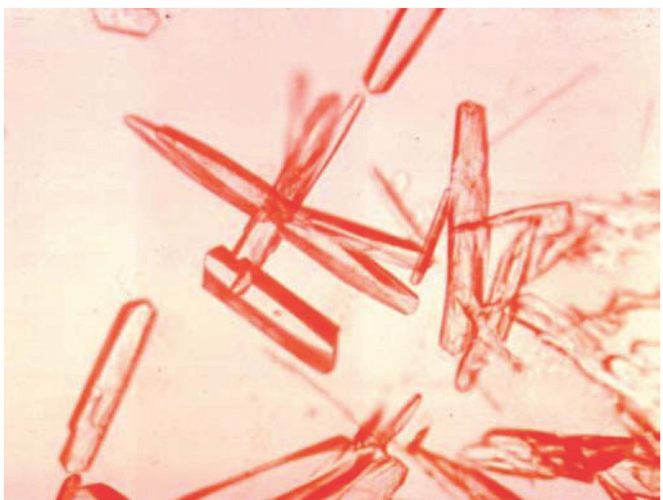

Figure 9-46. Aspirin crystals—stellate form. (Modified from The Roche Historical Collection and Archive.)

Figure 9-47. Acetophenetidin—stellate or "fir tree" forms. (Modified from The Roche Historical Collection and Archive.)

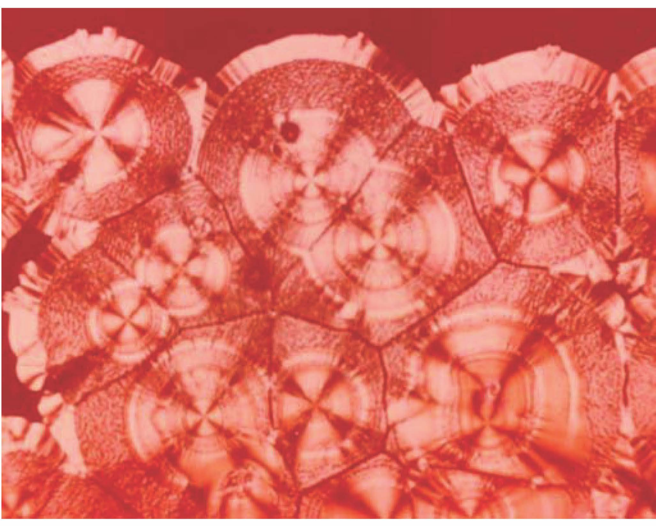

Figure 9-48. Ascorbic acid-spherulites (polarized light). (Modified from The Roche Historical Collection and Archive.)

Triple Phosphates

Triple phosphate (ammonium magnesium phosphate) crystals can be present in neutral and alkaline urines. Triple phosphate crystals are colorless prisms with from three to six sides that frequently have oblique ends (Fig. 9-51). Triple phosphate crystals have been described as resembling coffin lids or picture frames. Triple phosphates may sometimes precipitate as feathery or fernlike crystals. Both forms polarize light. Triple phosphate crystals are soluble in acetic acid.

Triple phosphate crystals are frequently found in normal urine but can also form urinary calculi. Pathologic conditions in which they may be found include chronic pyelitis, chronic cystitis, enlarged prostate. Triple phosphate crystals can also form when the urine is retained in the bladder.

Amorphous Phosphates

Amorphous phosphates are noncrystalline salts frequently present in urine (Fig. 9-52). These granular particles have no

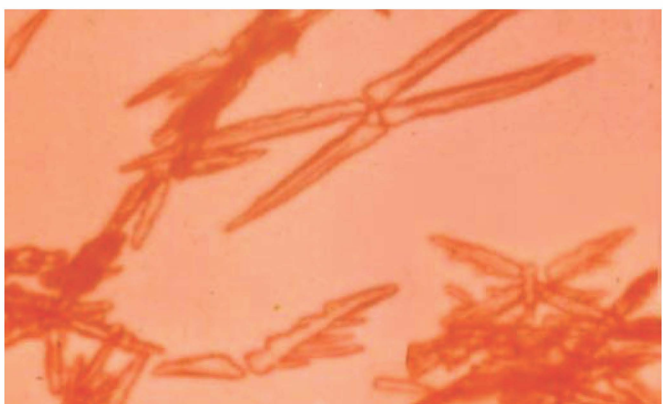

Figure 9-49. Caffeine crystals (bright field) (Modified from The Roche Historical Collection and Archive.)

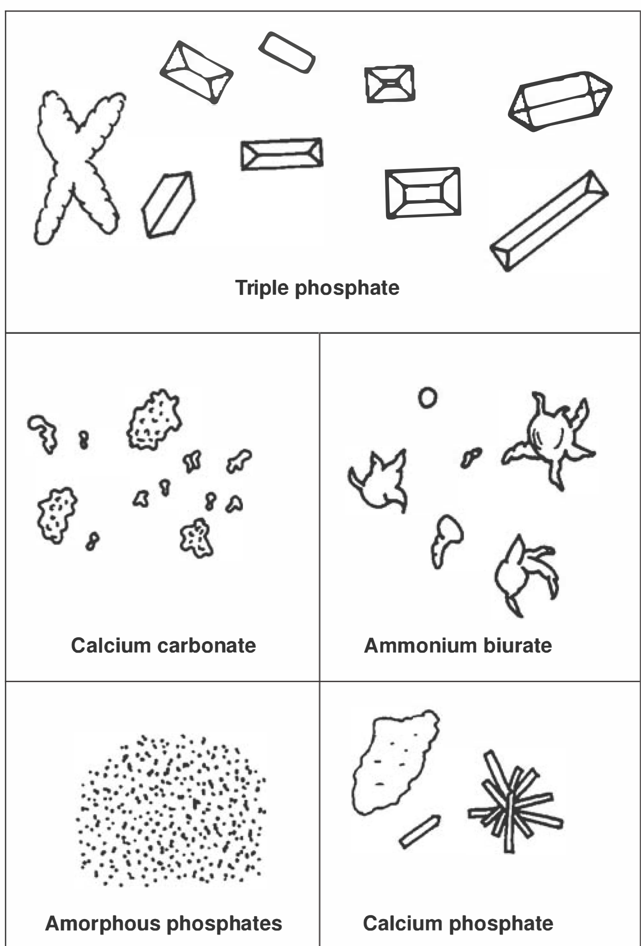

Figure 9-50. Crystals found in alkaline urine.

definite shape and they are usually visibly indistinguishable from amorphous urates. The pH of the urine helps distinguish between these two amorphous deposits as well as does their solubility properties. Amorphous phosphates are soluble in acetic acid, whereas amorphous urates are insoluble. Amorphous phosphates have no clinical significance.

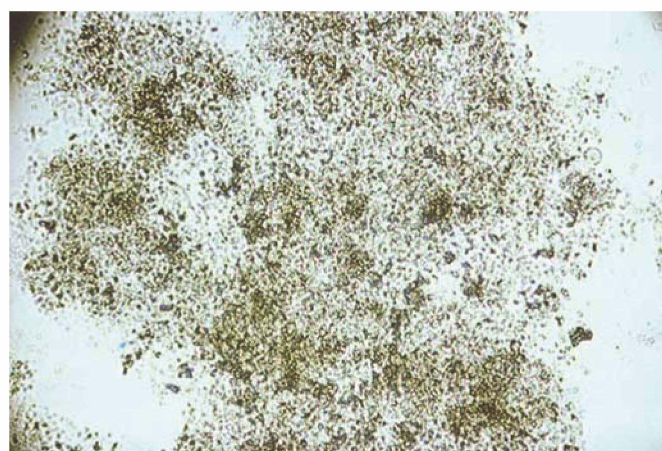

Figure 9-52. Amorphous phosphate (400×).

Calcium Carbonate

Calcium carbonate crystals are small, colorless crystals appearing in dumbbell or spherical forms and rarely rhombohedral forms (Fig. 9-53), or in large granular masses (Fig. 9-54). Calcium carbonate crystals are larger than amorphous and, when in clumps, they may appear to have a dark color. The mass of calcium carbonate crystals, as opposed to a clump of amorphous phosphates, will also be connected together around the edges.

Calcium carbonate crystals will dissolve in acetic acid with the resulting evolution of carbon dioxide gas. Calcium carbonate crystals have no clinical significance.

Calcium Phosphate

Calcium phosphate crystals are long, thin, colorless prisms and can have one pointed end, be arranged as rosettes or stars (stellar phosphates), or appear as needles (Fig. 9-55). Calcium phosphate crystals may also form large, thin, irregular plates that may float on the surface of the urine (Fig. 9-56). Calcium

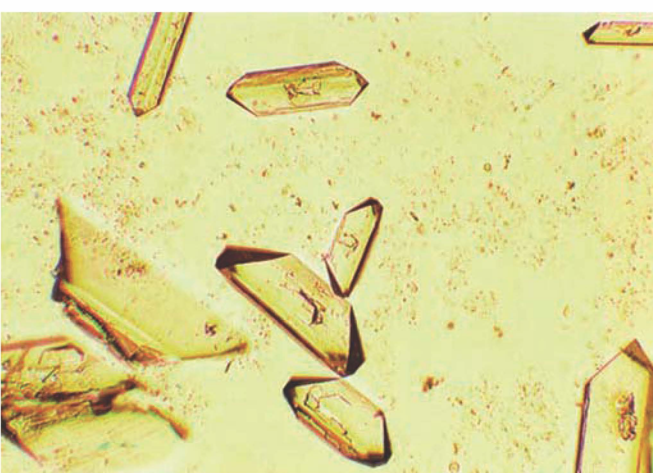

Figure 9-51. Triple phosphate crystals. Note the oblique ends of the prisms (200×).

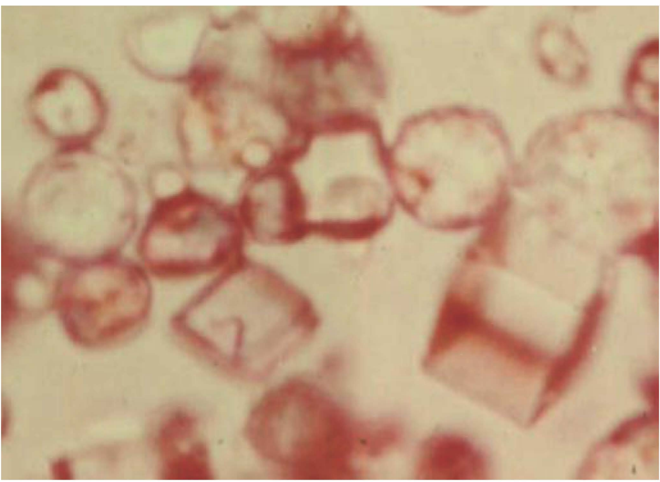

Figure 9-53. Calcium carbonate crystals, spheroid and rhombohedral forms. (Modified from The Roche Historical Collection and Archive.)

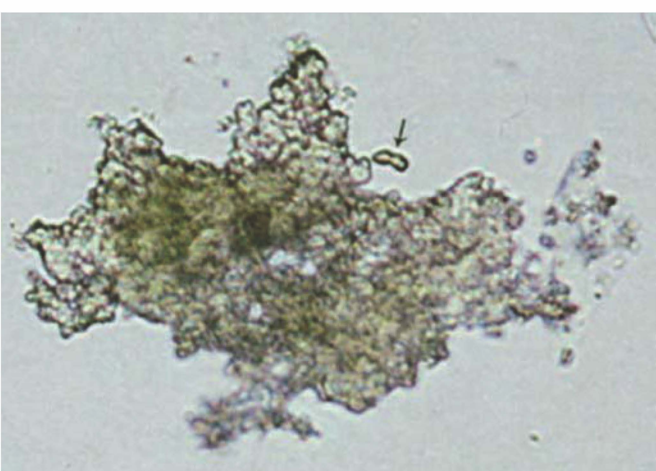

Figure 9-54. Calcium carbonate crystals. The *small arrow* points out the typical "dumbbell" form which is next to a large mass of calcium carbonate crystals (400×).

Figure 9-55. Calcium phosphate crystals (400×).

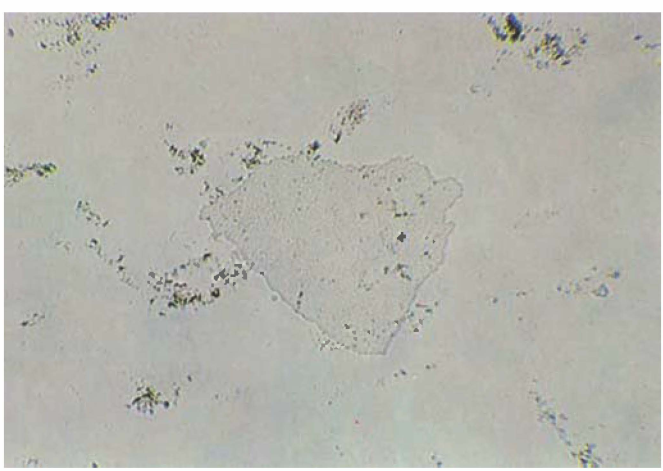

Figure 9-56. Calcium phosphate plate or phosphate sheath (200×).

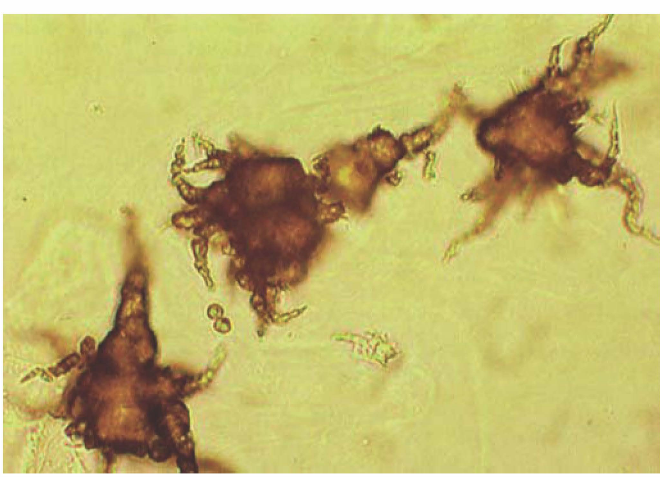

Figure 9-57. Ammonium biurate crystals (500×).

phosphate crystals are soluble in dilute acetic acid. These crystals may be present in normal urine, but they may also form calculi.

Ammonium Biurates

Ammonium biurate crystals, also referred to as ammonium urates, are found in alkaline and neutral urine. However, they may occasionally be found in acidic urine. Ammonium biurates are yellow-brown spherical bodies with long, irregular spicules (Fig. 9-57). Their appearance is often described with the term "thorn apple." Ammonium biurates may also occur as yellow-brown spheroids without spicules (Fig. 9-58) although this form is not so common.

Ammonium biurates dissolve by warming and are soluble in acetic acid, with the formation of colorless uric acid crystals after standing. The addition of sodium hydroxide will liberate ammonia. Ammonium biurates are abnormal only if found in freshly voided urine.

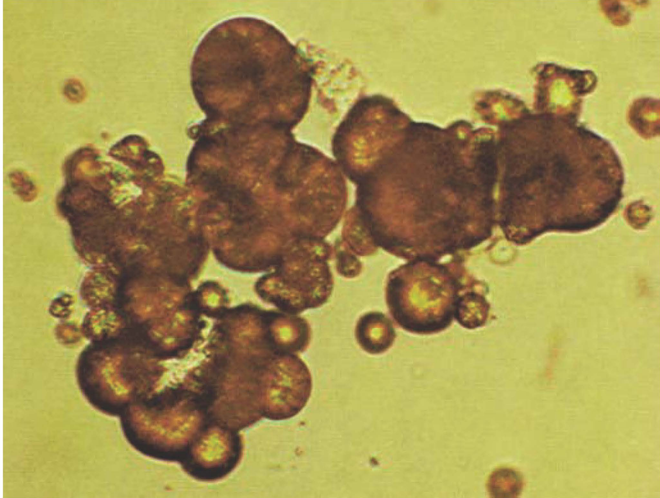

Figure 9-58. Ammonium biurate crystals without spicules (500×).

Casts

The presence of protein detected by urine chemistry methods may indicate the presence of casts, but casts are not always present when protein is detected and may also be present in small enough numbers as to not be detected by these methods. Therefore, microscopic evaluation of urine is important for detection of casts.

Urinary casts are formed in the lumen of the tubules of the kidney. They are so named because they are molded in the tubules. The renal tubules secrete a mucoprotein **Tamm–Horsfall protein**, which is believed to form the basic matrix of casts. Casts can form as the result of the precipitation or gelation of Tamm–Horsfall mucoprotein, the clumping of cells or other material within a protein matrix, the adherence of cells or material to the matrix, or by conglutination of material within the matrix. Some casts may also contain serum proteins but they are usually confined to the cast granules. In waxy casts, serum proteins are present in a homogeneous distribution.

Factors that are involved in cast formation include urinary stasis (marked decrease in urine flow), increased acidity, high solute concentration, and the presence of abnormal ionic or protein constituents. Cast formation usually takes place in the distal and collecting tubules because there the urine reaches its maximum concentration and acidification. Casts will dissolve in alkaline urine and in neutral urine having a specific gravity of 1.003 or less. The presence of casts in the urine is frequently accompanied by proteinuria. However, casts can be seen in the absence of protein, making microscopic examination of urine an important tool in the diction of casts.

Casts have nearly parallel sides and rounded or blunted ends, and they vary in size and shape according to the tubules in which they were formed. They may be convoluted, straight, or curved, and they may vary in length. The width of the cast indicates the diameter of the tubule responsible for its formation. Broad casts, which can be from two to six times wider than ordinary casts, are formed either in pathologically dilated or atrophied tubules or in collecting tubules. Broad casts are frequently referred to as renal failure casts.

Casts are always renal in origin, and they are important indicators of intrinsic renal disease. Disorders in which cast maybe present include glomerular damage, tubular damage, renal inflammation, and renal infection. Classification of casts is made on the basis of their appearance and the cellular components that they contain. The different types of casts are hyaline, red cell, white cell, epithelial cell, granular (coarse and fine), waxy, and fatty.

At times, it may be difficult to distinguish the various casts because of degeneration, or because the cast may contain a variety of structures (mixed casts). It has been proposed that as cellular casts degenerate they form granular casts that in turn degenerate, forming waxy casts (Fig. 9-59).

Casts are cylindrical in shape and do not have dark edges. Occasionally, waxy casts may appear to have a thin dark edge but only because the shiny surface of the cast comes to an abrupt ending. Usually, this thin dark edge will disappear when the fine adjustment is turned slightly. Any structure, therefore, that has dark edges is most likely a piece of fiber. In addition, any structure with parallel sides that is flat in the middle with thick edges is probably also a fiber. Remember, renal tubules are round, so casts will be more or less circular and will be thicker in the middle.

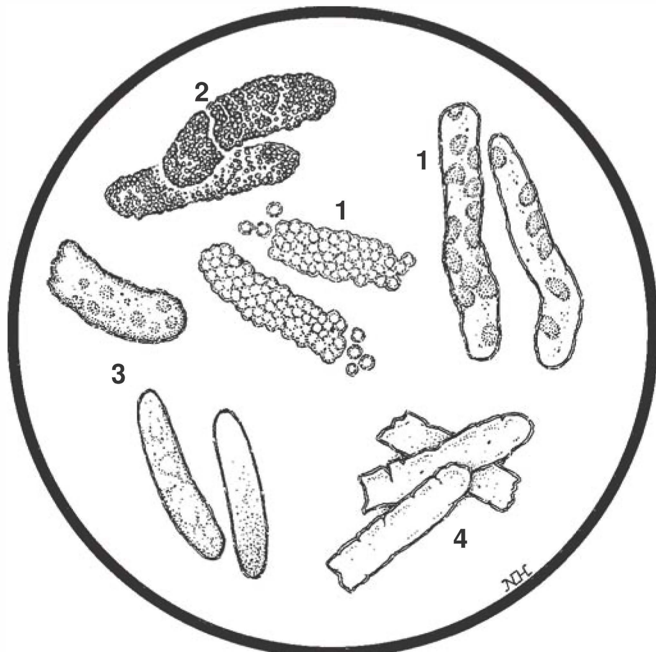

Figure 9-59. Sequence of urinary cast degeneration: (1) cellular casts; (2) coarse granular cast; (3) fine granular cast; (4) waxy cast. (Courtesy of Neil O. Hardy, Westpoint, CT.)

Casts are reported according to type and the number that is present per low-power field (100×). Ranges reported are usually none seen, 0–2, 2–5, 5–10, 10–20/LPF.

HYALINE CASTS

Hyaline casts are the most frequently occurring casts in urine. They may show positive protein reagent strip reaction and may contain some inclusions which were incorporated while in the kidney. Because they are composed of only protein, they have a very low refractive index and must be viewed under low light. They are colorless, homogeneous, and transparent, and usually have rounded ends (Fig. 9-60), and a smooth surface (Fig. 9-61). Hyaline casts can be seen in even the mildest kind of renal disease and are not associated with any one disease in particular. A few hyaline casts may be found in the normal urine, and increased amounts are frequently present following physical exercise and physiologic dehydration.

RED BLOOD CELL CASTS

Red blood cell casts mean renal hematuria and they are always pathologic. They are usually diagnostic of glomerular

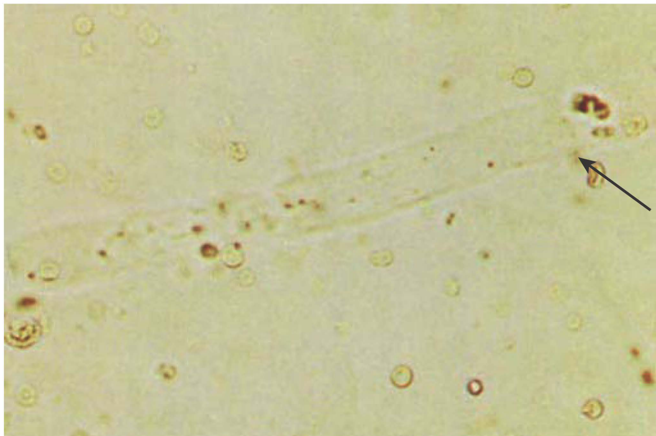

Figure 9-60. Hyaline cast and red blood cells. Note the low refractive index of the cast (400×).

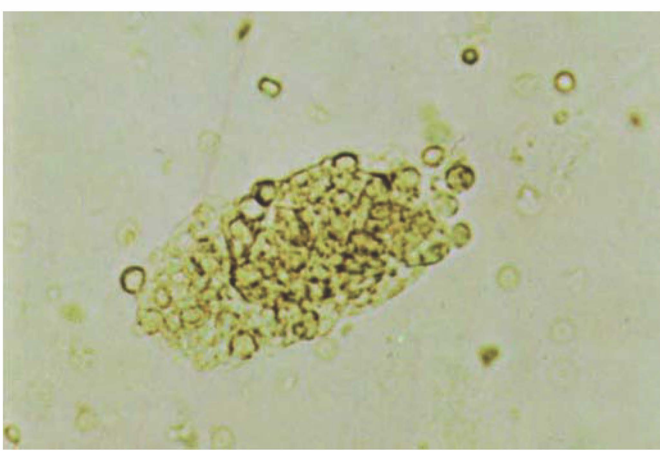

Figure 9-62. Red cell cast and RBCs (400×).

disease being found in acute glomerulonephritis, lupus nephritis, Goodpasture syndrome, subacute bacterial endocarditis, and renal trauma. Red cell casts can also be present in renal infarction, severe pyelonephritis, right-sided congestive heart failure, renal vein thrombosis, and periarteritis nodosa.

Red blood cell casts may appear brown to almost colorless (Fig. 9-62). The cast may contain only a few RBCs in a protein matrix, or there may be many cells packed close together with no visible matrix. If the red cells are still intact and the outlines are still detectable, then the cast is termed a red cell cast. If the cast has degenerated to a reddish-brown granular cast, then the cast is a hemoglobin or blood cast. Figure 9-63 shows red blood cell cast using DIT microscopy.

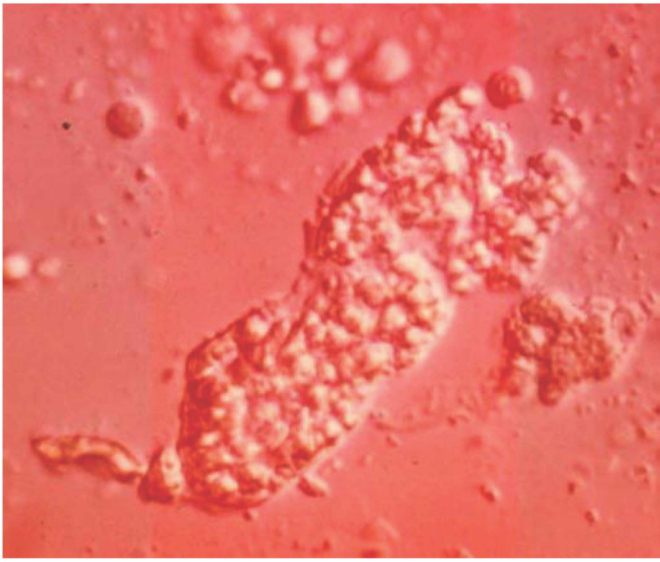

Figure 9-63. Red cell cast and RBCs (DIT microscopy) (400×). (Modified from The Roche Historical Collection and Archive.)

WHITE BLOOD CELL CASTS

White blood cell casts are present in renal infection and in noninfectious inflammation, such as acute pyelonephritis, interstitial nephritis, and lupus nephritis. They may also be present in glomerular disease. The majority of white blood cells that appear in casts are polymorphonuclear neutrophils; and may be few in number, or there may be many cells tightly packed together (Fig. 9-64). If the cells are still intact,

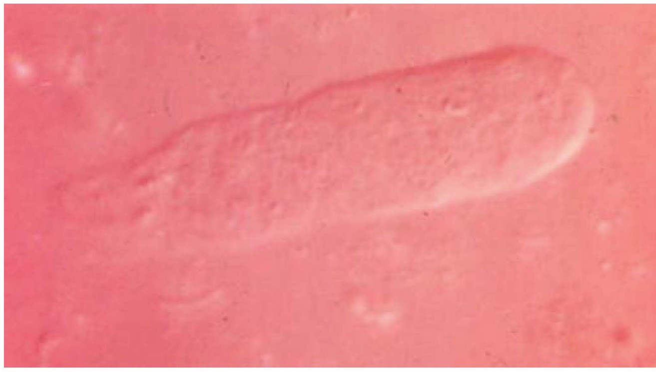

Figure 9-61. Hyaline cast. (DIT microscopy) (600×). (Modified from The Roche Historical Collection and Archive.)

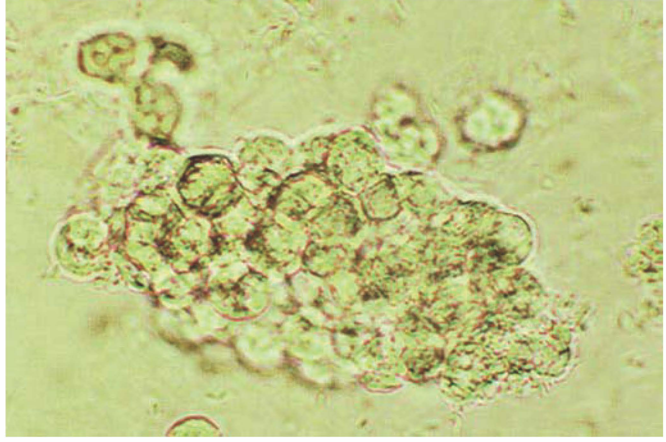

Figure 9-64. White cell cast and WBCs (500×).

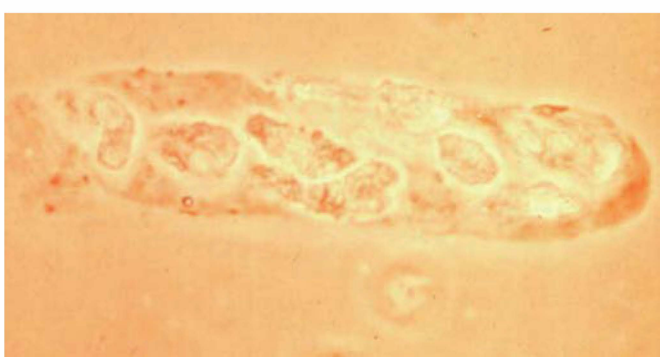

Figure 9-65. Epithelial cell cast. (Phase) (800×). (Modified from The Roche Historical Collection and Archive.)

the nuclei may be clearly visible, but disappear, as the cast becomes granular in appearance.

EPITHELIAL CELL CASTS

Epithelial cell casts form as the result of stasis and the desquamation of renal tubular epithelial cells. These casts are only rarely seen in the urine because of the infrequent occurrence of renal diseases which primarily affect the tubules (necrosis). Epithelial cell casts degeneration (Figs. 9-65 and 9-66) may be present in urine after exposure to nephrotoxic agents or viruses (e.g., cytomegalovirus, hepatitis virus), which cause damage that accompanies glomerular injury, and in the rejection of a kidney allograft.

The epithelial cells may either be arranged in parallel rows in the cast or may be arranged haphazardly and vary in size, shape, and stage of degeneration. The cells in the former type of arrangement are believed to come from the same segment of the tubule, whereas the irregular arrangement seems to indicate that the cells came from different portions of the tubule.

GRANULAR CASTS

Granular casts may be the result of the degeneration of cellular casts or they may represent the direct aggregation of

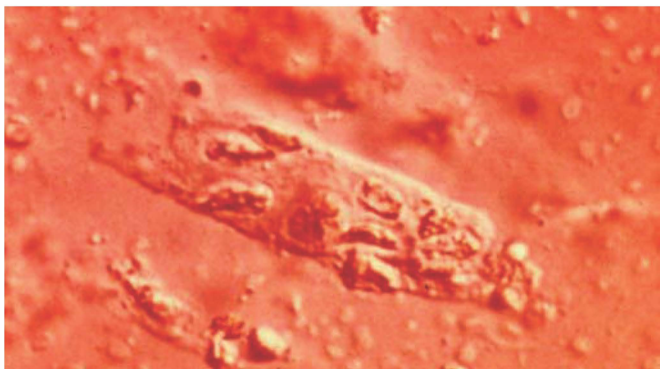

Figure 9-66. Epithelial cell cast. (DIT microscopy) (800×). (Modified from The Roche Historical Collection and Archive).

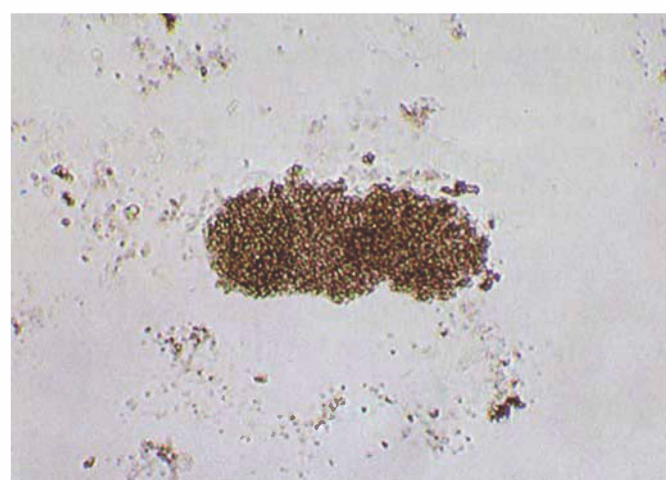

Figure 9-67. Coarsely granular cast (200×).

serum proteins into a matrix of Tamm–Horsfall mucoprotein. Initially, the granules are large and coarse, but when urine stasis is prolonged, these granules break down to fine granules. Granular casts almost always indicate significant renal disease, however, granular casts may be present in the urine for a short time following strenuous exercise.

Determining whether a cast is coarsely or finely granular is of no clinical significance, although the distinction is not hard to make. Coarsely granular casts contain larger granules that are darker in color and often appear black because of the density of the granules (Fig. 9-67). Finely granular casts contain fine granules which may appear gray or pale yellow in color (Fig. 9-68).

WAXY CASTS

Waxy casts have a very high refractive index, are yellow, gray, or colorless, and have a smooth homogeneous appearance (Fig. 9-69). Waxy casts frequently occur as short broad casts with blunt or broken ends, and they often have cracked or serrated edges (Fig. 9-70). It has been postulated that waxy casts result from the degeneration of granular casts. Waxy casts are

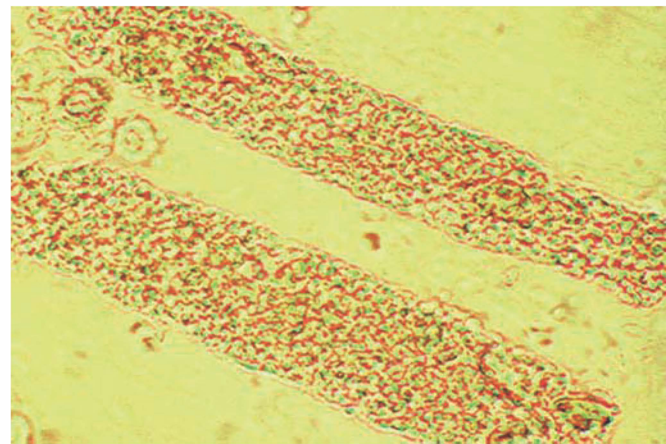

Figure 9-68. Finely granular casts. Note the RBCs between the two casts (500×).

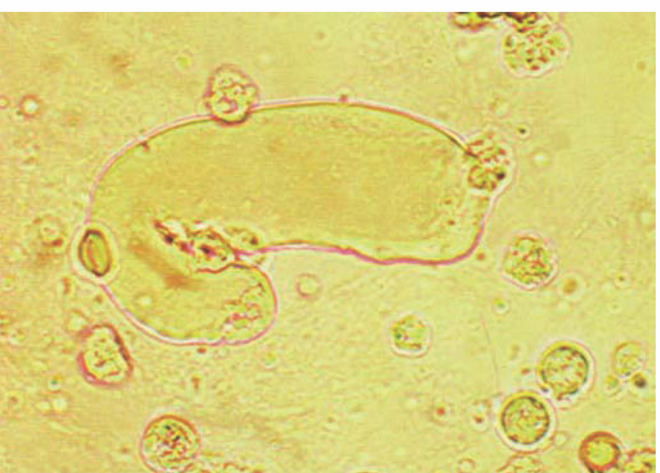

Figure 9-69. Waxy cast, WBCs, and bacteria (400×).

found in patients with severe chronic renal failure, malignant hypertension, renal amyloidosis, and diabetic nephropathy. They may also be found in acute renal disease, tubular inflammation and degeneration, and during renal allograft rejection.

Fatty Casts

Fatty casts are casts which have incorporated either free fat droplets or oval fat bodies (refer to the section on Oval Fat Bodies). These casts may contain only a few fat droplets, or the cast may be composed almost entirely of fat droplets of various sizes. Figure 9-71 shows a typical fatty cast with large fat droplets in half of the cast and smaller yellow-brown droplets in the other half. If the fat is cholesterol, the droplets will be anisotropic, and under polarized light will demonstrate a characteristic "Maltese-cross" formation (Fig. 9-72). Isotropic droplets, which consist of triglycerides, will not polarize but will stain with Sudan III or Oil Red O.

Fatty casts are seen when there is fatty degeneration of the tubular epithelium, as in degenerative tubular disease. They are frequently seen in the nephrotic syndrome and may occur in diabetic glomerulosclerosis, lipoid nephrosis, chronic glomerulonephritis, Kimmelstiel–Wilson syndrome, lupus, and toxic renal poisoning.

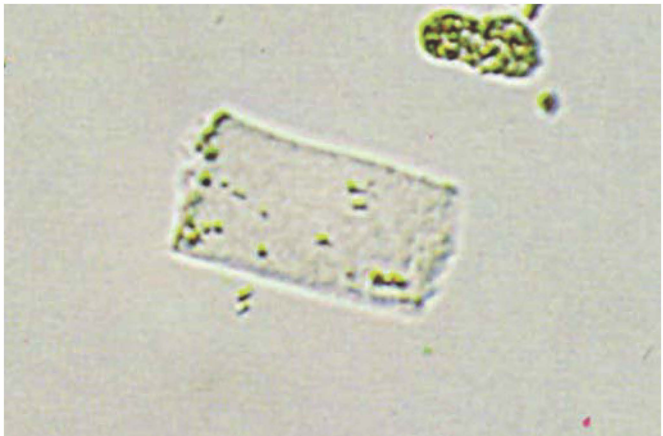

Figure 9-70. Waxy cast and WBCs (200×).

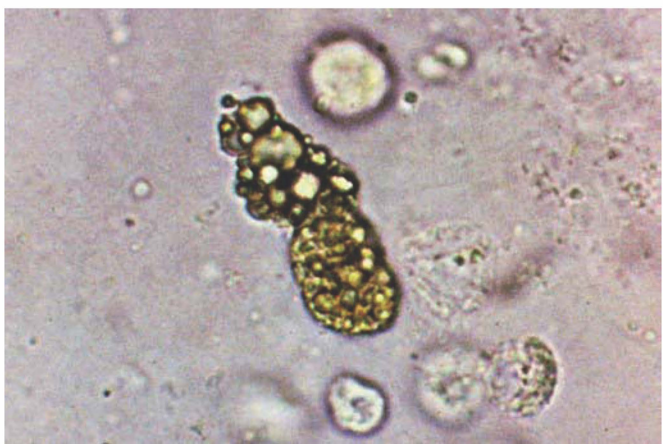

Figure 9-71. Fatty cast (400×).

Miscellaneous Structures

Chemical analysis does not detect most of these types of sediment. Microscopic evaluation of urinary sediment is important if these structures are to be detected.

OVAL FAT BODIES AND FREE FAT DROPLETS

Fat is not detected by chemical tests; therefore, microscopic examination for the detection of fat and oval fat bodies is necessary. Fat may be present in the urine as free droplets or globules, within degenerating or necrotic cells (**oval fat bodies**), or incorporated in a cast. Oval fat bodies are usually defined as being renal tubular cells which contain highly refractile fat droplets (Fig. 9-73). They are either the result of the incorporation

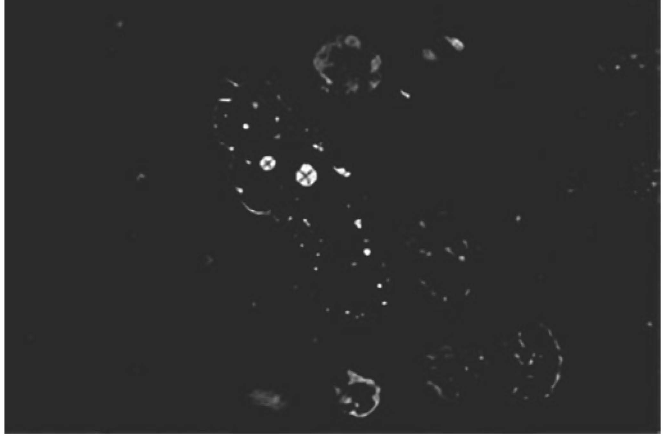

Figure 9-72. Fatty cast. (Polarization computer added) (400×).

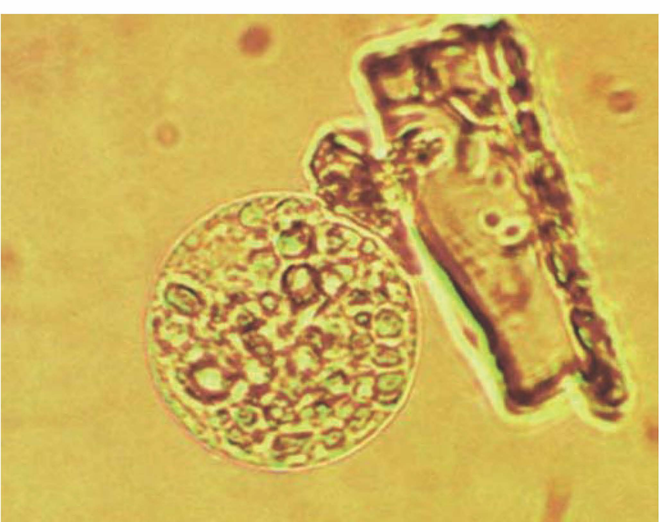

Figure 9-73. Oval fat body and fiber (500×).

of fat that has been filtered through the glomerulus or they are renal tubular cells which have undergone fatty degeneration. Oval fat bodies may also be macrophages or polymorphonuclear leukocytes that have either ingested lipids or degenerated cells, or have undergone fatty degeneration.

Lipids may also appear in the urine as free fat droplets (Fig. 9-74). These droplets frequently vary in size, since the fat globules can coalesce together. Fat droplets are highly refractile, are globular in shape, and frequently have a yellow-brown appearance, although under low power and under subdued light they may sometimes appear to be black because of their high refractive index. In lipiduria (the excretion of lipids into the urine), the free fat droplets may be found floating on the surface of the urine.

Free-floating fat, or fat incorporated in a cell or cast, is composed of cholesterol esters or free cholesterol. This form of fat is anisotropic and will form "Maltese crosses" under polarized light (Fig. 9-75), but they will not stain with fat stain. If they consist of triglycerides, or neutral fat, they will not polarize but will stain with Sudan III or Oil Red O (Fig. 9-76).

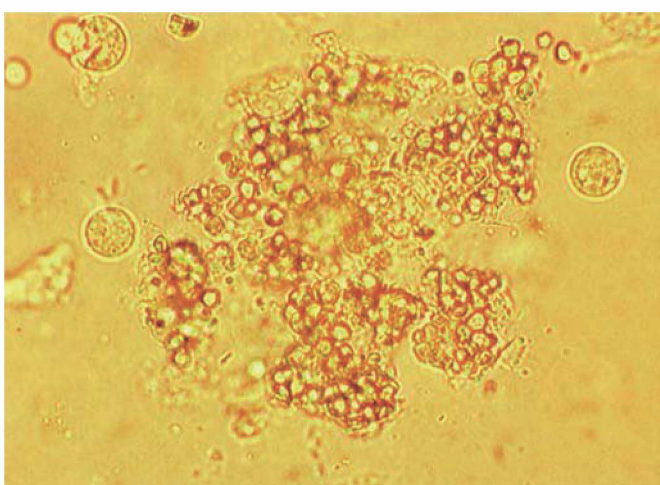

Figure 9-74. Fat droplets. Field also contains WBCs (500×).

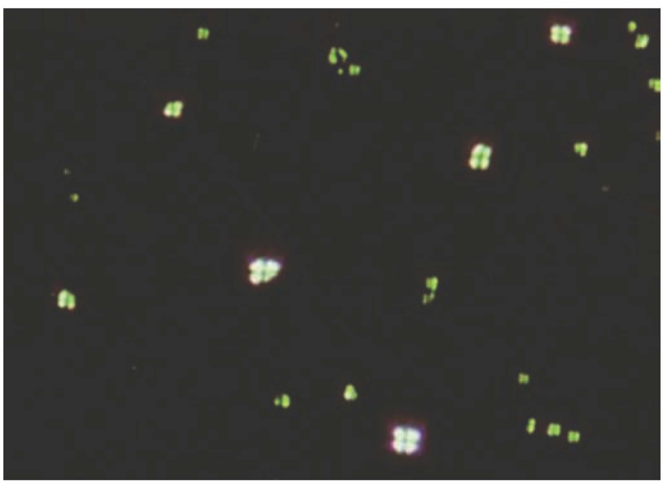

Figure 9-75. Polarized anisotropic fat droplets. Note the "Maltese-cross" formation (160×).

Anisotropic fat globules which manifest the "Maltese-cross" formation are termed "doubly refractile fat bodies". Fat may be present in the urine as the result of fatty degeneration of the tubules. It is frequently found in the nephrotic syndrome and may also be present in diabetes mellitus, eclampsia, toxic renal poisoning, chronic glomerulonephritis, lipoid nephrosis, fat embolism, and following extensive superficial injuries with crushing of the subcutaneous fat. Lipiduria may also occur following fractures of the major long bones or pelvis, and in multiple fractures in which fat may be released from the bone marrow into the circulation and then filtered through the glomerulus.

CYLINDROIDS

Cylindroids resemble casts but have one end which tapers out like a strand of mucus. The exact site and mechanism of their formation are not known, but since they usually occur in conjunction with casts, they are considered to have the same significance. Separate classification of cylindroids from that

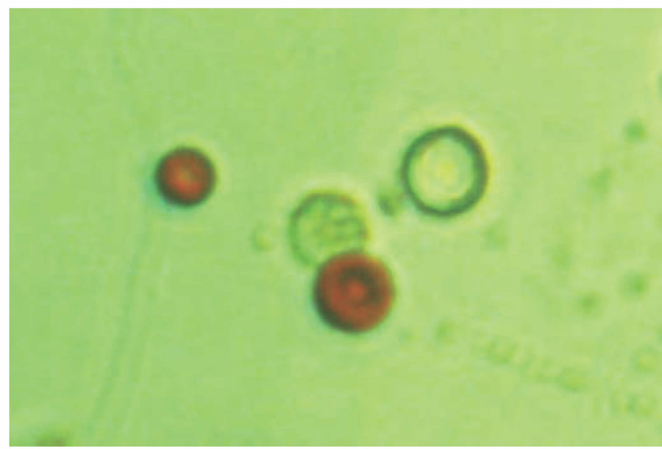

Figure 9-76. Neutral fats and triglycerides stain with Sudan III, while esterified cholesterol remains colorless (400×).

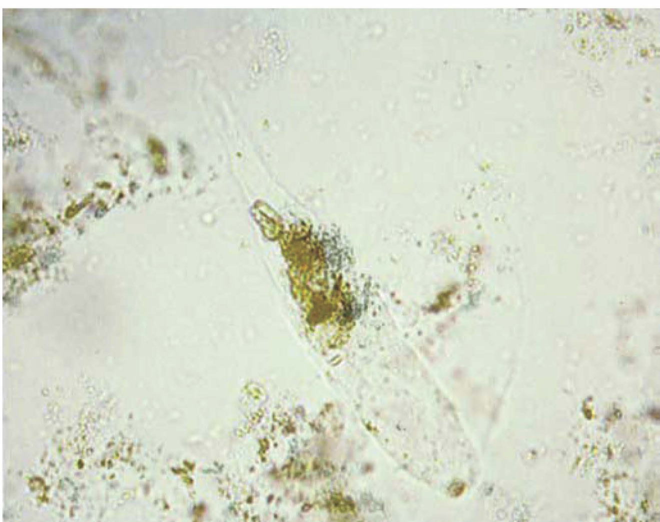

Figure 9-77. Cylindroid. Note the tapering tail (400×).

of casts is no longer needed. Cylindroids are frequently hyaline, but like the one pictured in Figure 9-77, they may also incorporate other material.

MUCOUS THREADS

Mucous threads are long, thin, wavy threads of ribbon-like structures which may show faint longitudinal striations (Fig. 9-78). Mucous threads are present in normal urine in small numbers, but they may be very abundant in the presence of inflammation or irritation of the urinary tract. Some of the wider threads may be confused with cylindroids or hyaline casts. Heavy mucous threads tend to incorporate WBCs.

Artifacts and Contaminants

Artifacts (objects not originating in the urinary tract) can find their way into the urine specimen during collection, transportation, chemical analysis or microscopic examination. It is important for the technologist to recognize these objects as being extraneous structures.

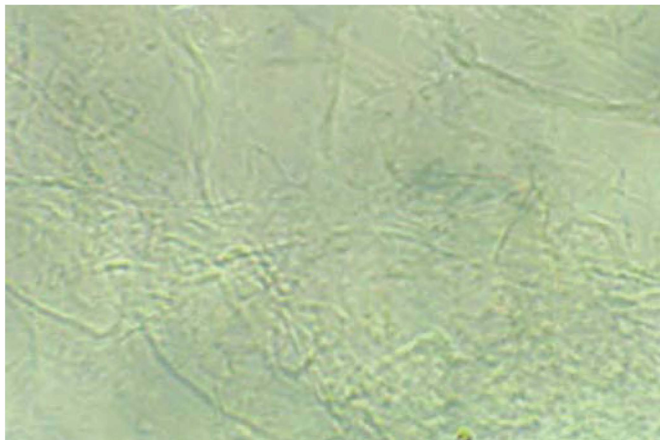

Figure 9-78. Mucus (100×).

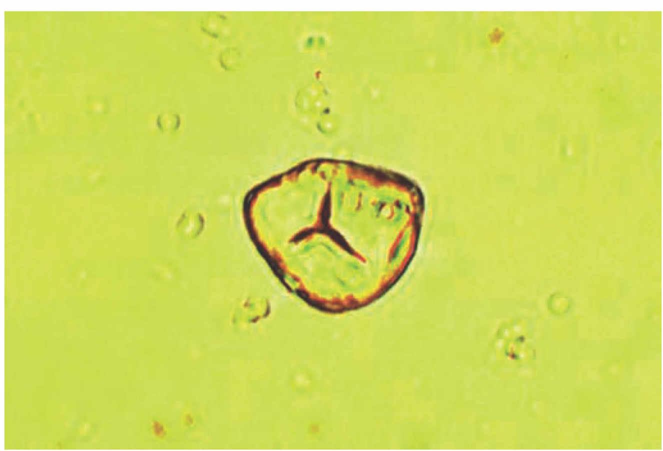

Figure 9-79. Starch crystal (500×).

STARCH CRYSTALS

Starch crystals can be found in urine. They are round or oval, are highly refractive, and vary in size. The most common type of starch which can be present in the urine is cornstarch, possibly because some brands of powder contain cornstarch. Cornstarch crystals are almost hexagonal in shape, and they contain an irregular indentation in the center (Fig. 9-79). Under polarized light these starch crystals will appear as "Maltese crosses" (Fig. 9-80). Anisotropic fat and starch are the only structures that will form these crosses under polarized light. Lycopodium is similar in appearance to cornstarch and is used as a dusting powder.

CLOTH FIBERS

Cloth fibers are undoubtedly the most frequently occurring type of artifact found in the urine. They may come

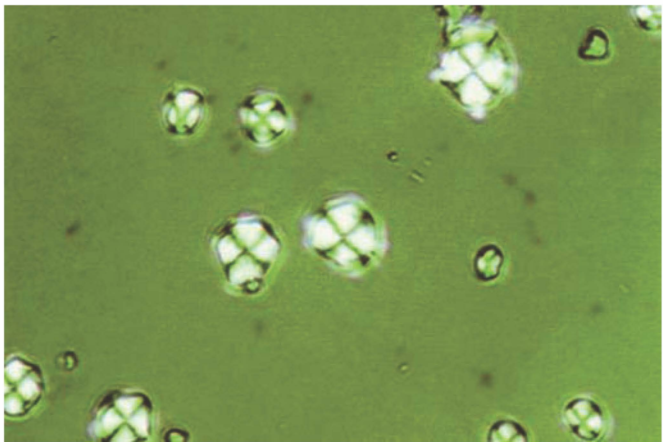

Figure 9-80. Starch crystals. (Polarized light). Note the "Maltese-cross" formation (400×).

Figure 9-81. Cloth fibers (160×).

from clothing, diapers, toilet paper, lens paper, or they may be pieces of lint from the air. Fibers which are long and flat are easily recognizable (Fig. 9-81). However, fibers those are short and are approximately the same size as casts can be mistaken for casts. This error can be avoided by exposing the technologist to the various types of fibers, because there are certain characteristics of the different fibers that can be easily recognized. One way to do this is to take a disposable diaper, cut out a small square, wet the section with water, squeeze it out into a test tube, and examine the sediment (Fig. 9-82).

Artifacts of this sort can be seen in sediment that is obtained by squeezing the urine out of a diaper (a method that should not be practiced). Disposable diapers contain many of the varieties of fibers that appear as contaminants in infant urine specimens. When observing fibers, a few characteristics can be readily noticeable. First of all, they usually have dark edges; casts do not have dark edges. Second, most of the fibers are flat; casts are cylindrical. The fiber in Figure 9-83 is frequently encountered in the urine

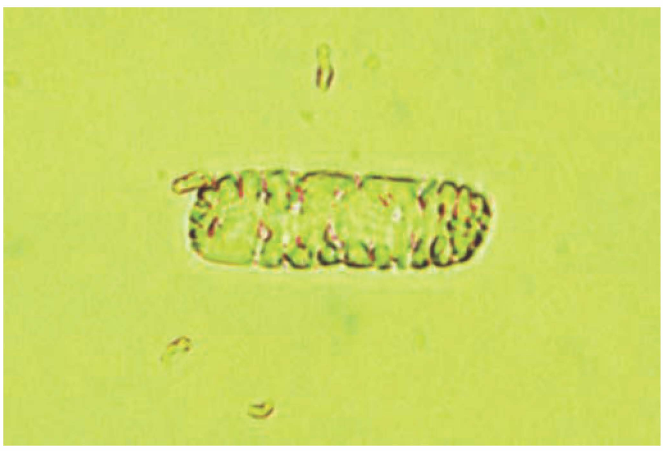

Figure 9-83. This fiber is a common contaminant (400×).

sediment but may be recognized by the thick, nodular edges and the nodular indentations on both ends of the fiber. This fiber is thicker on the edges than in the middle and is usually flat.

OIL DROPLETS

Oil droplets in the urine are the result of contamination from lubricants. Oil droplets may also be seen if immersion oil is present on the slide or coverslip. They are spherical and can vary in size (Fig. 9-84).

OTHER ARTIFACTS

Some of the other types of debris or extraneous material which may be found in the urinary sediment include hair (Fig. 9-85); glass fragments (Fig. 9-86), as well as scratches on the microscope slide; air bubbles (Fig. 9-87); pollen granules; and talcum powder, which is usually formed from silicate

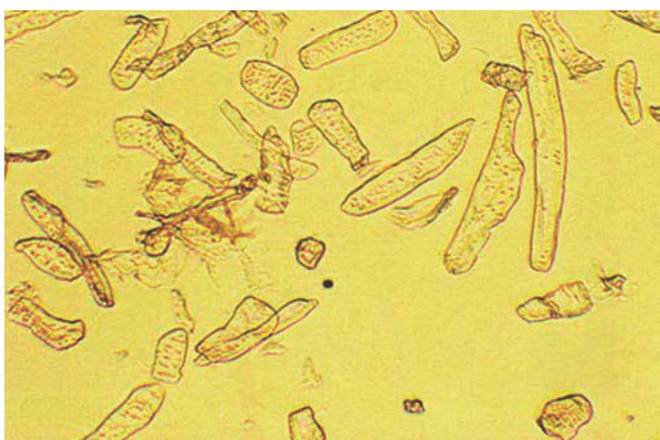

Figure 9-82. Fibers. Debris from a diaper. This squeezed-out specimen rendered microscopic examination useless. Note the various types of fibers present (200×).

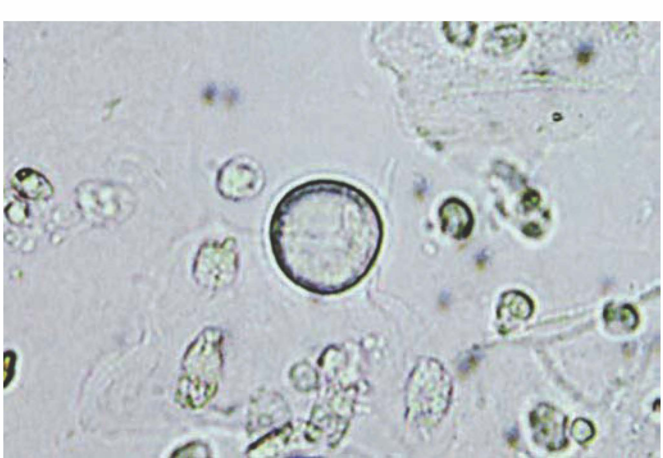

Figure 9-84. This field of view shows an oil droplet (center), several WBCs and part of squamous cell (400×).

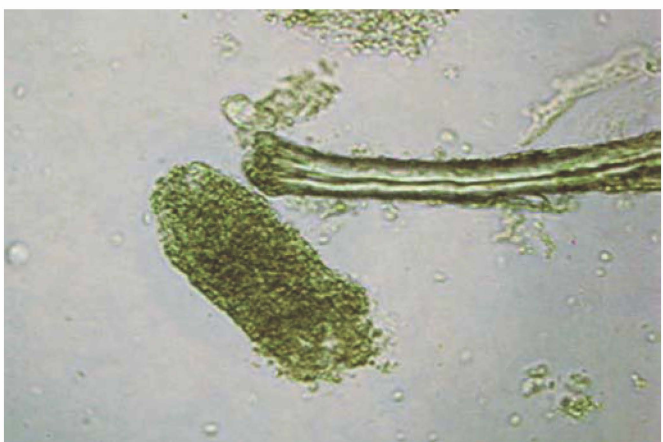

Figure 9-85. Hair **(right)** and coarsely granular cast **(left)**, viewed using an 80A filter (400×).

Figure 9-86. Glass fragments are frequently present if a glass pipette is used to transfer sediment to the slide (400×).

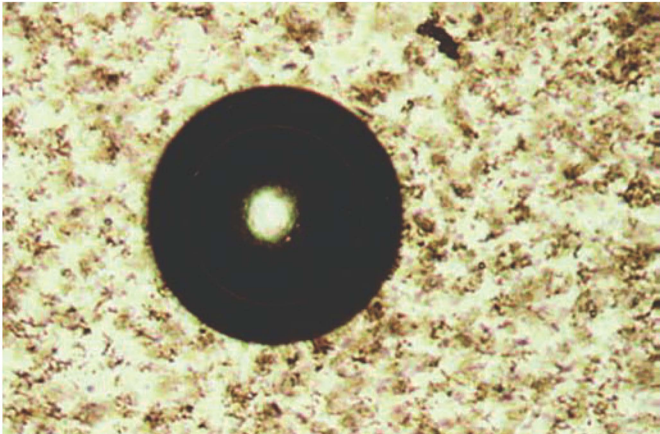

Figure 9-87. Air bubble and amorphous urates (160×).

sources giving the particles rather angular shapes (Fig. 9-88). Urine may be contaminated with fecal material and may, therefore, contain vegetable fibers, muscle fibers, and tissue strands (Fig. 9-89). These structures should be recognized as being fecal contaminants.

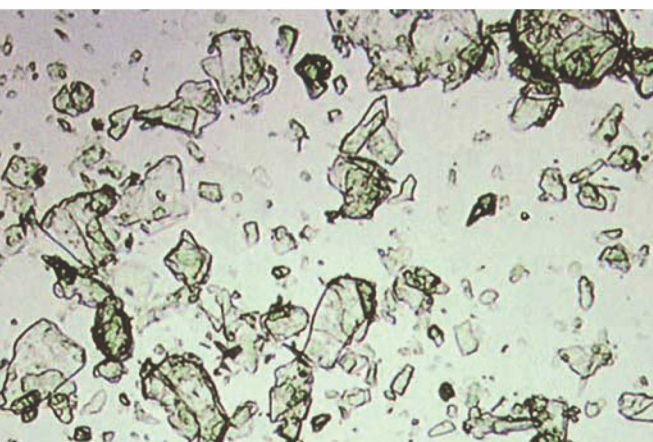

Figure 9-88. Talcum powder particles (160×).

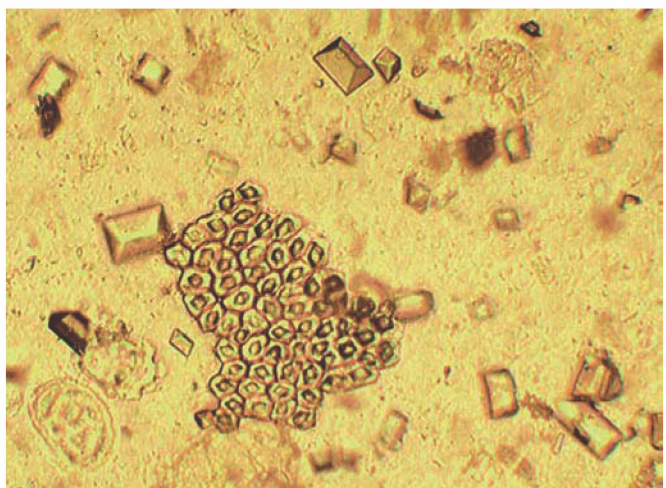

Figure 9-89. Fecal contamination. The field also contains triple phosphate crystals (100×).

STUDY QUESTIONS

1. Which of the following methods is NOT commonly performed in the microscopic examination of urine sediment?
   - **a.** Bright field with the use of stains
   - **b.** Differential interference contrast
   - **c.** Phase contrast microscopy
   - **d.** Polarized and compensated light
2. What changes in urine sediment can take place over time if the urine is not examined as soon after collection as possible? (Select all that apply.)
   - **a.** Bacteria lyse
   - **b.** Casts dissolve
   - **c.** Crystals dissolve
   - **d.** Crystals form

e. Erythrocytes crenate
f. Microorganisms multiple

3. How will erythrocytes appear in hypertonic urine?
   a. Biconcave discs
   b. Crenated
   c. Lysed
   d. Swollen

4. Glitter cells are:
   a. Crenated erythrocytes
   b. Infected tubular cells
   c. Macrophages with inclusions
   d. Swollen leukocytes

5. Prime conditions for cast formation include (select all that apply):
   a. Marked decrease in urine flow
   b. Acidic pH
   c. Alkaline pH
   d. High solute concentration
   e. Presence of abnormal ions

6. In what part of the nephron does cast formation NOT take place?
   a. Collecting tubules
   b. Distal convoluted tubule
   c. Distal portion in loop of Henle
   d. Proximal convoluted tubule

7. Casts are classified on the basis of their:
   a. Color
   b. Contents
   c. Length
   d. Site of formation

8. The order of cast degradation is:
   a. Cellular > granular > waxy
   b. Cellular > hyaline > waxy
   c. Hyaline > cellular > waxy
   d. Hyaline > granular > waxy

9. Crystals in the urine are NOT:
   a. Confirmed by reagent strip tests
   b. Dependent upon pH and temperature
   c. Formed during pathologic processes
   d. Observed in normal specimens

10. Parasites seen in the urine are:
    a. Confirmed by reagent strip tests
    b. Confused for red blood cells
    c. Unstainable with Sternheimer–Malbin
    d. Usually fecal or vaginal contaminants

Match these crystals to their associated pH.

A. Acid
B. Alkaline

11. Ammonium biurate

12. Ammonium magnesium phosphate

13. Bilirubin

14. Calcium carbonate

15. Calcium oxalate

16. Cystine

17. Hippuric acid

18. Leucine

19. Tyrosine

20. Uric acid

21. In Figure 9-90
    a. What is the name of the shape displayed in this image?

    b. What method of microscopy is used in visualizing this phenomenon?

    c. Name three structures that can be found in urinary sediment which create this effect.

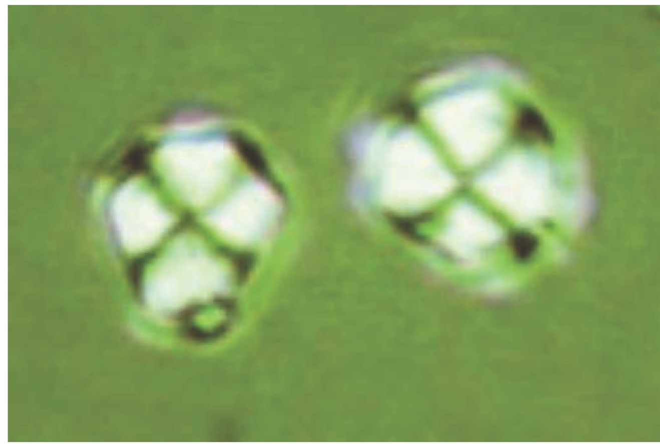

Figure 9-90. Image for Question 21.

22. In Figure 9-91
    a. Identify these structures.

    b. What term describes their presence in urine?

    c. What reagent strip test may indicate their presence?

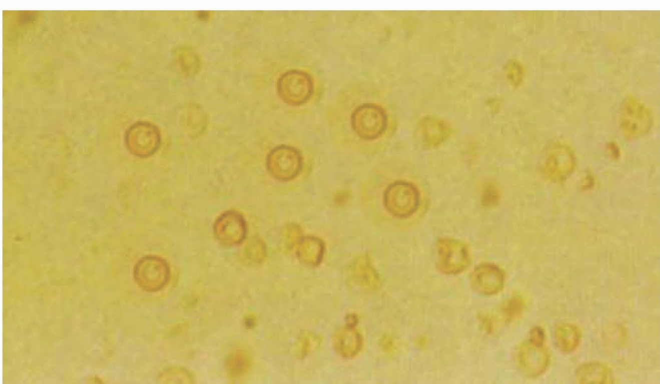

Figure 9-91. Image for Question 22.

CASE STUDY

Case 9-1 The urinalysis result below is on a specimen from a patient with metastatic carcinoma.

Color:	Amber
Appearance:	Hazy
Specific gravity:	1.015
pH:	6.5
Protein:	Trace
Glucose:	Negative
Ketone:	Negative
Bilirubin:	Positive
Blood:	Negative
Urobilinogen:	8.0
Nitrite:	Negative
Leukocyte est:	Negative
WBCs:	Rare
RBCs:	None seen
Bacteria:	None seen
Casts	None seen
Crystals:	Many (Fig. 9-92)

1. Identify these crystals.
2. Which other crystals appear similar to those seen in this urine and may cause confusion in identification?
3. How can these two crystals be differentiated?
4. What chemical findings are consistent with the presence of these crystals?
5. What organ system may be affected by this patient's carcinoma?

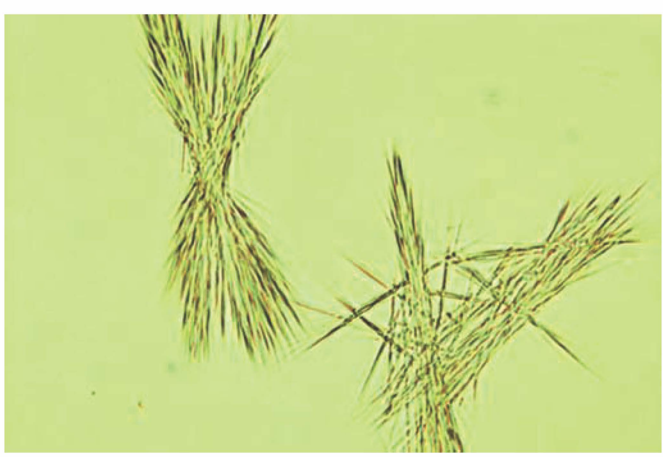

Figure 9-92. Image for Case Study 9-1. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc; 1999.)

Case 9-2 The following results were obtained on urine from an adult.

Color:	Yellow
Appearance:	Hazy
Specific gravity:	1.016
pH:	6.0
Protein 2:	+
Glucose:	Negative
Ketone:	Negative
Bilirubin:	Positive
Blood:	Trace
Urobilinogen:	1.0
Nitrite:	Negative
Leukocyte est:	Trace
WBCs:	5–10/HPF
RBCs:	2–5/HPF
Bacteria:	2+
Casts:	2–5/LPF (Fig. 9-93)
Crystals:	Moderate (Fig. 9-94)

1. Identify these casts and crystals.
2. Explain how microscopic findings correlate with physical and chemical findings.
3. Explain any discrepancies observed between the microscopic and chemical findings.

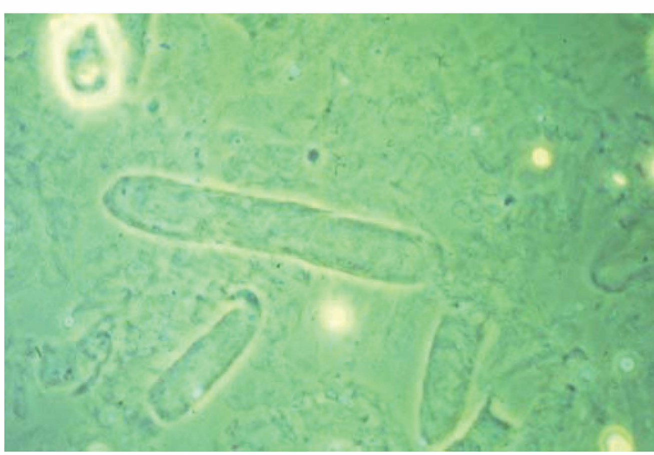

Figure 9-93. Image for Case Study 9-2. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc; 1999.)

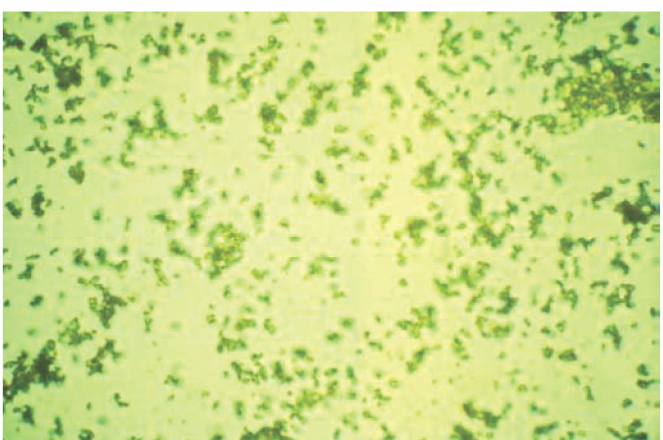

Figure 9-94. Image for Case Study 9-2. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc; 1999.)

Case 9-3 A urinalysis on a 70-year-old male revealed the findings below. He has recently undergone urological procedures.

Color:	Brown
Appearance:	Cloudy
Specific gravity:	1.011
pH:	8.0
Protein 3:	+
Glucose:	Negative
Ketone:	Negative
Bilirubin:	Positive
Blood:	2+
Urobilinogen:	0.2
Nitrite:	Negative
Leukocyte est:	Negative
WBCs:	Rare
RBCs:	20–50/HPF
Bacteria:	Rare
Casts:	None seen
Crystals:	Moderate (Fig. 9-95)

1. Identify these crystals.
2. Explain how microscopic findings correlate with physical and chemical findings.
3. Discuss the pathophysiology behind the microscopic findings in this case.

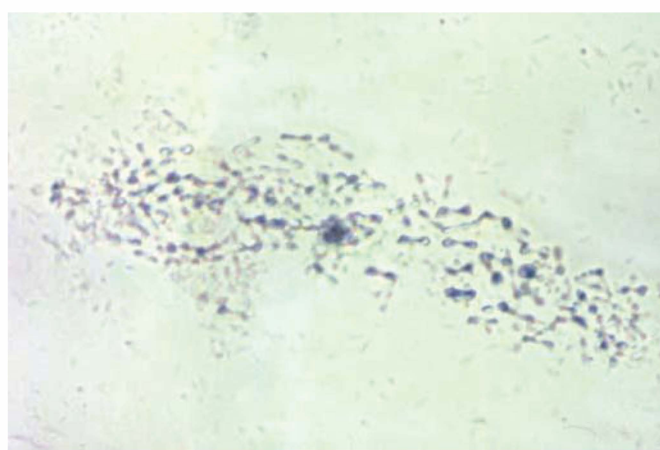

Figure 9-95. Image for Case Study 9-3. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc; 1999.)

Case 9-4 Figures 9-96 and 9-97 display the microscopic findings on urine from a 54-year-old female.

1. Identify the structures shown in the images.
2. Explain what reagent strip findings would suggest their presence.
3. Suggest some disorders in which these structures may be found in the urine.

Case 9-5 Figure 9-98 displays the microscopic findings on urine from a 21-year-old female.

1. Identify the structures shown in the figures.
2. Suggest sources of error in identification of urinary sediment that these structures may present.

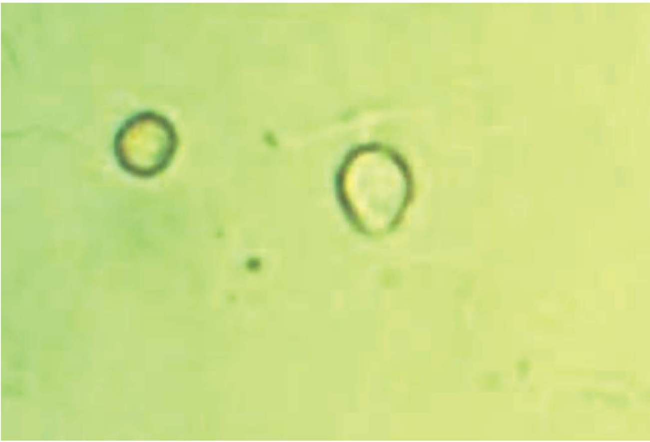

Figure 9-96. Image for Case Study 9-4. Unstained sediment. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc; 1999.)

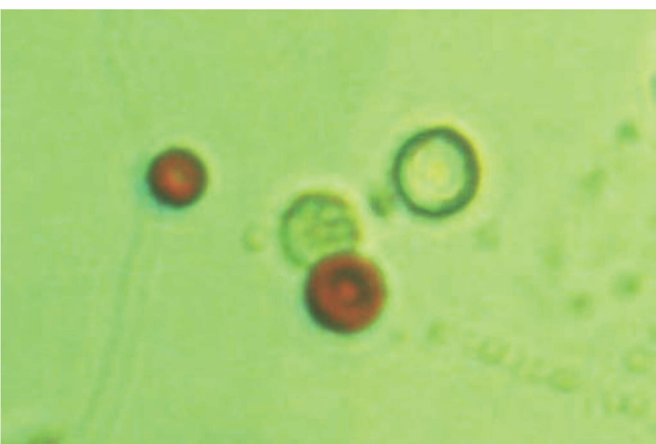

Figure 9-97. Image for Case Study 9-4 Sudan III-stained sediment. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc; 1999.)

Figure 9-98. Image for Case Study 9-5. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc; 1999.)

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Atlas of Urinary Sediment

10
Chapter

LEARNING OBJECTIVES

1. Identify cells that may be found in urinary sediment.
2. Identify crystals that may be found in acidic urine.
3. Identify crystals that may be found in alkaline urine.
4. Identify casts that may be found in urinary sediment.
5. Identify microorganisms that may be found in urinary sediment.
6. Identify artifacts that may complicate the identification of urinary sediment.
7. Recognize when bright field, phase contrast, polarized light, and interference contrast microscopy have been used.
8. Recognize when Stemheimer–Malbin staining and other staining have been used.
9. Compare and contrast urinary sediment viewed using bright field, phase contrast, polarized light, and interference contrast microscopy.
10. Compare and contrast urinary sediment viewed using bright field, Stemheimer–Malbin staining, and Sudan III staining.
11. Recognize when sediment has been stained by bilirubin.
12. Differentiate between true urinary sediment and artifacts.

The first edition of this text included an atlas of urine sediment that provided a valuable reference for many laboratory scientists at the bench. This edition contains not only most of the images from previous editions but also images from other sources. Having a tool such as a detailed atlas is essential to proper identification of uncommon urine sediment. The images in this chapter are organized into cells, crystals, casts, and other urinary sediment as well as artifacts. Some of the images where photographed after staining with Sternheimer–Malbin stain and are noted as "SM-stained."

CELLS

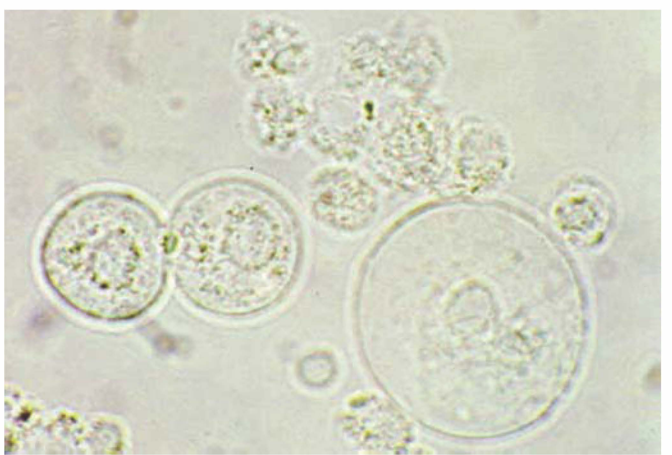


Figure 10-1. Hypotonic urine containing an RBC, several WBCs, two renal epithelial cells, and a transitional epithelial cell (500×).

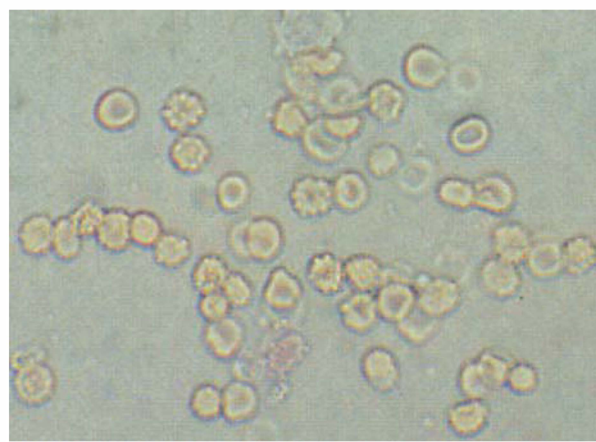


Figure 10-4. SM-stained RBCs, some crenated (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

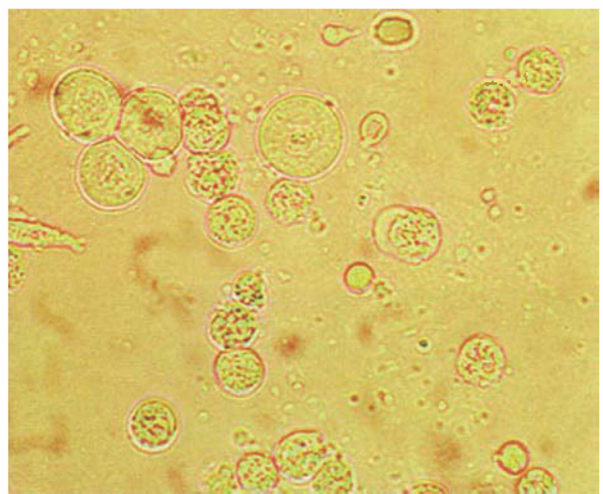


Figure 10-2. Renal epithelial cells, WBCs, RBCs, and bacteria (500×).

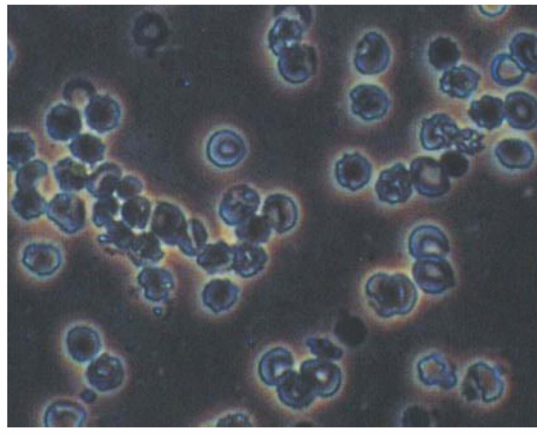


Figure 10-5. Same field of view as previous figure under phase contrast microscopy (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

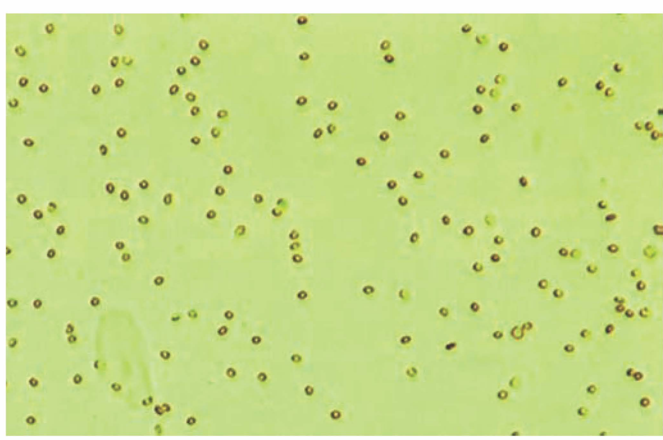


Figure 10-3. Many RBCs and a squamous epithelial cell (160×).

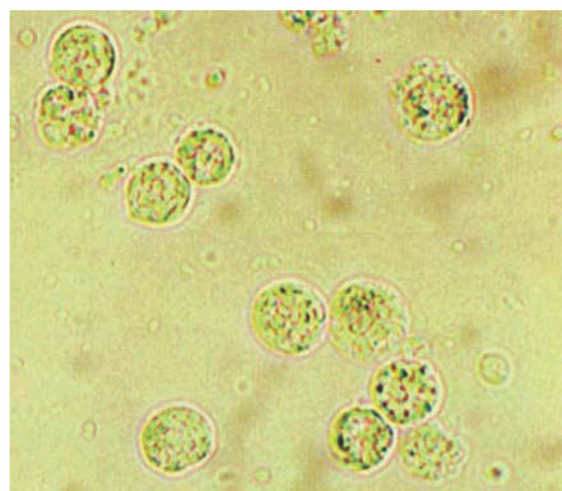


Figure 10-6. WBCs, a few RBCs, and bacteria (500×).

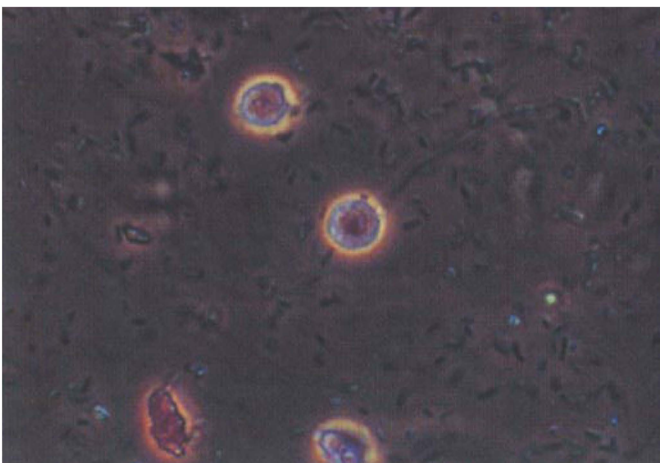


Figure 10-7. SM-stained WBCs and bacteria under phase contrast microscopy (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

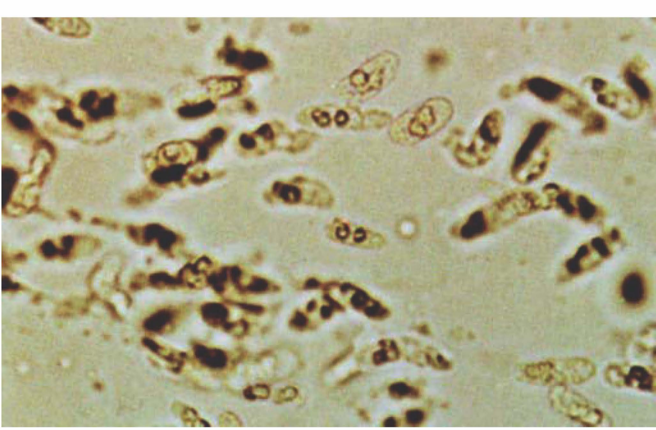


Figure 10-10. Distorted WBCs. Acetic acid (2%) was added to the slide to accentuate the nuclei, thereby confirming that the distorted cells are WBCs. The reason for this distortion is unknown (400×).

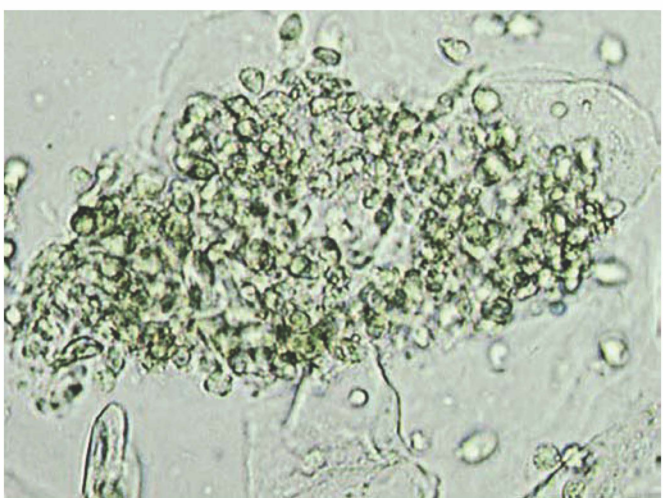


Figure 10-8. Large clump of WBCs and many squamous epithelial cells (400×).

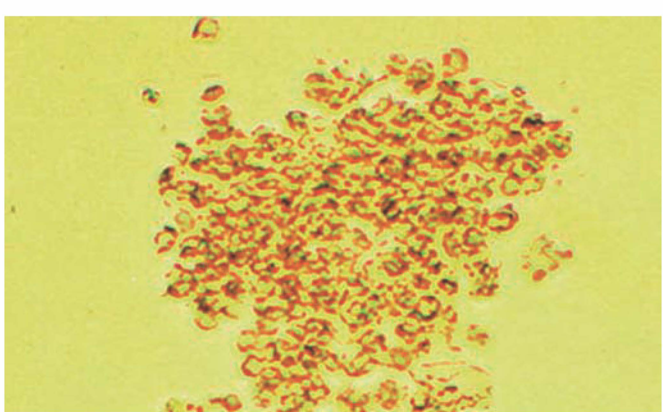


Figure 10-11. Clump of WBCs, stained by bilirubin (200×).

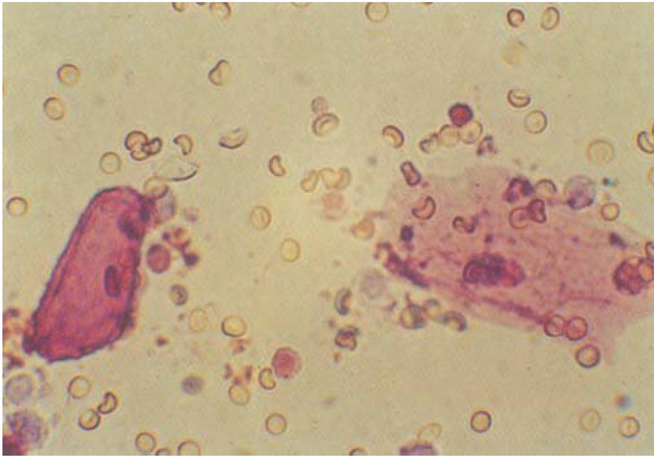


Figure 10-9. SM-stained RBCs, WBCs, and squamous epithelial cells (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

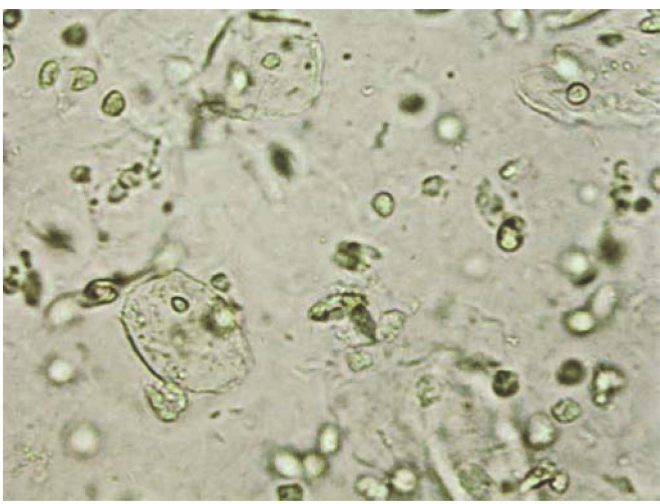


Figure 10-12. WBCs and squamous epithelial cells (400×).

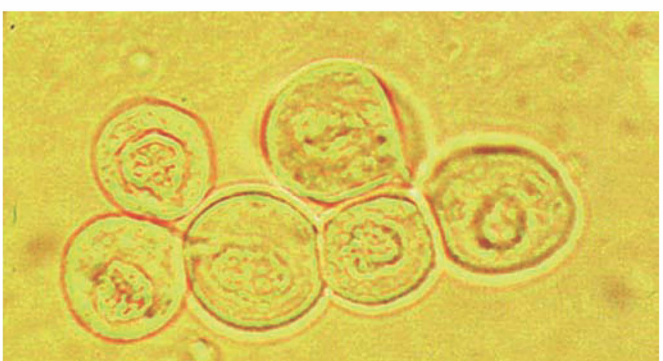


Figure 10-13. Renal epithelial cells (500×).

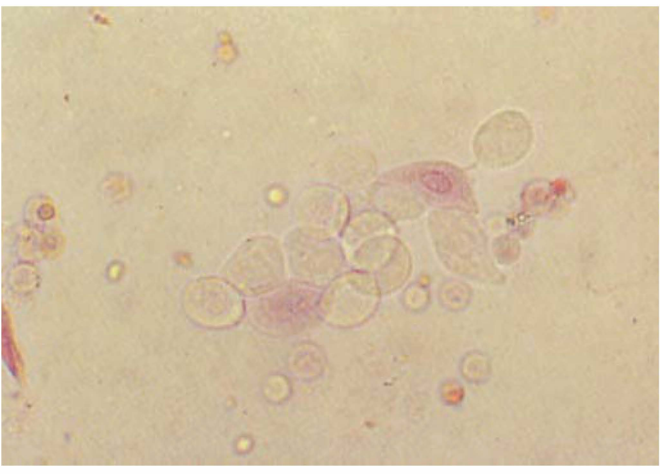


Figure 10-16. SM-stained WBCs and transitional epithelial cells (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

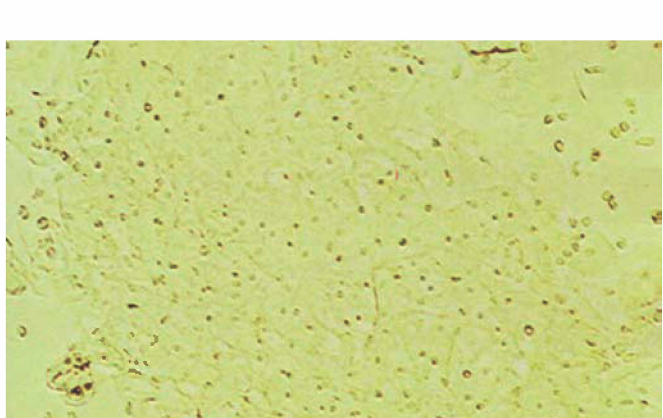


Figure 10-14. Sheet of squamous epithelial cells; most likely vaginal contamination (160×).

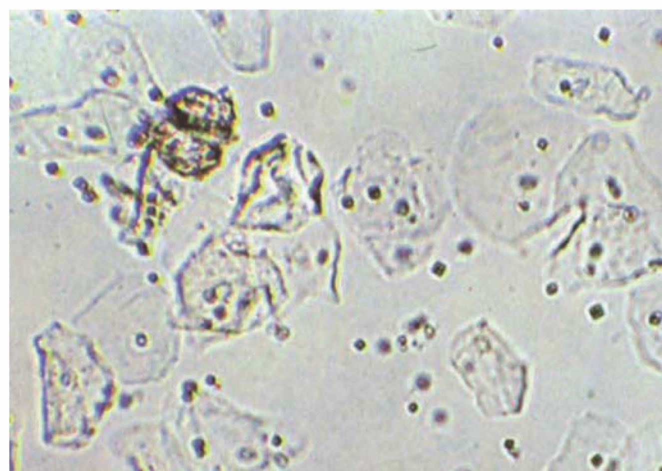


Figure 10-17. Squamous epithelial cells.

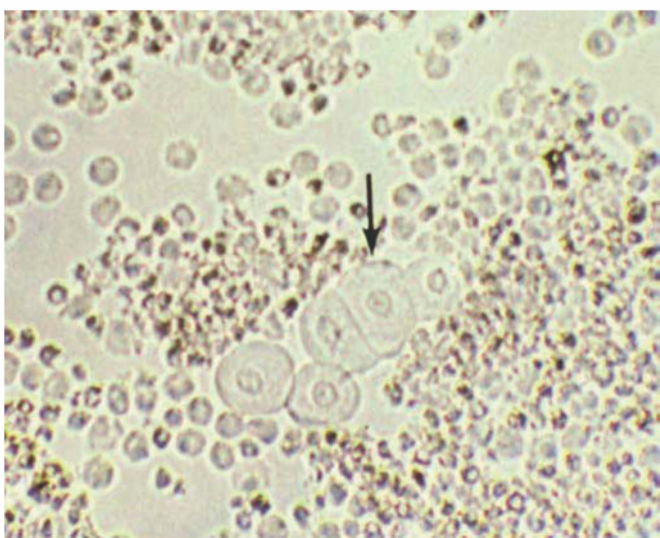


Figure 10-15. Numerous WBCs and few transitional cells (*arrow*) (200×).

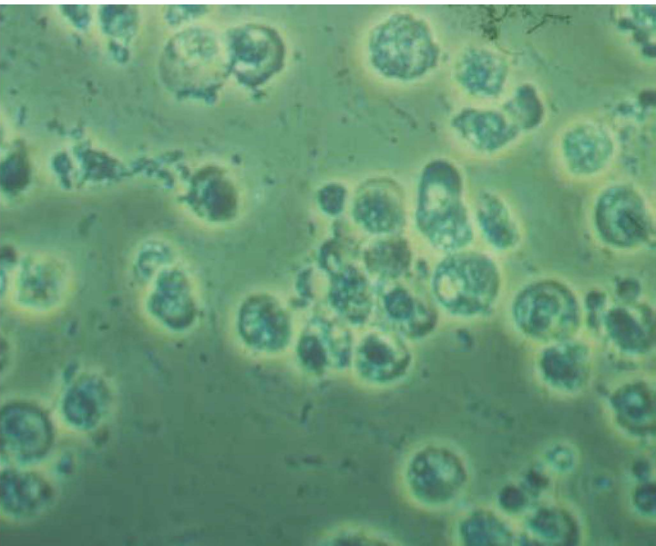


Figure 10-18. WBCs and bacteria. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

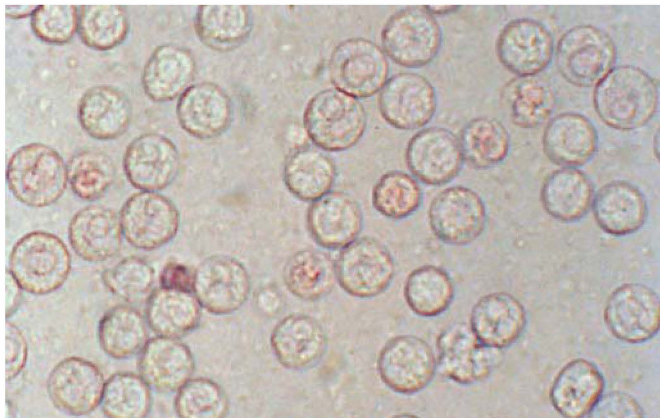


Figure 10-19. SM-stained WBCs and bacteria (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

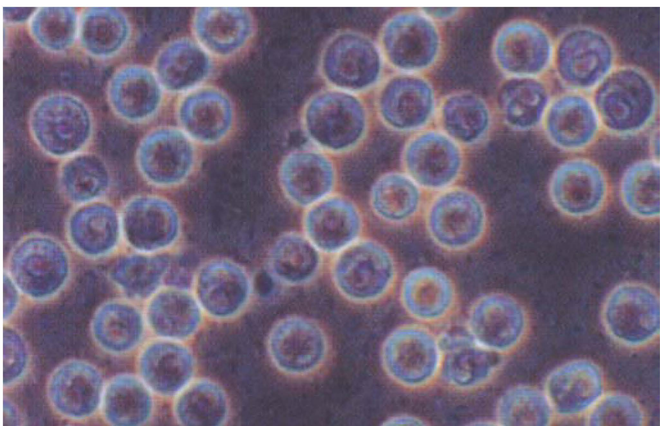


Figure 10-20. Same field of view as previous figure under phase contrast microscopy (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

CRYSTALS FOUND IN ACIDIC URINE

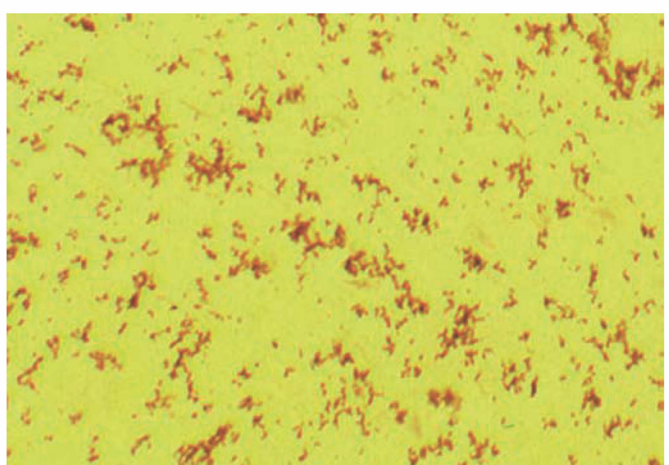


Figure 10-21. Amorphous urates (100×).

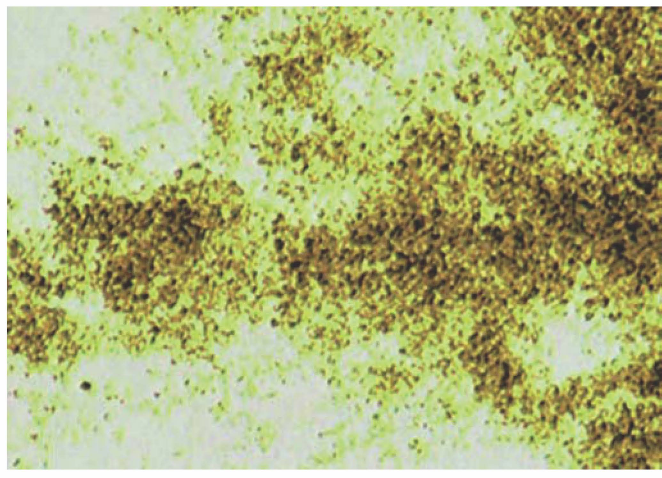


Figure 10-22. Amorphous urates. The urates in this field are clumped close together. Note the characteristic color (100×).

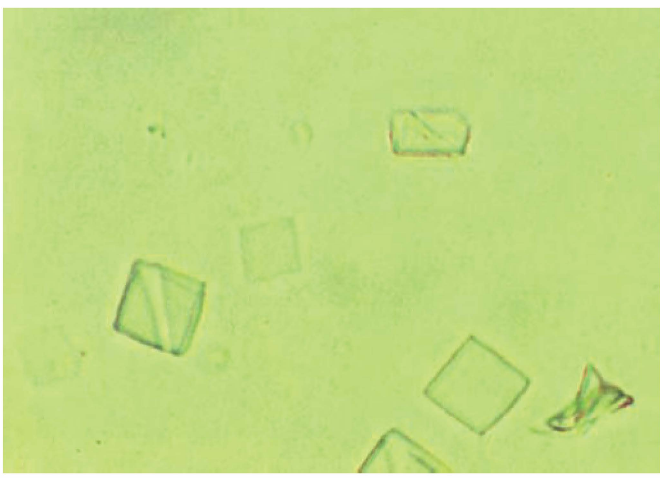


Figure 10-23. Uric acid crystals, diamond or rhombic form. These crystals are very thin and almost colorless (400×).

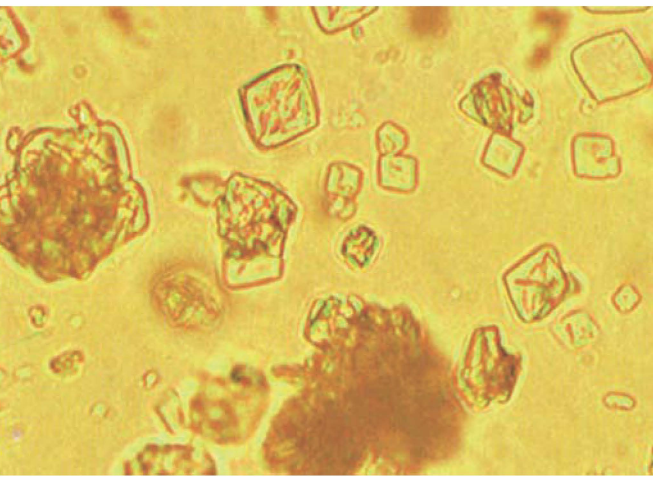


Figure 10-24. Uric acid crystals in the urine of a patient with a kidney stone. Note the heavy clumps of crystals that were present, even in the fresh specimen (400×).

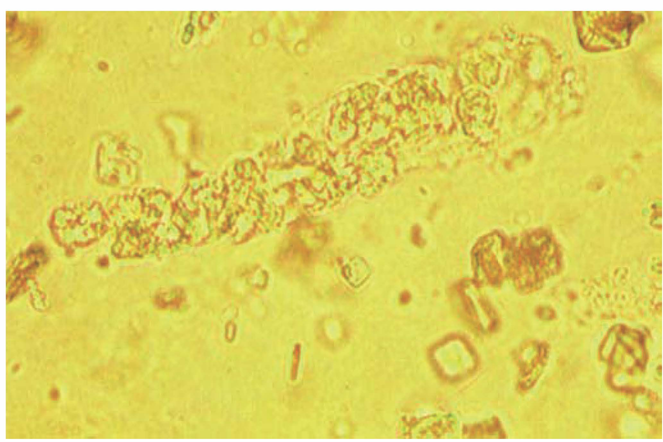


Figure 10-25. WBC cast and uric acid crystals. Same patient as in previous figure (400×).

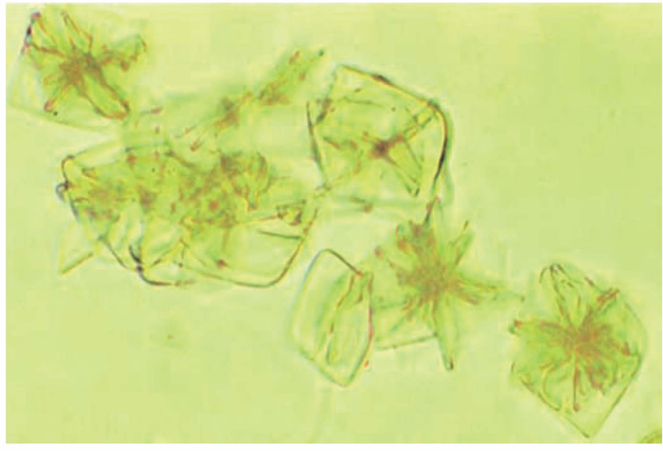


Figure 10-26. Uric acid crystals in rosette formation (400×).

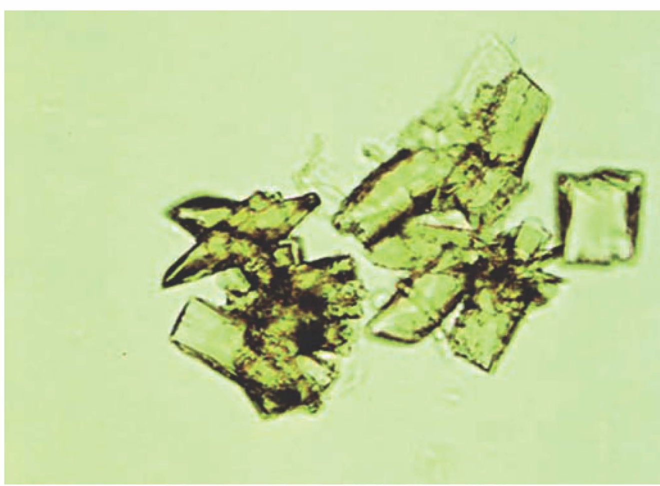


Figure 10-27. Uric acid crystals, atypical form (400×).

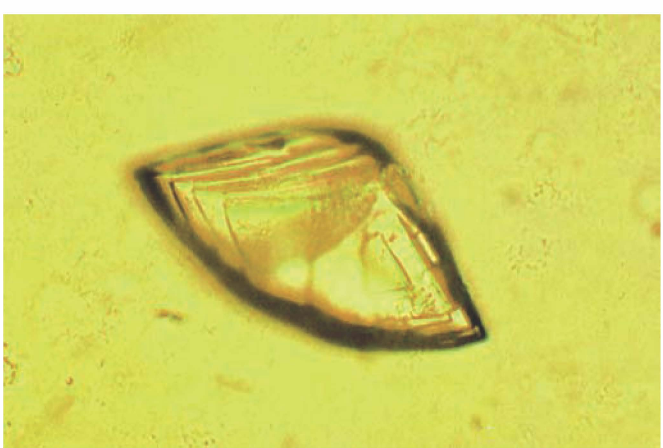


Figure 10-28. Uric acid crystals, layered formation (500×).

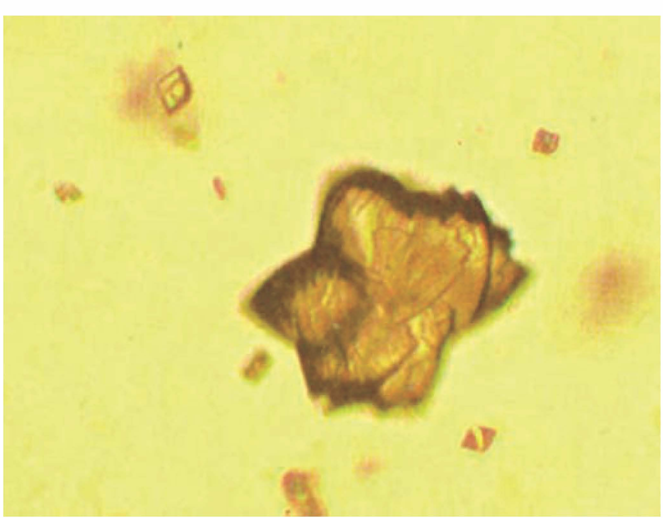


Figure 10-29. Uric acid crystals, thick rosette formation (200×).

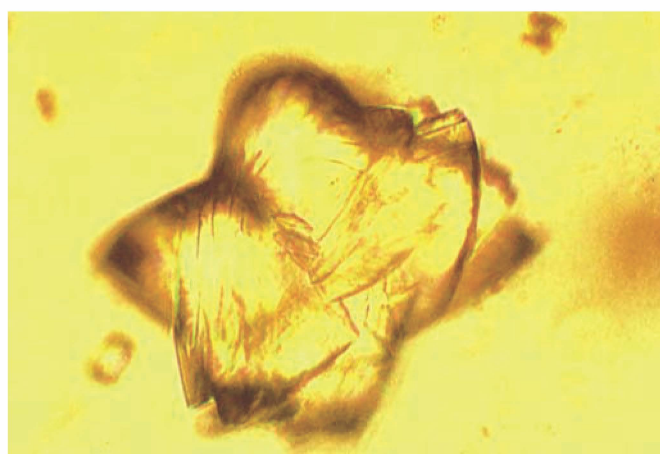


Figure 10-30. Uric acid, thick rosette formation under higher power. Note the many layered crystals (500×).

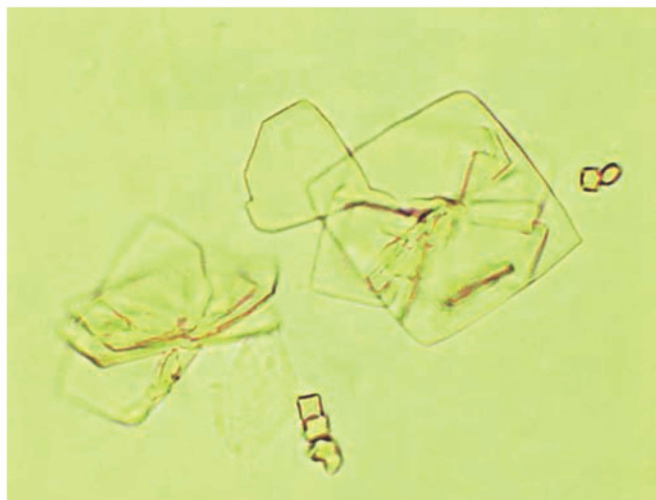


Figure 10-31. Uric acid and calcium oxalate crystals (500×).

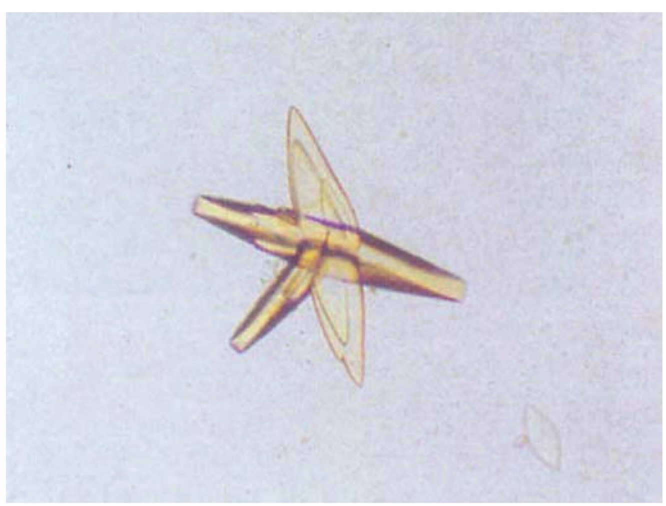


Figure 10-34. Uric acid crystals (100×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

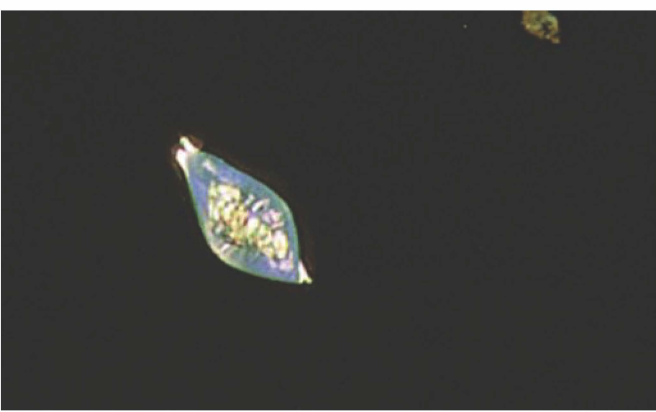


Figure 10-32. Uric acid crystals under polarized light. Note the small crystal (400×).

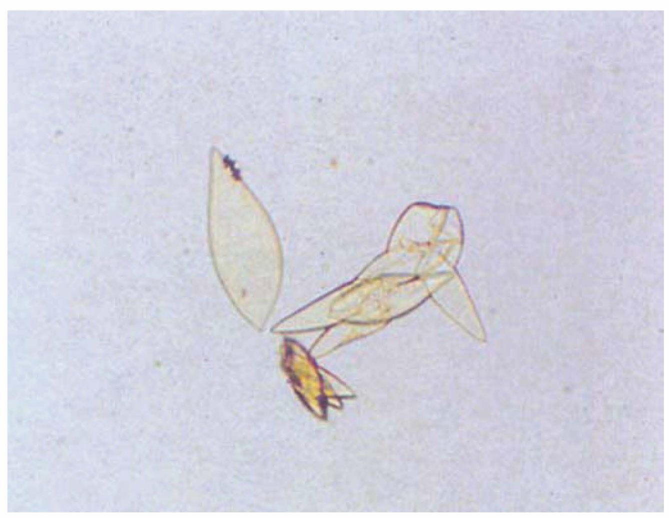


Figure 10-35. Uric acid crystals (100×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia: Lippincott, 1998.)

Figure 10-33. Uric acid under polarized light (400×).

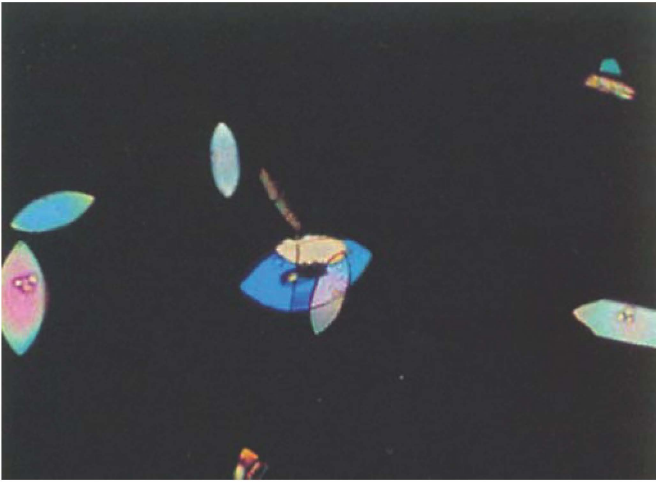


Figure 10-36. Uric acid crystals under polarized light (100×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott, 1998.)

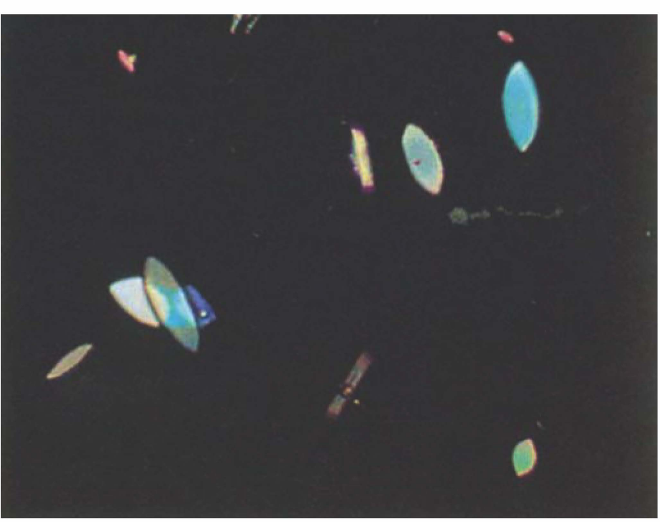


Figure 10-37. Uric acid crystals under polarized light (100×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

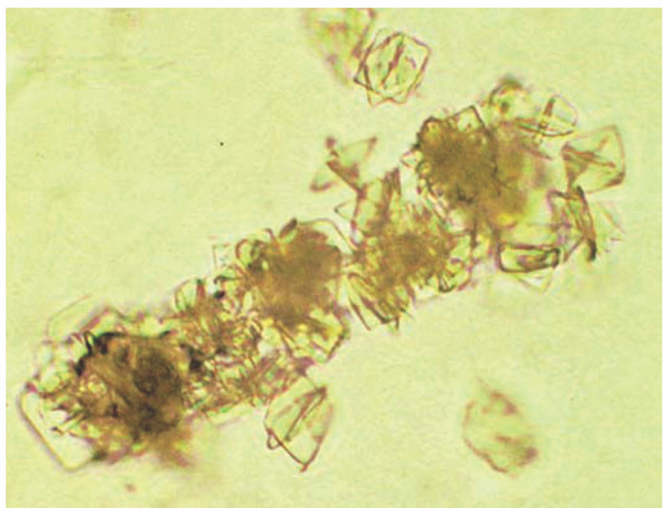


Figure 10-38. Uric acid crystals in pseudocast formation (400×).

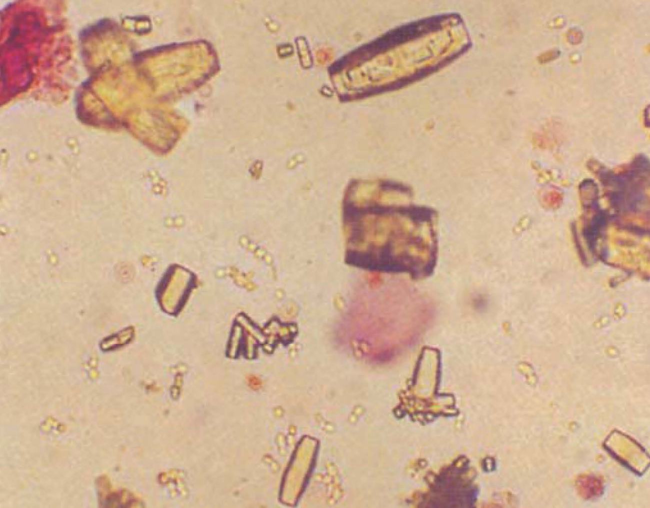


Figure 10-39. Uric acid, barrel shape, and yeast in the background (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

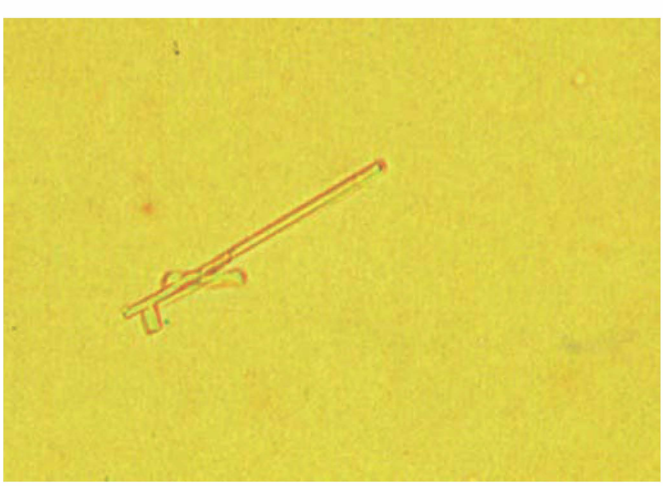


Figure 10-40. Sodium urate crystals. Note the square ends on each needle-like crystal (400×).

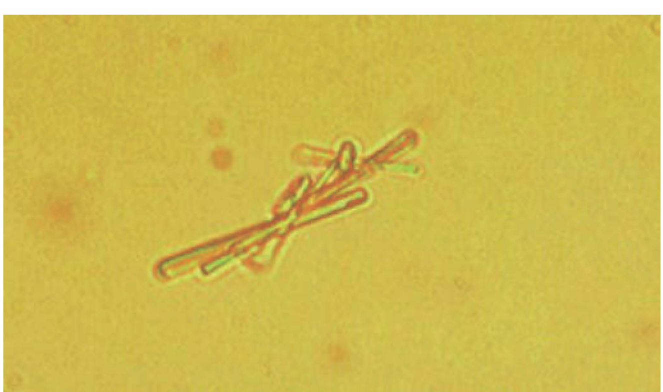


Figure 10-41. Sodium urates. Notice how narrow these crystals are (400×).

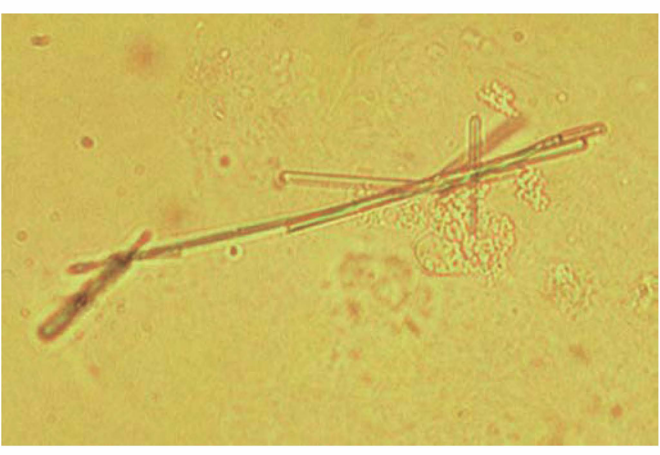


Figure 10-42. Sodium urate crystals (400×).

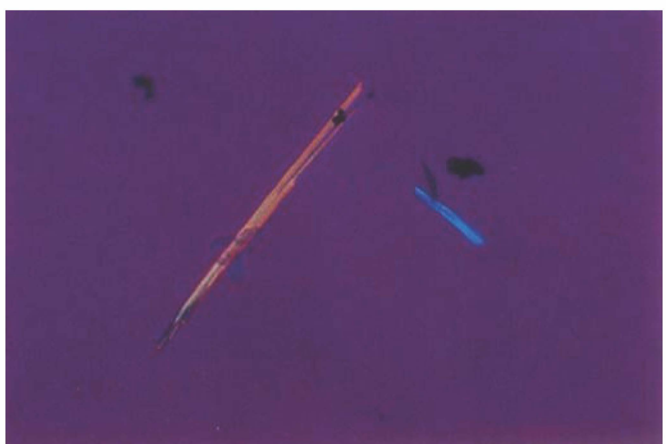


Figure 10-43. Uric acid, needle shape under polarized light with red compensator (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

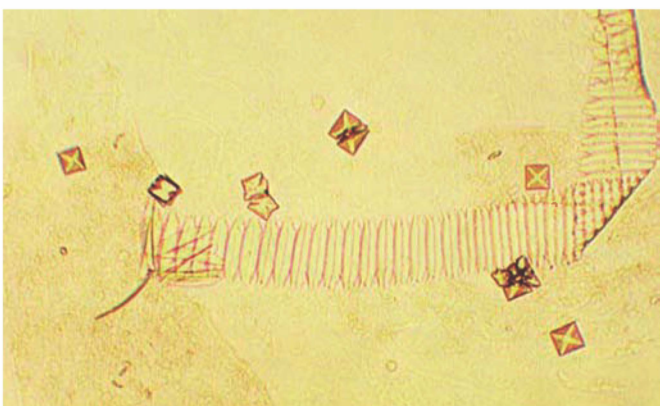


Figure 10-46. Calcium oxalates, amorphous urates, and a piece of debris. Some of the crystals cracked when the coverslip was touched (200×).

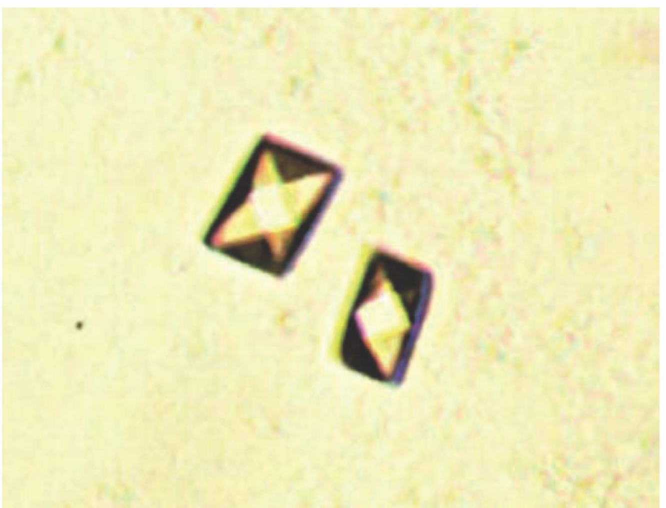


Figure 10-44. Calcium oxalate crystals (200×).

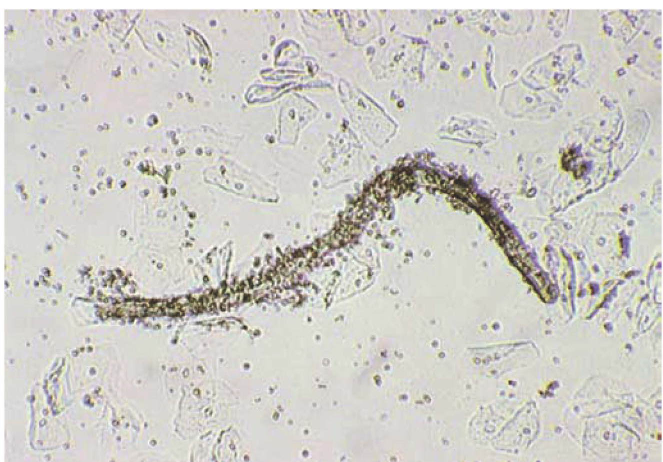


Figure 10-47. Calcium oxalate crystals clustered around a piece of debris. The field also contains squamous epithelial cells as well at many calcium oxalates (100×).

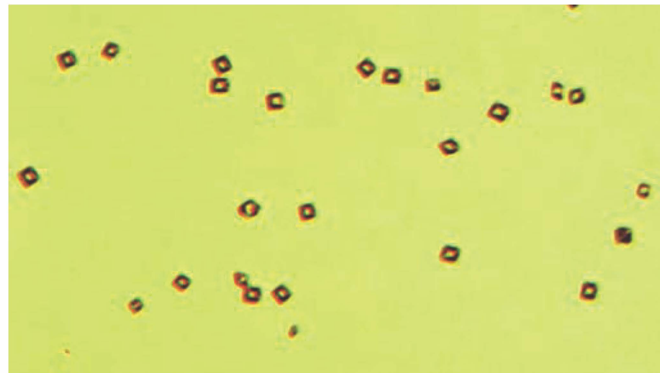


Figure 10-45. Calcium oxalate crystals. Even under low-power magnification, the characteristics of the crystals are easily recognized (160×).

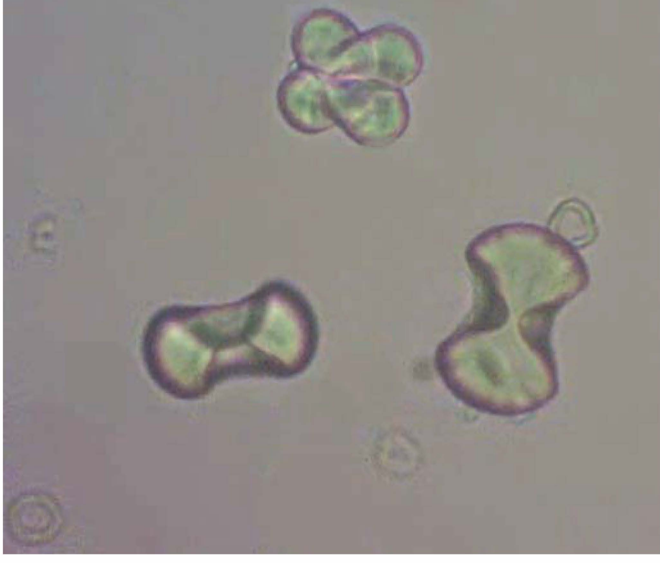


Figure 10-48. Calcium oxalate forms seen in ethylene glycol poisoning.

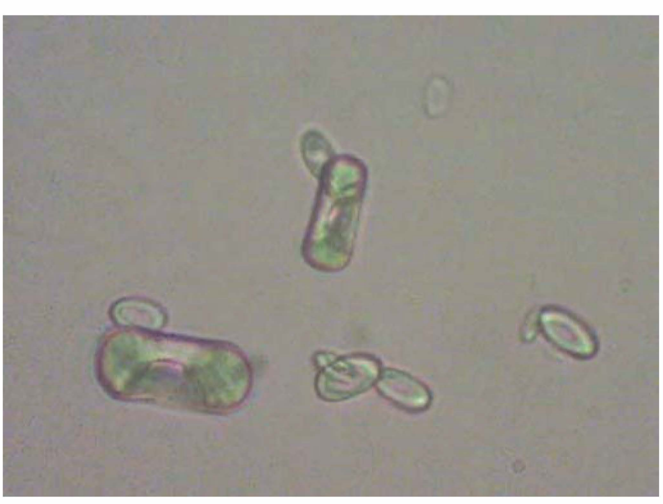


Figure 10-49. More forms of calcium oxalate seen in ethylene glycol poisoning.

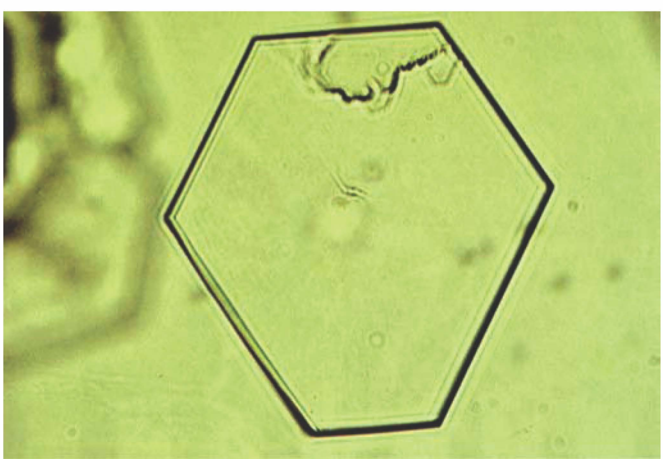


Figure 10-52. Cystine crystal with unequal sides (1,000×).

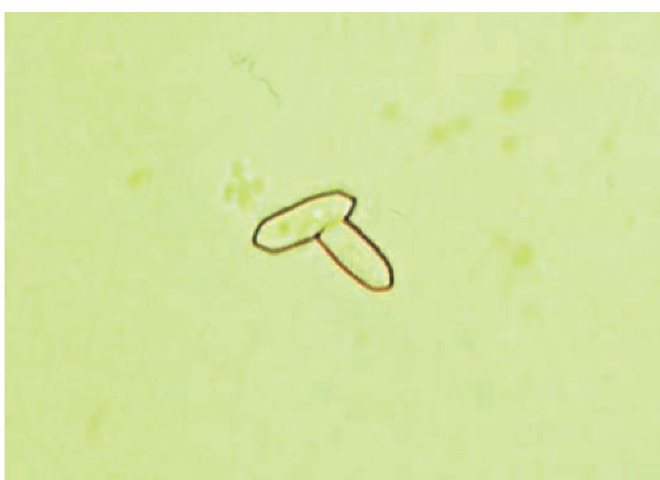


Figure 10-50. Hippuric acid crystals (400×).

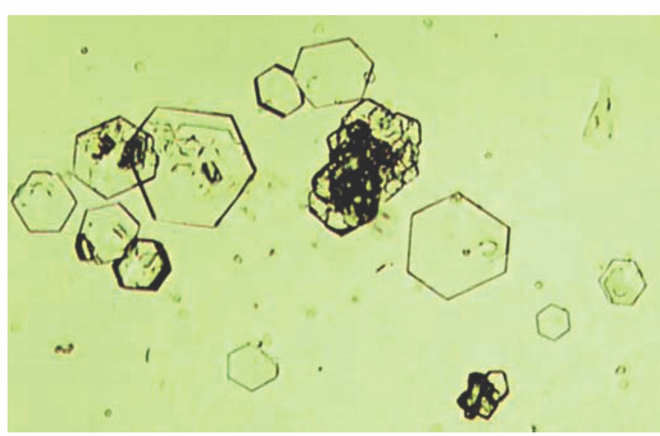


Figure 10-53. Cystine crystals. Note how these crystals can form clusters (160×).

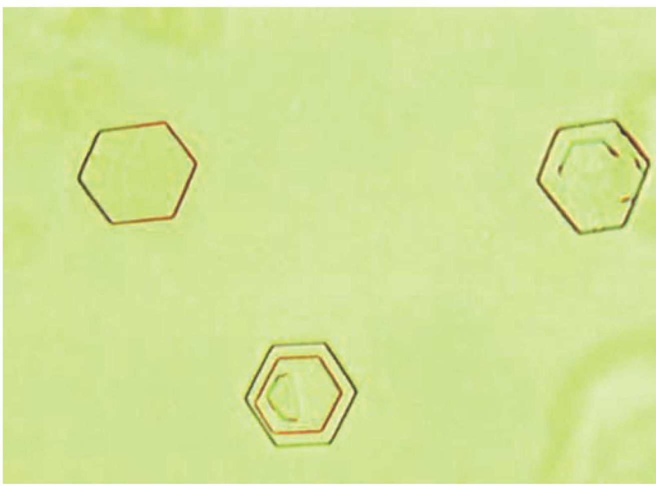


Figure 10-51. Cystine crystals (160×).

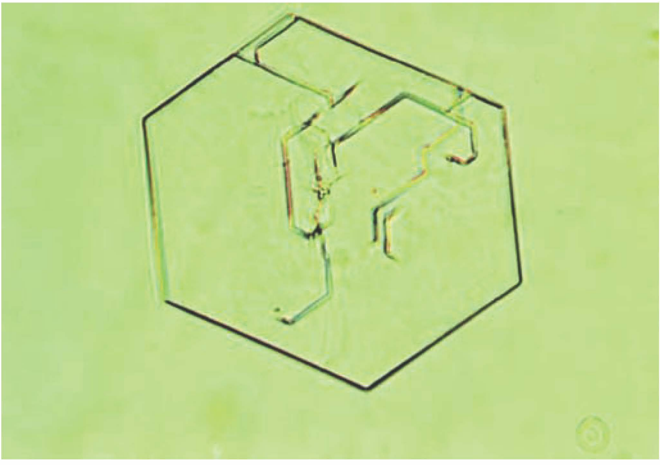


Figure 10-54. Cystine crystal with layered or laminated surface (1,000×).

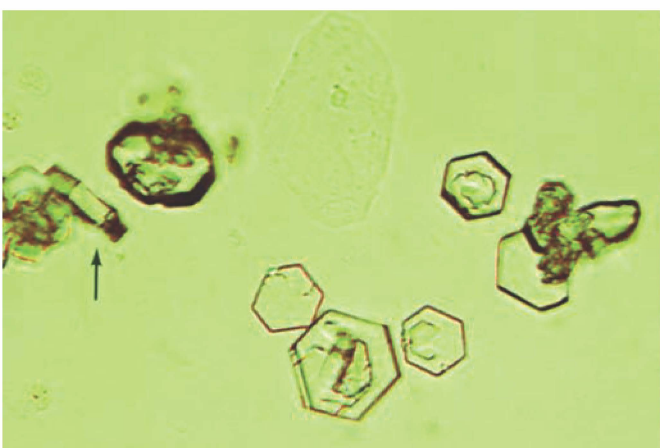


Figure 10-55. Cystine crystals and a squamous epithelial cell. Some crystals have laminated surfaces and others are quite thick. The *arrow* shows a thick crystal that is turned on its edge (400×).

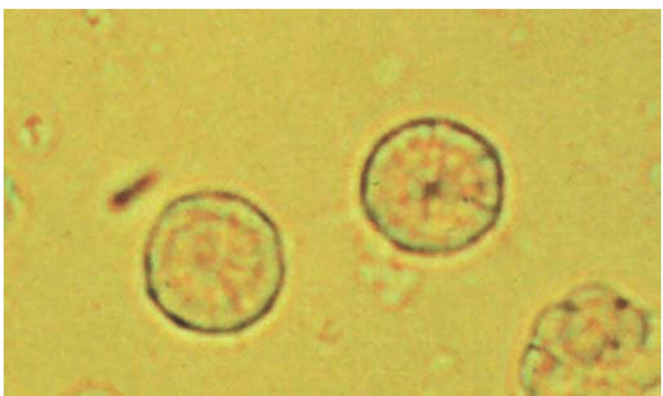


Figure 10-56. Leucine crystals. Note what appears to be a thick double wall and a striated center.

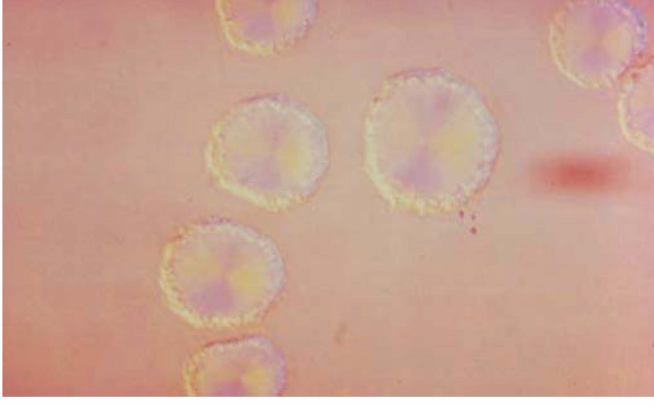


Figure 10-57. Leucine crystals under interference contrast microscopy. (Courtesy of Smith B, Foster KA. *The Urine Microscopic.* 5th ed. Educational Material for Health Professionals Inc.; 1999.)

Figure 10-58. Tyrosine crystals. Note how black the crystals appear on low power (160×).

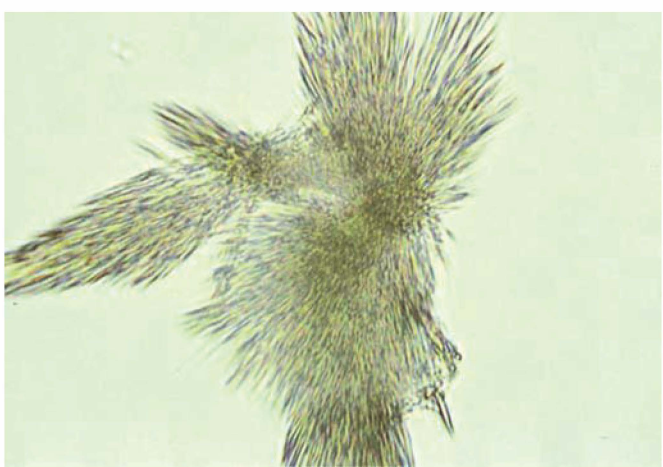


Figure 10-59. Tyrosine crystals. Note the fine, very pointy needles (1,000×).

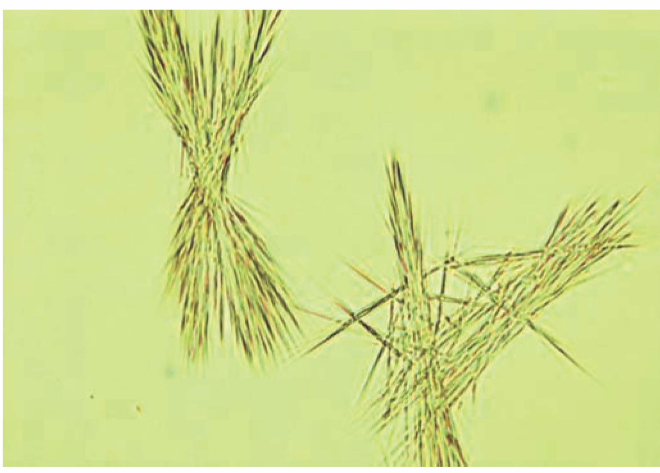


Figure 10-60. Tyrosine crystals (1,000×).

Figure 10-61. Tyrosine crystals under polarized light. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

Figure 10-62. Tyrosine crystals (1,000×).

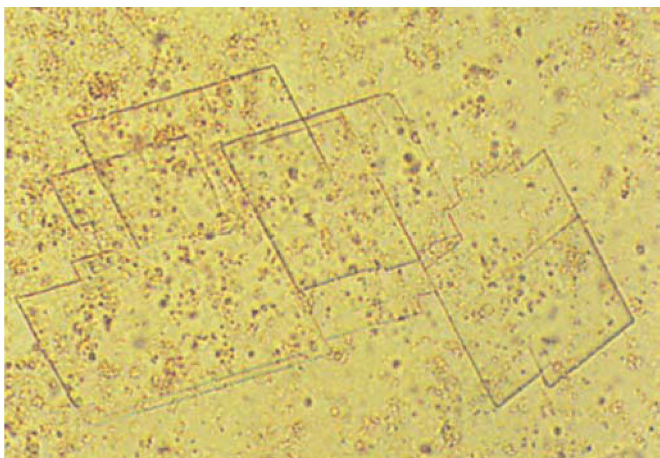


Figure 10-63. Cholesterol crystals from "kidney fluid" (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia: Lippincott, 1998.)

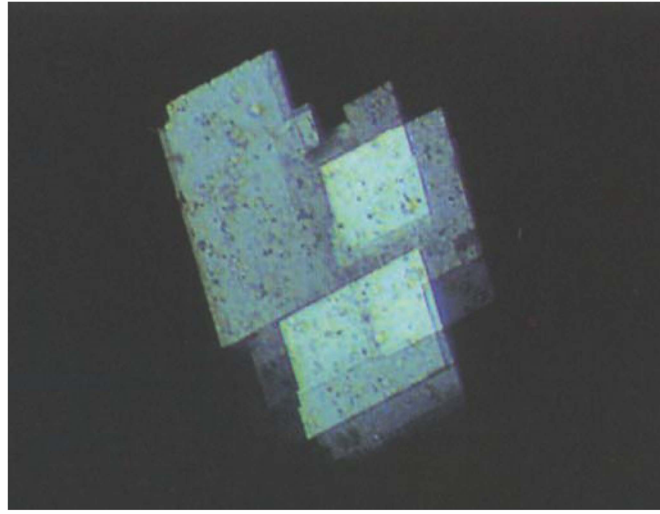


Figure 10-64. Same specimen as the previous figure under polarized light. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia: Lippincott, 1998.)

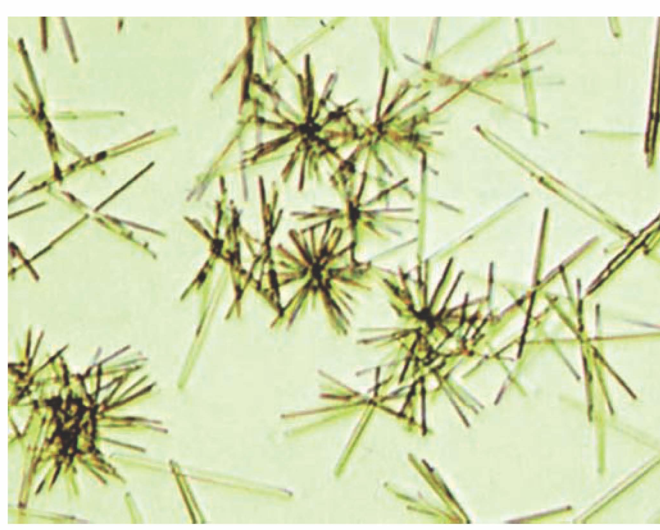


Figure 10-65. X-ray dye crystals. Specific gravity of the specimen was 1.070 (160×).

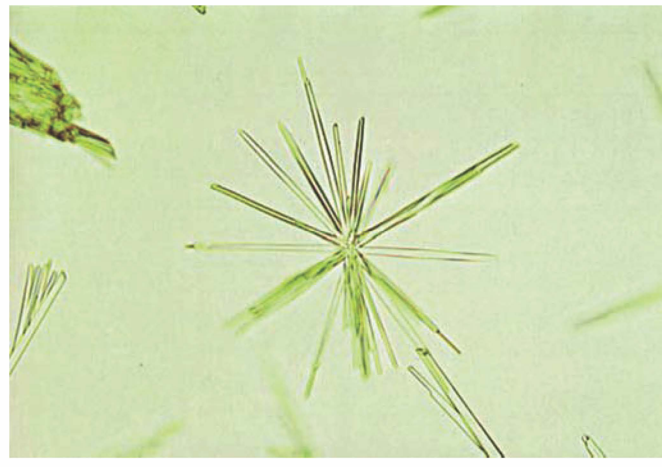


Figure 10-66. X-ray dye crystals (400×).

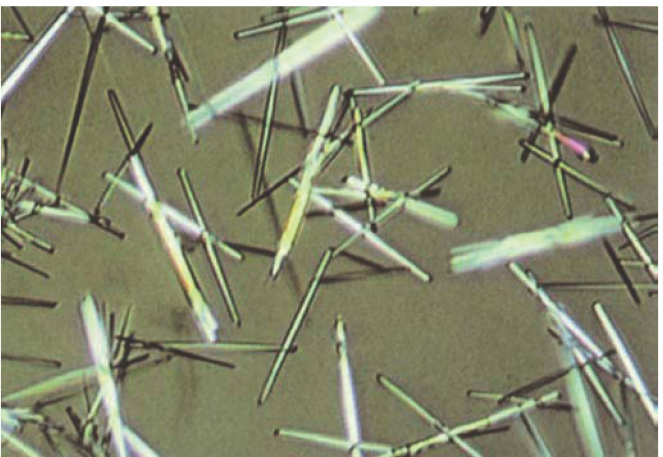


Figure 10-67. X-ray dye crystals under polarized light (160×).

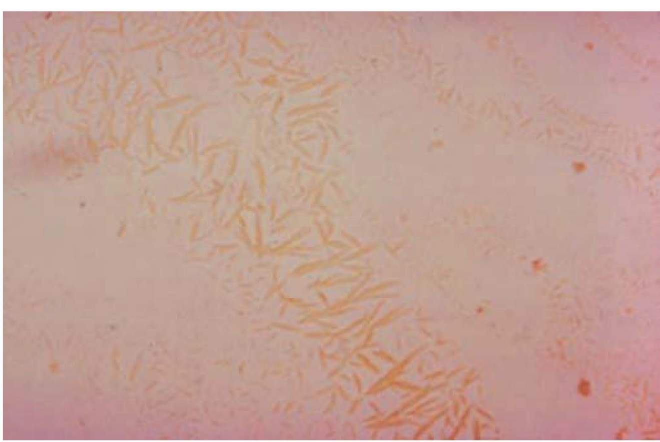


Figure 10-70. Bilirubin crystals. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

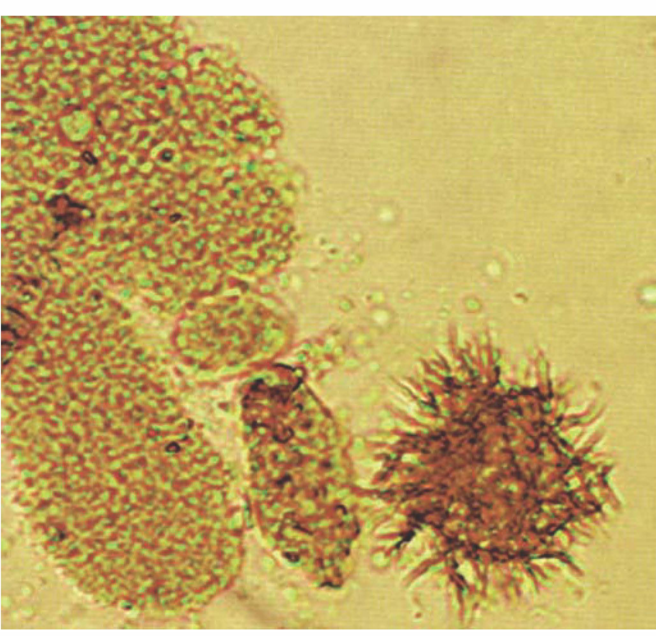


Figure 10-68. Bilirubin crystals and bilirubin-stained WBC and granular cast (500×).

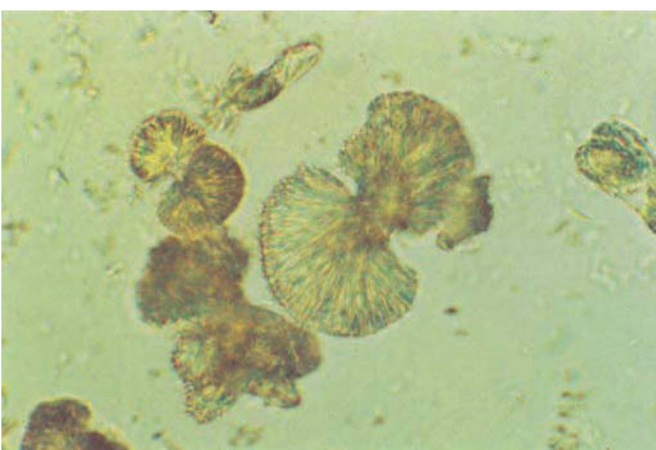


Figure 10-71. Sulfonamide crystals (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

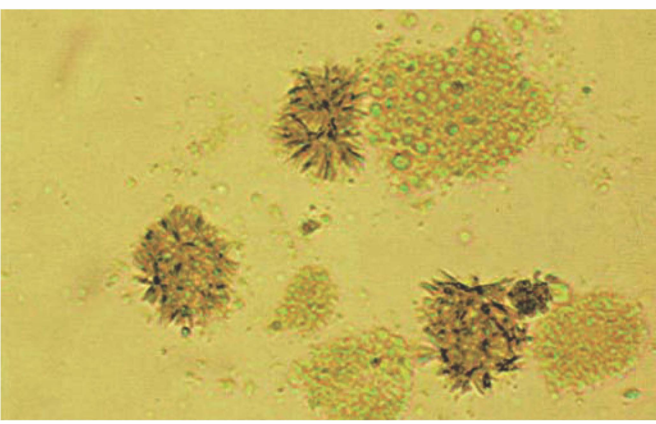


Figure 10-69. Bilirubin crystals and bilirubin-stained sediment (500×).

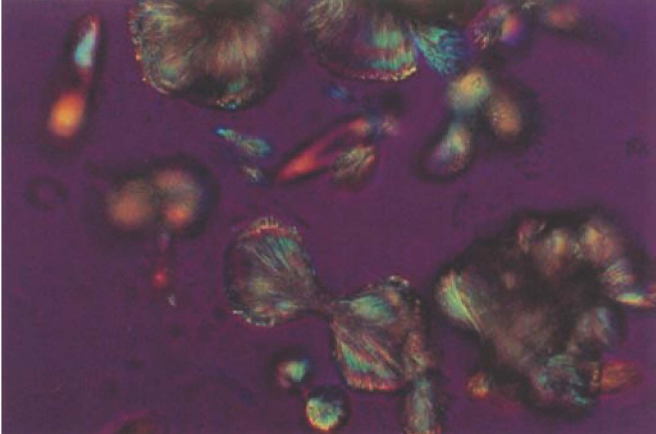


Figure 10-72. Sulfonamide crystals under polarized light with red compensator. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

CRYSTALS FOUND IN ALKALINE URINE

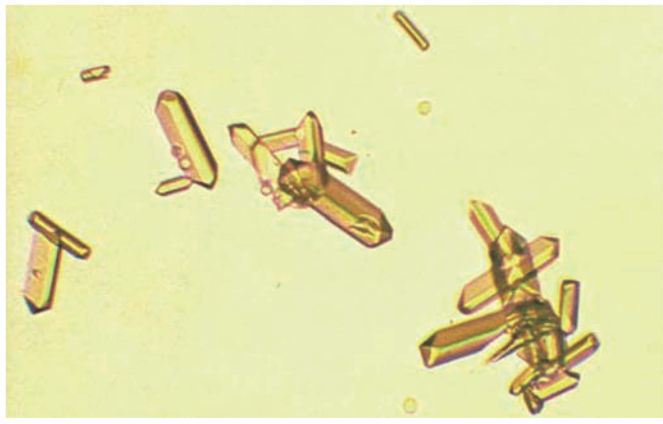


Figure 10-73. Triple phosphate crystals. Many of these prisms are six-sided (200×).

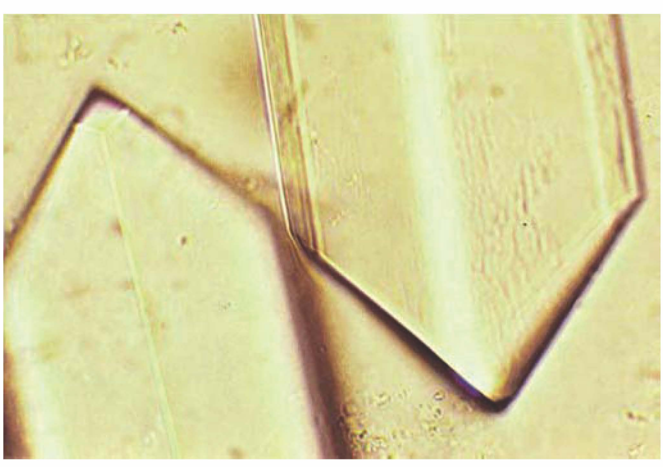


Figure 10-76. Triple phosphate crystals (500×).

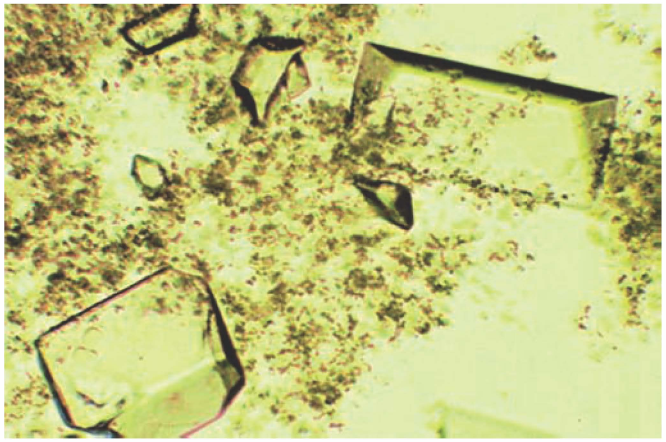


Figure 10-74. Triple phosphate crystals and amorphous phosphates (200×).

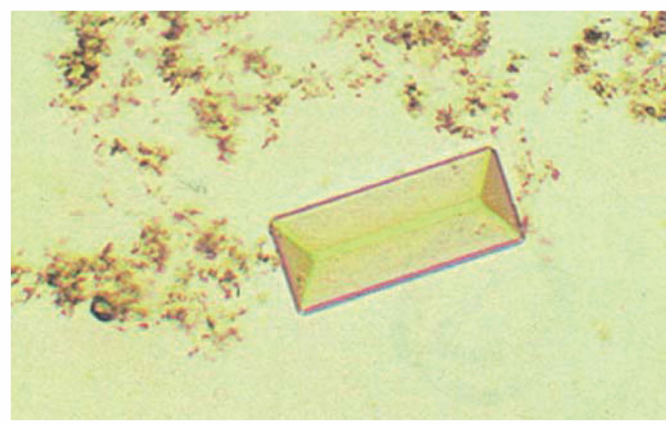


Figure 10-77. Triple phosphates crystal and amorphous phosphates (200×).

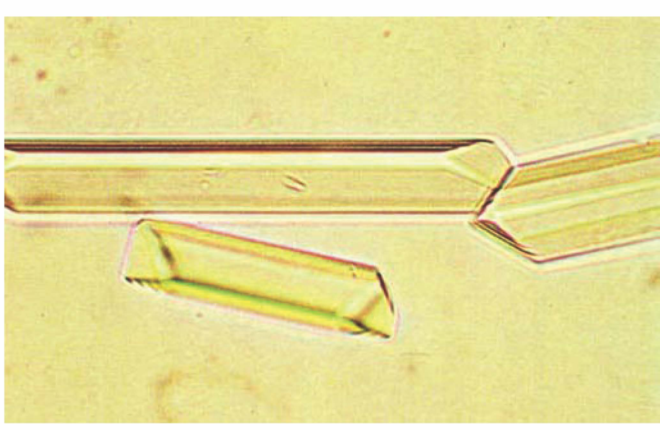


Figure 10-75. Triple phosphate crystals (400×).

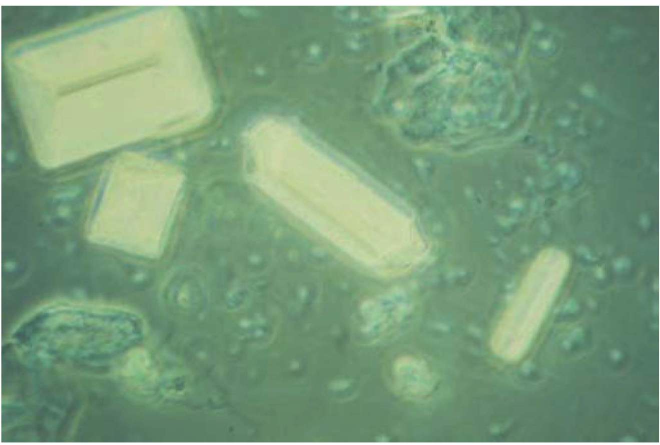


Figure 10-78. Triple phosphate crystals under polarized light. (Courtesy of Smith B, Foster KA. *The Urine Microscopic.* 5th ed. Educational Material for Health Professionals Inc.; 1999.)

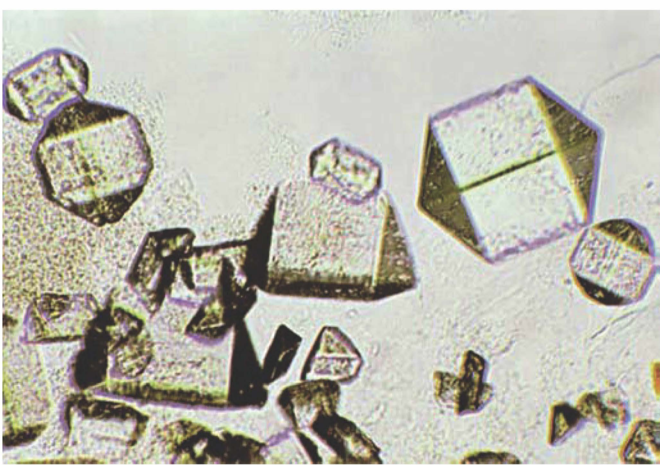


Figure 10-79. Triple phosphates crystal and amorphous phosphates. When crystals take on this grayish-black color, it usually means that they are beginning to dissolve (200×).

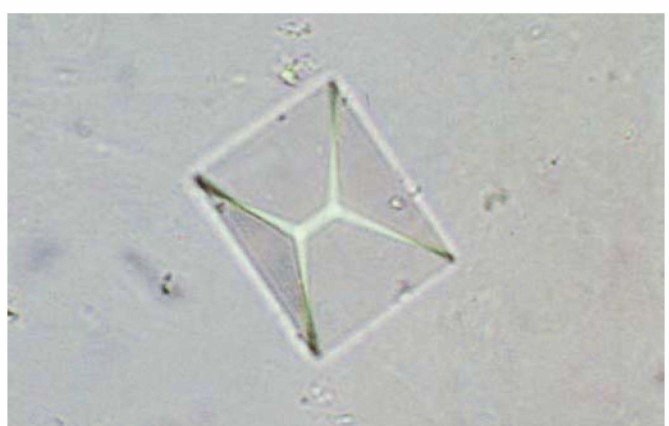


Figure 10-82. Triple phosphate crystal. This crystal could be mistaken to be calcium oxalate, but the "X" does not cross exactly in the middle (400×).

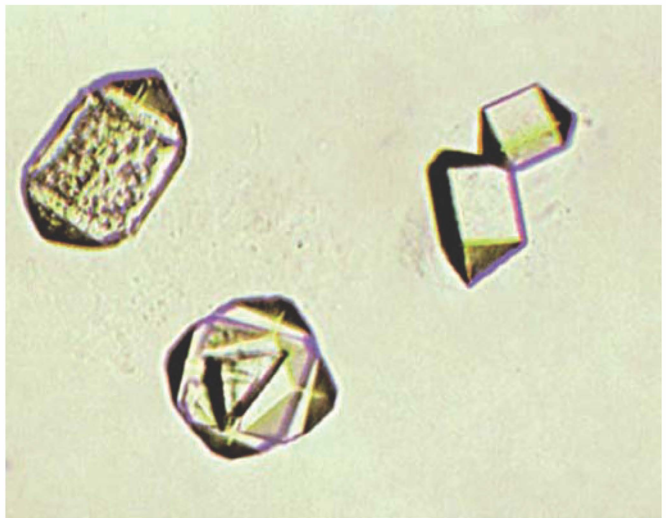


Figure 10-80. Triple phosphates crystal and amorphous phosphates. Note the unique formation of the center crystal (200×).

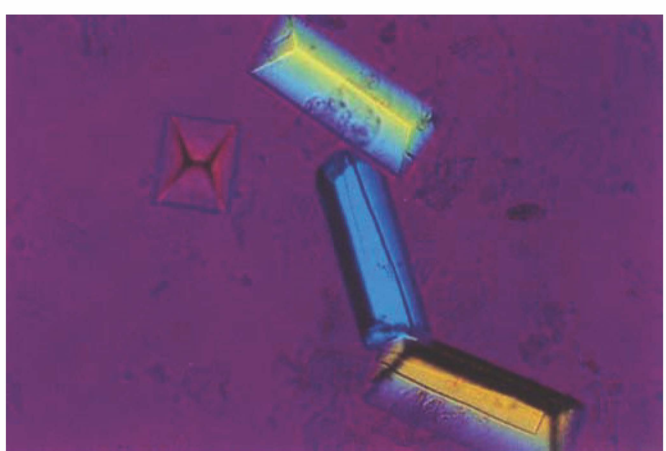


Figure 10-83. Triple phosphate crystals under polarized light with red compensator (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott; 1998.)

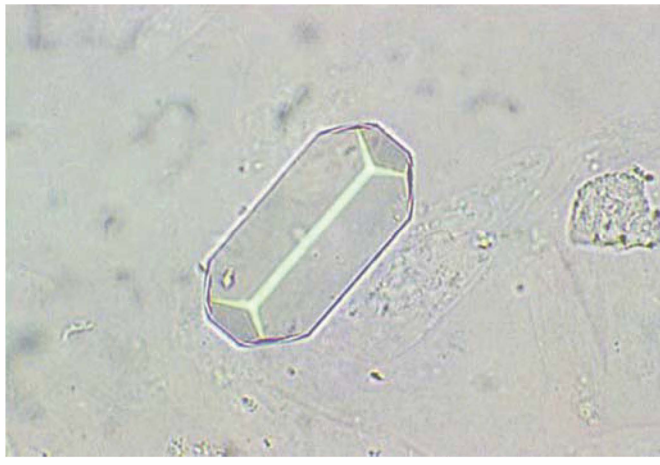


Figure 10-81. Triple phosphates crystal and mucus (400×).

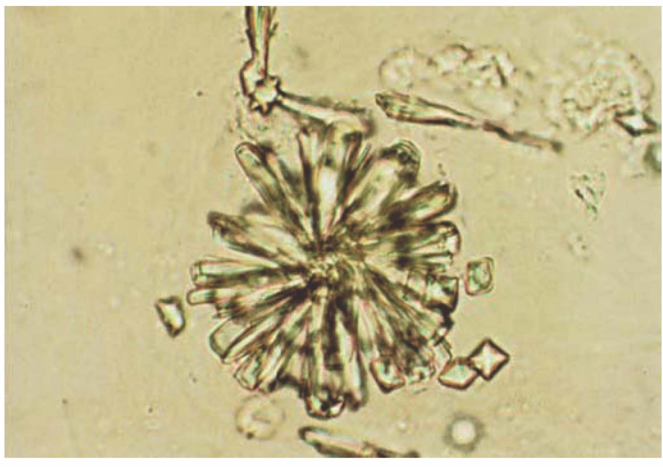


Figure 10-84. Calcium phosphate crystals (400×).

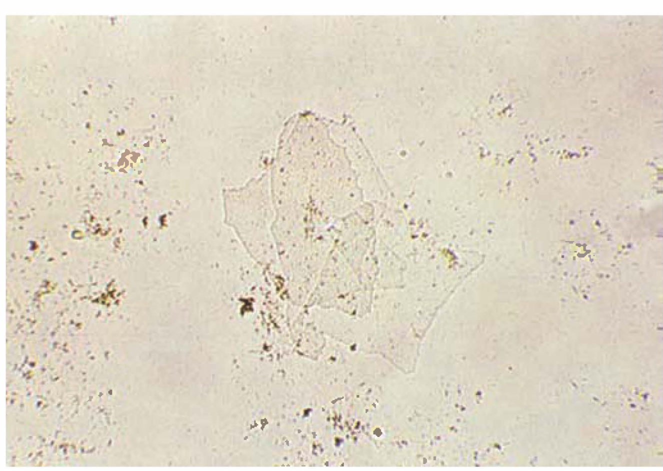


Figure 10-85. Calcium phosphate plates and amorphous phosphates. Notice the thin granular plates (200×).

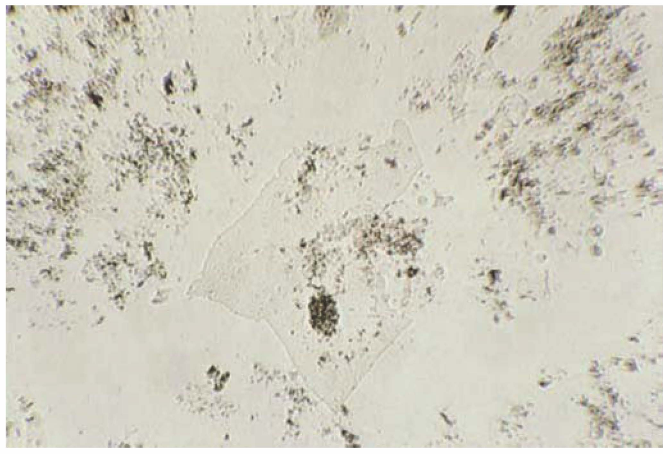


Figure 10-86. Calcium phosphate plate (or phosphate sheath) and amorphous phosphates (200×).

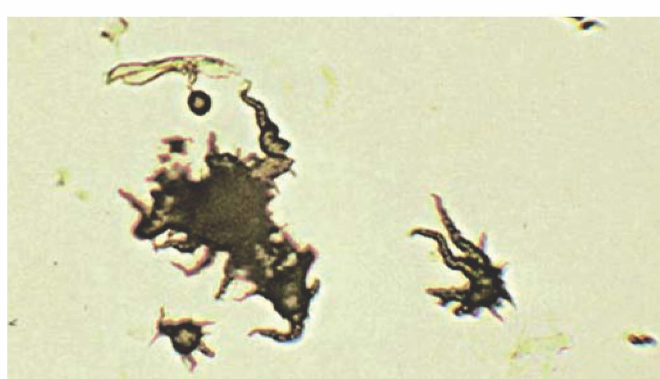


Figure 10-87. Ammonium biurate crystals (200×).

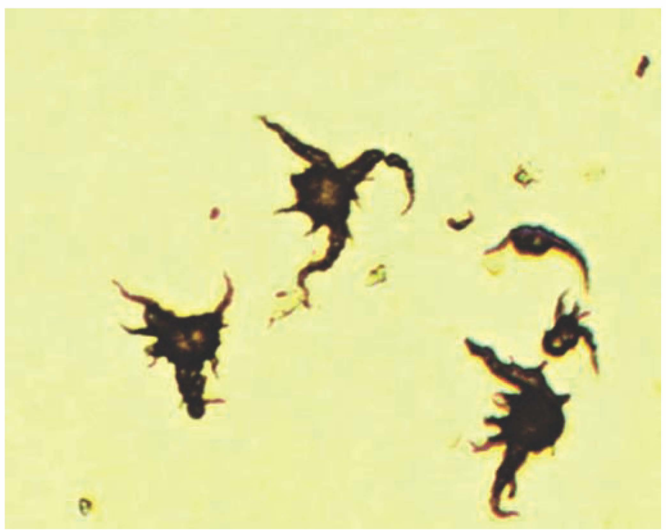


Figure 10-88. Ammonium biurate crystals (200×).

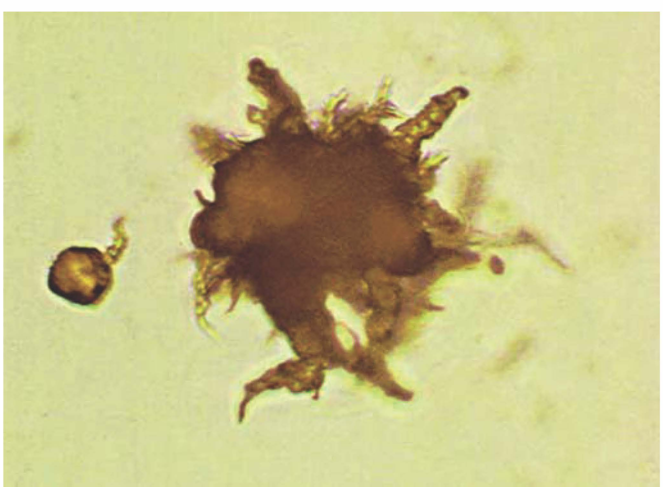


Figure 10-89. Ammonium biurate crystals (500×).

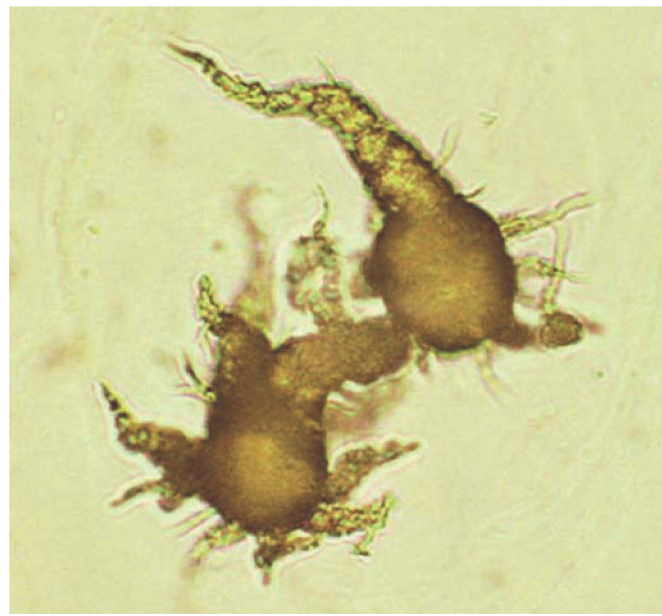


Figure 10-90. Ammonium biurate crystals (500×).

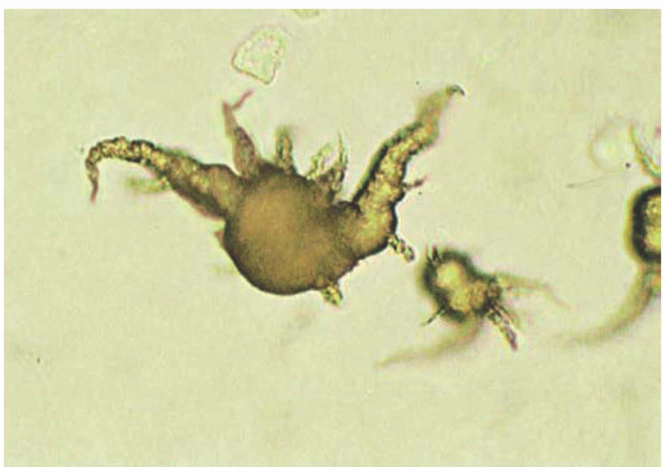


Figure 10-91. Ammonium biurate crystals (500×).

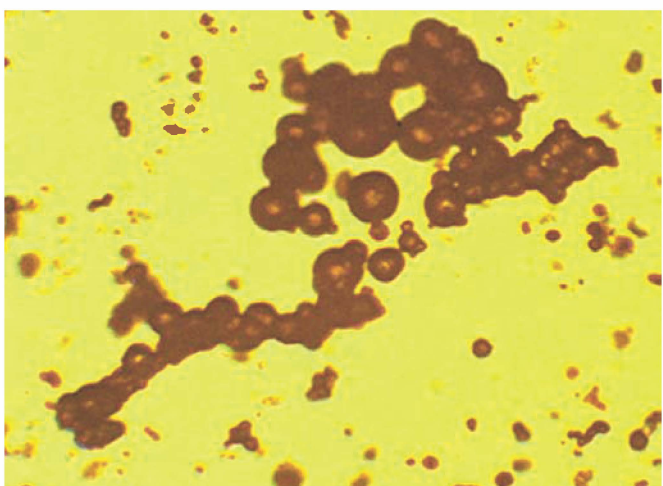


Figure 10-93. Ammonium biurate crystals. These are the spheroid form of the crystal (500×).

Figure 10-92. Ammonium biurate crystal and a squamous epithelial cell (500×).

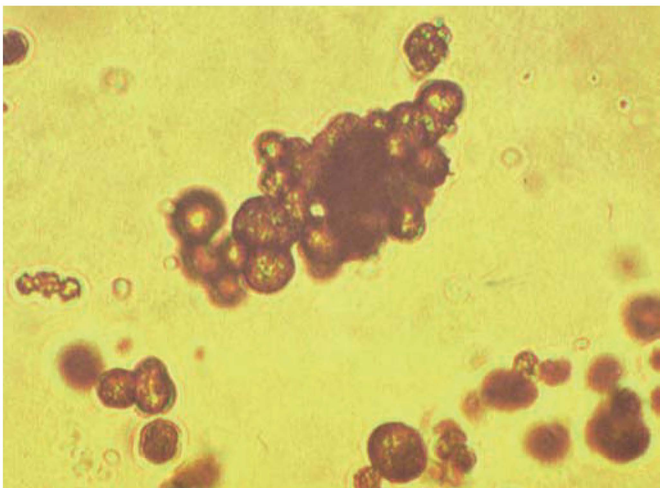


Figure 10-94. Ammonium biurate crystals. Spheroid form without spicules (400×).

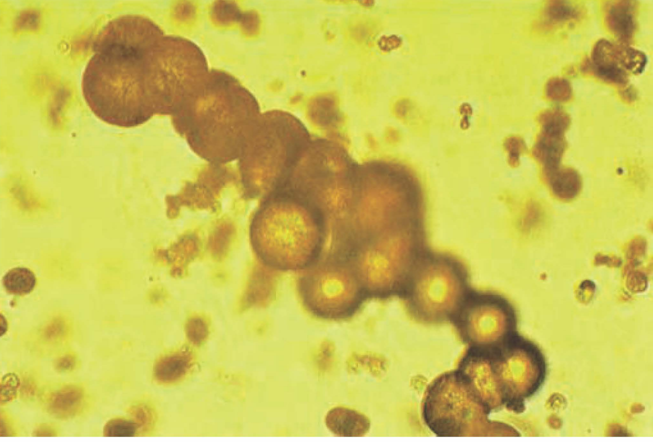


Figure 10-95. Ammonium biurate crystals (500×).

CASTS

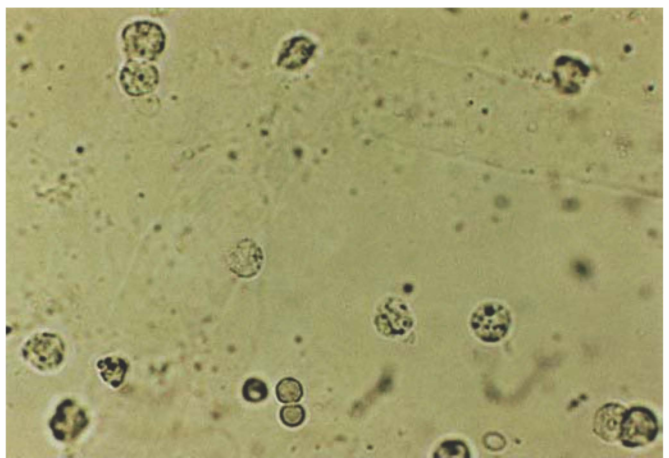


Figure 10-96. Hyaline cast, WBCs, RBCs, and bacteria. Can you see the bent cast? (500×).

Figure 10-99. Hyaline casts. Viewed with an 80A filter (400×).

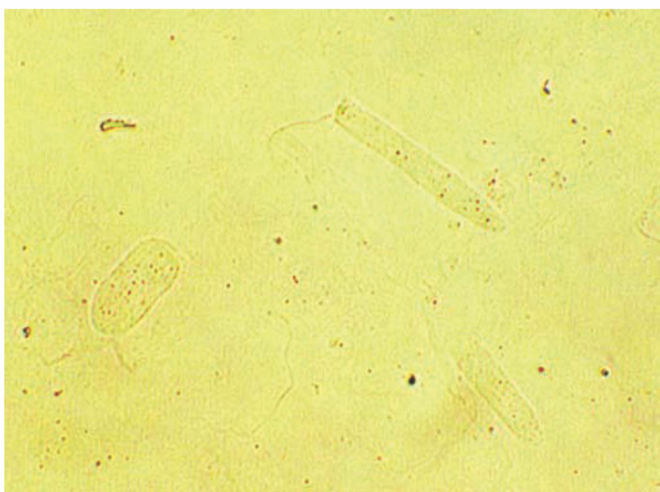


Figure 10-97. Hyaline casts. How many casts can you find? (200×).

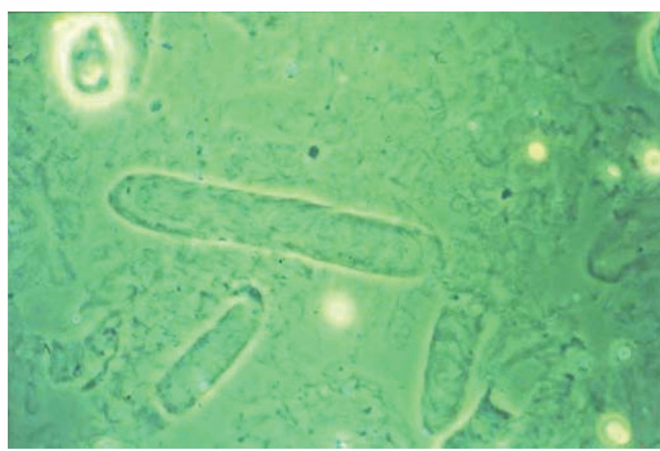


Figure 10-100. Hyaline casts using phase contrast microscopy. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

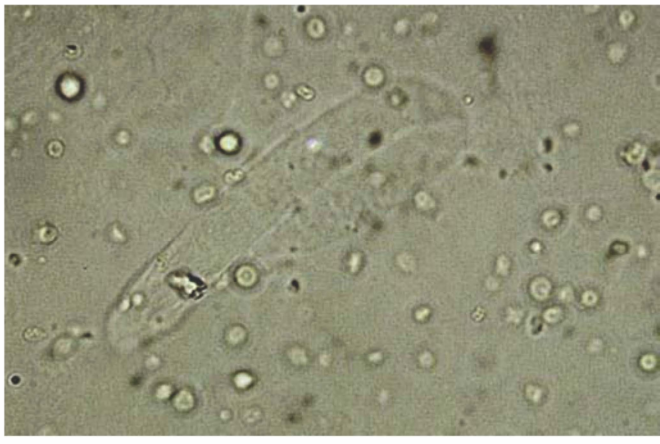


Figure 10-98. Hyaline cast that is bent back upon itself and many RBCs. Viewed with an 80A filter (400×).

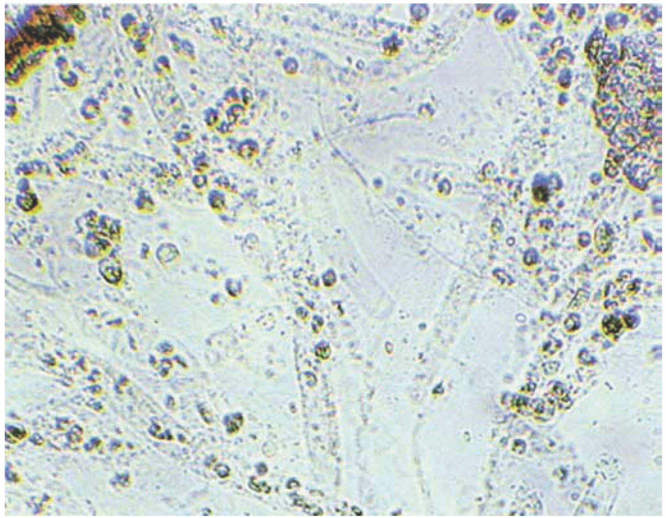


Figure 10-101. Many hyaline casts and WBC casts and rare RBC (200×).

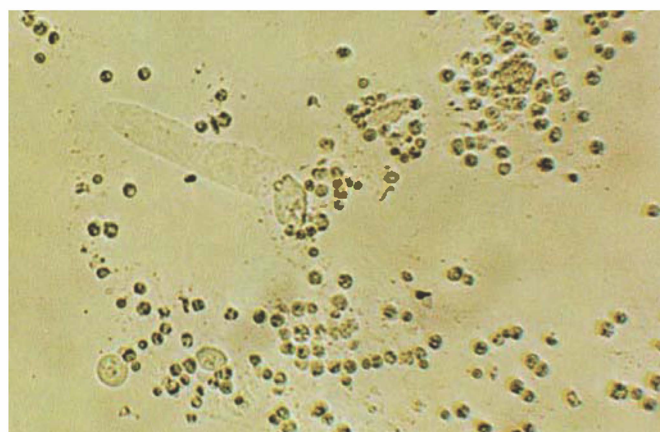


Figure 10-102. Hyaline cast, WBCs, RBCs, and epithelial cells (200×).

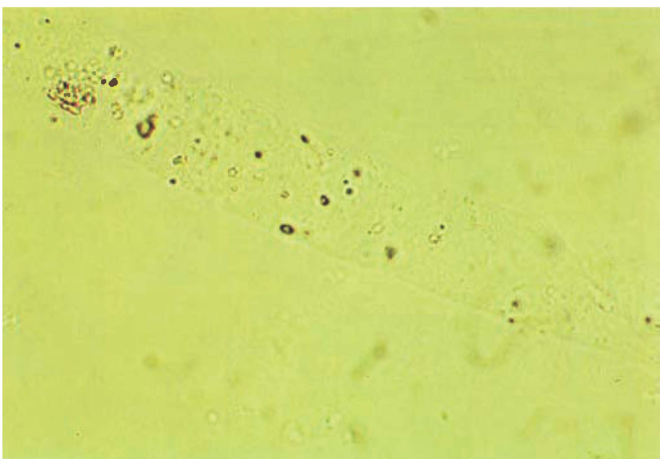


Figure 10-103. Hyaline cast with a few granular inclusions (500×).

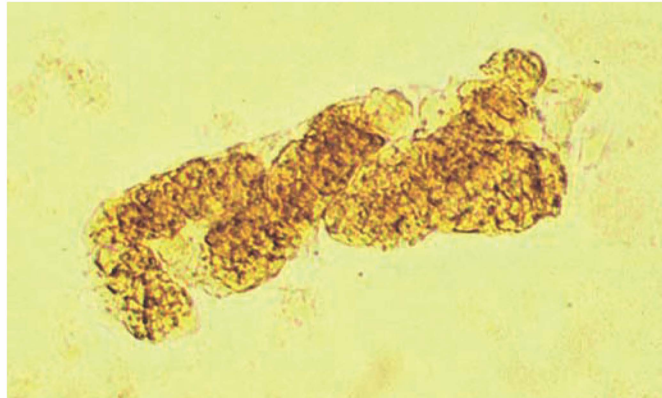


Figure 10-104. Convoluted red blood cell cast (500×).

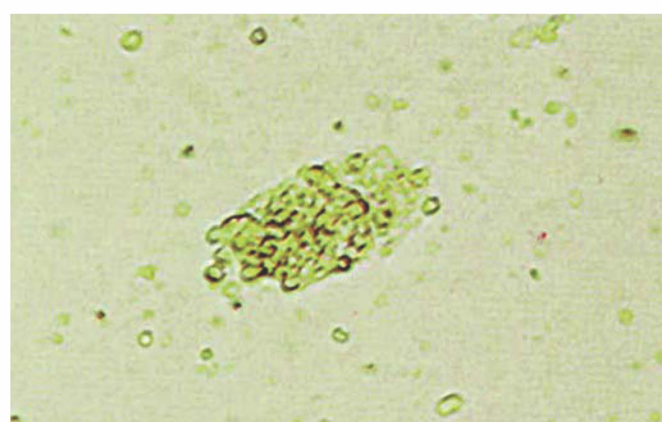


Figure 10-105. Red blood cell cast and many RBCs. The cells in the cast are still intact (500×).

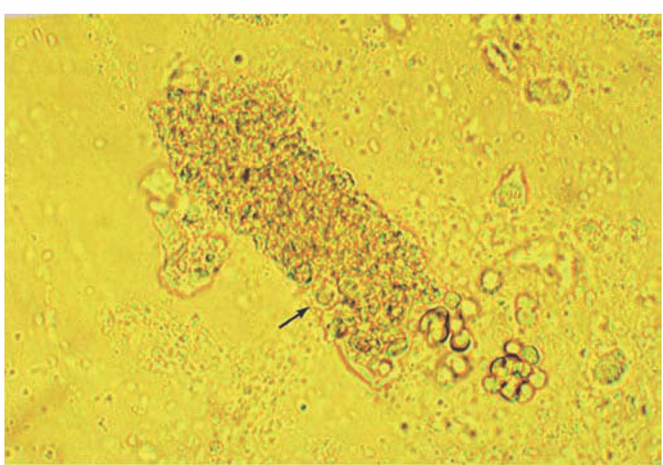


Figure 10-106. Red blood cell cast. There are still some intact cells in the cast (*arrow*), although many of the cells have begun to degenerate (500×).

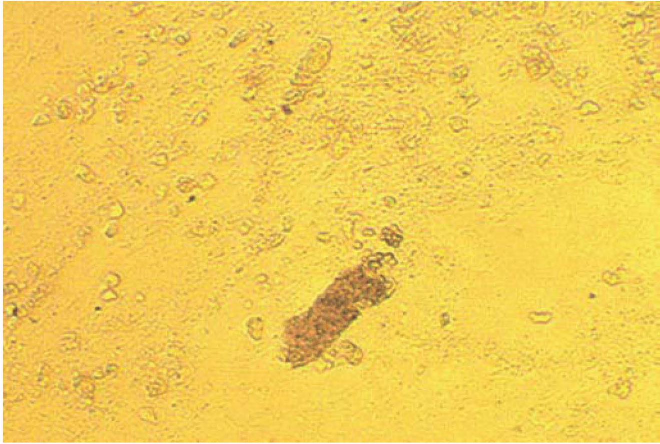


Figure 10-107. Red blood cell cast. When the cast in the previous figure is viewed under low power, the color of the cast is more prominent (200×).

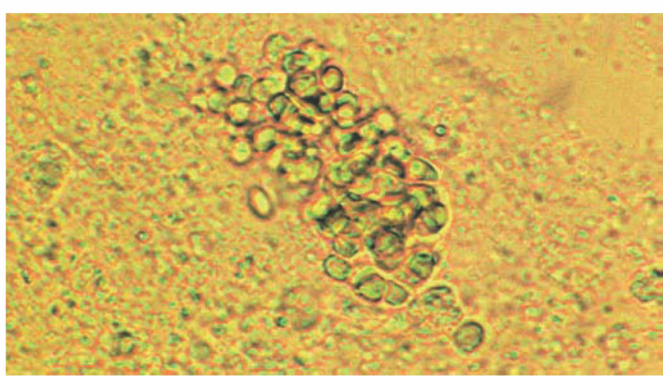


Figure 10-108. Red blood cell cast and amorphous urates (500×).

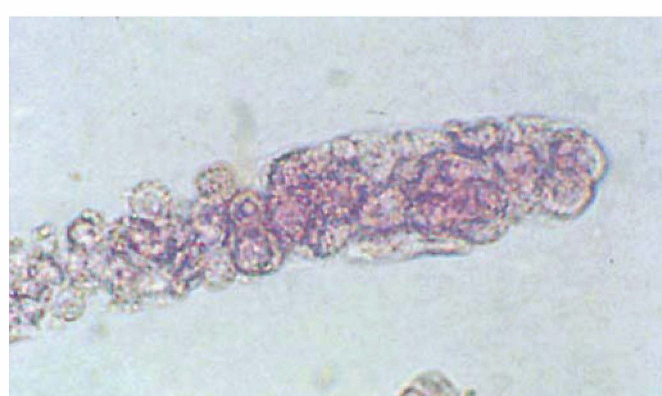


Figure 10-111. SM-stained WBC cast (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia: Lippincott, 1998.)

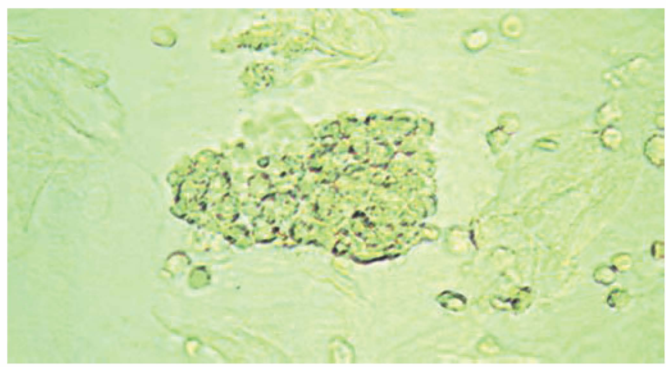


Figure 10-109. White blood cell cast, WBCs, squamous epithelial cells, and mucus (400×).

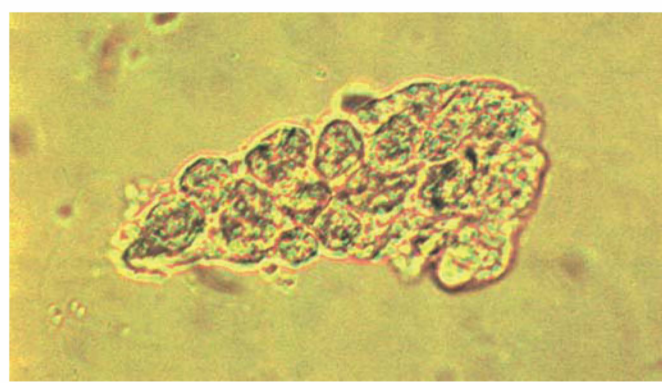


Figure 10-112. White blood cell cast (400×).

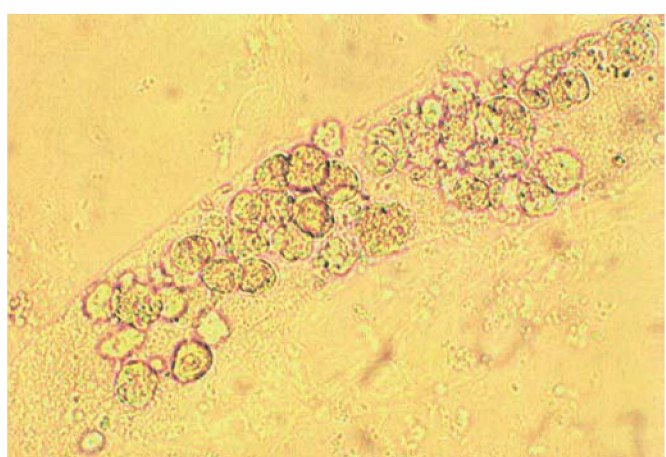


Figure 10-110. White blood cell cast. The protein matrix is clearly visible (500×).

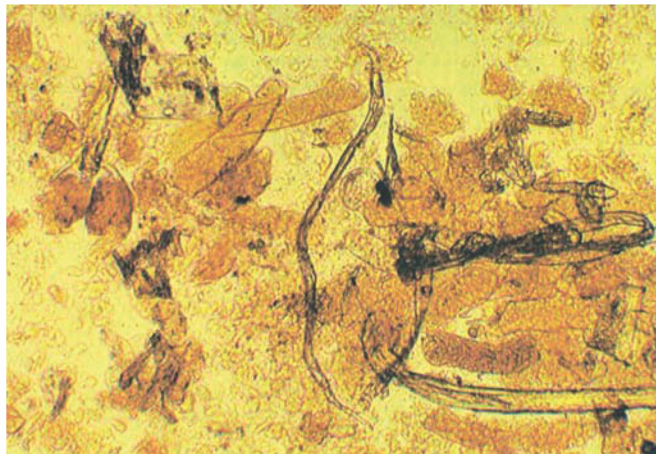


Figure 10-113. Bilirubin-stained casts, fibers, and sediment (200×).

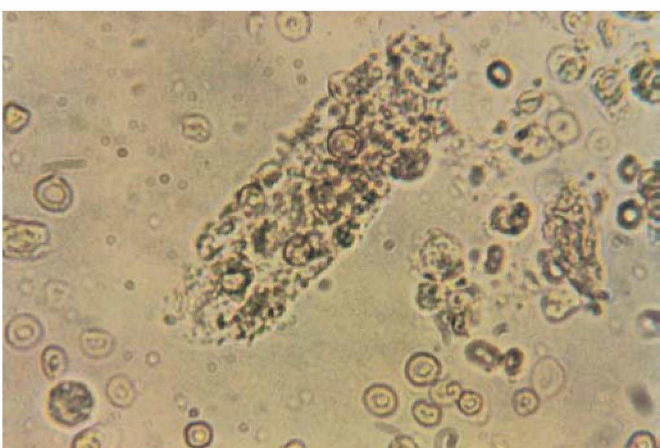


Figure 10-114. Mixed cell cast, WBCs, and RBCs. This cast contains degenerating WBCs and several RBCs (500×).

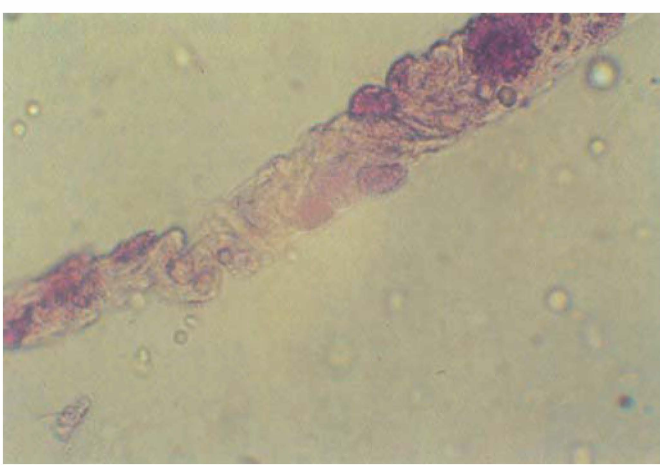


Figure 10-117. SM-stained mixed cellular cast including renal tubular epithelial cells (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

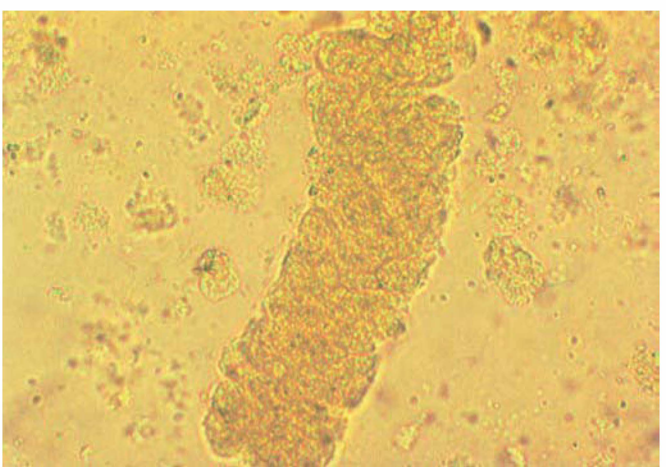


Figure 10-115. Bilirubin-stained WBC cast. Bilirubin staining can cause problems in identifying structures, but you can see some cell outlines. In addition, the WBCs are beginning to degenerate, creating a granular cast appearance (500×).

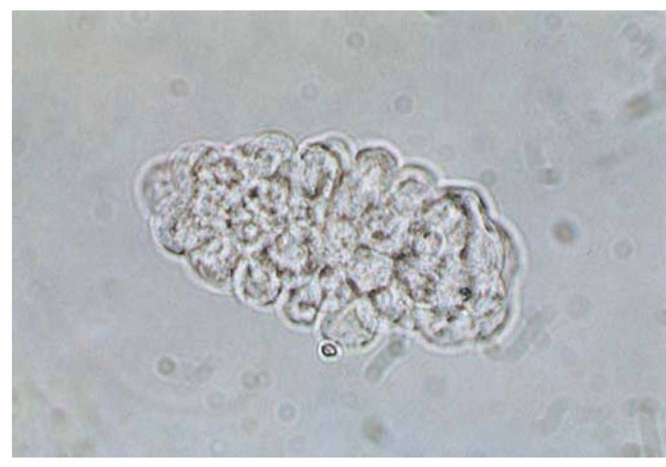


Figure 10-118. Epithelial cell cast. The nuclei are visible in some of the cells (500×).

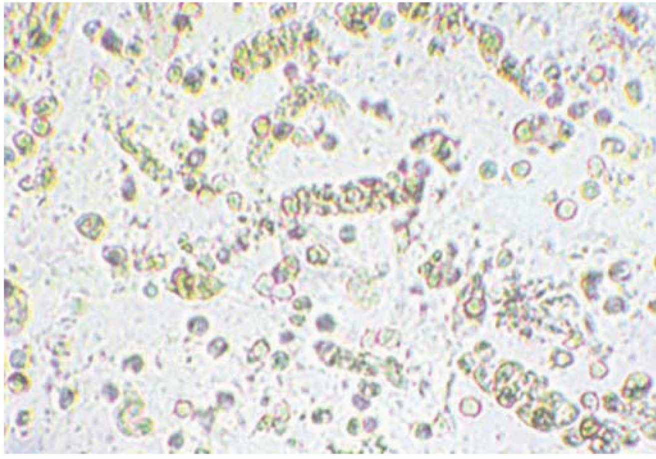


Figure 10-116. Many WBC casts and many WBCs (200×).

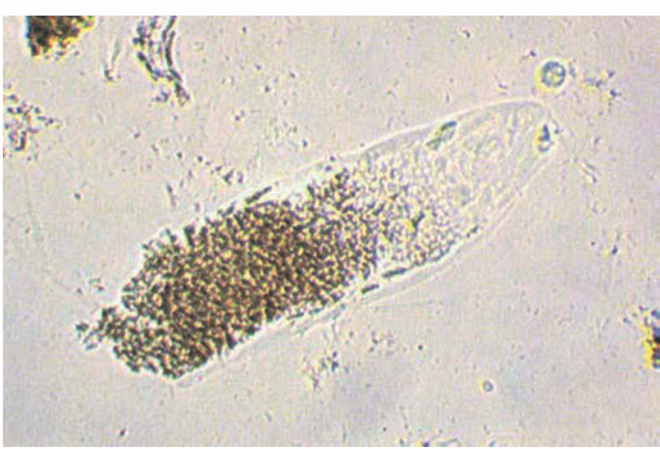


Figure 10-119. Mixed cast. This cast is half hyaline and half granular. Report as "hyaline" and/or "granular," but not "mixed" cast (400×).

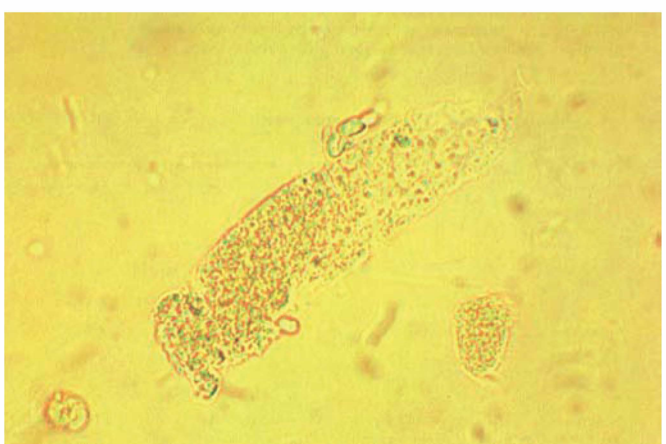


Figure 10-120. Mixed cast, yeast, and a WBC. This cast is also half hyaline and half granular (500×).

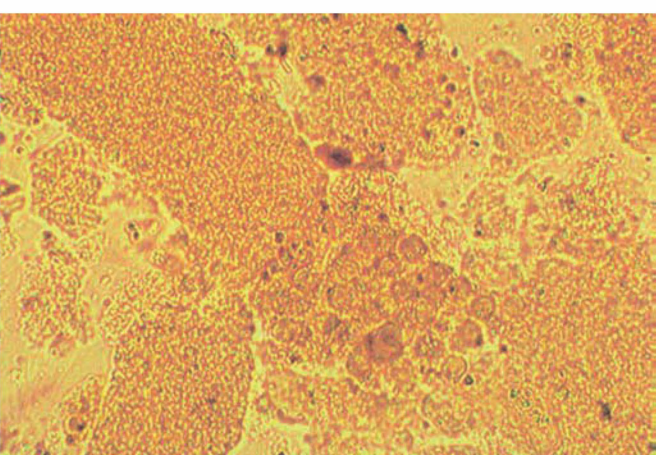


Figure 10-123. Broad mixed granular and RBC cast, and a broad granular cast. Higher magnification of previous figure. This specimen is from a patient with Wilson disease (500×).

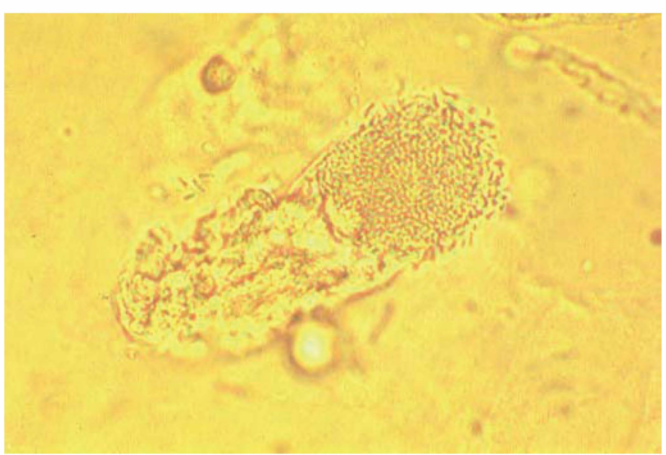


Figure 10-121. Mixed cast. Note bacteria in one-half of the cast. Bacterial casts are not very common (500×).

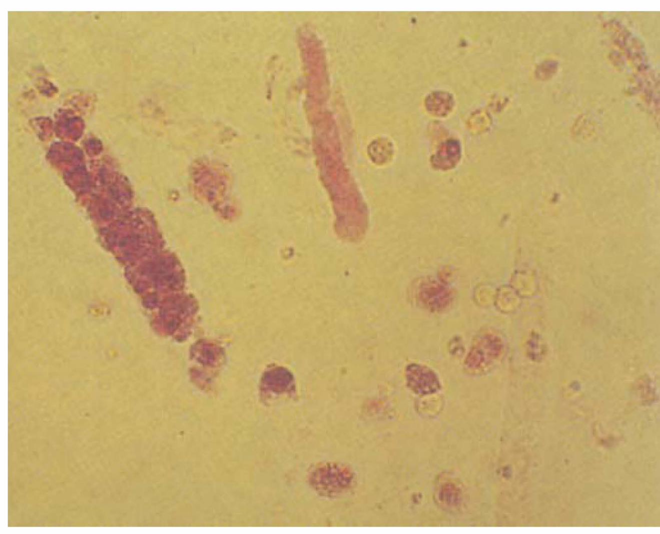


Figure 10-124. SM-stained hyaline cast, granular cast, mixed cellular cast, and partially degenerated renal tubule epithelial cells (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

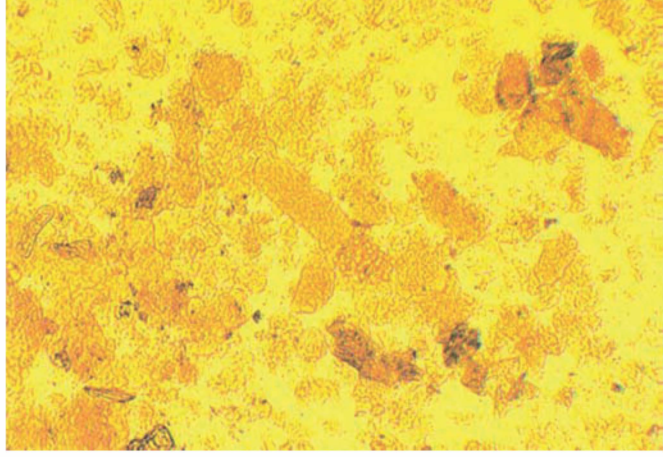


Figure 10-122. Many casts, WBCs, RBCs, and amorphous sediment, all of which are stained with bilirubin (200×).

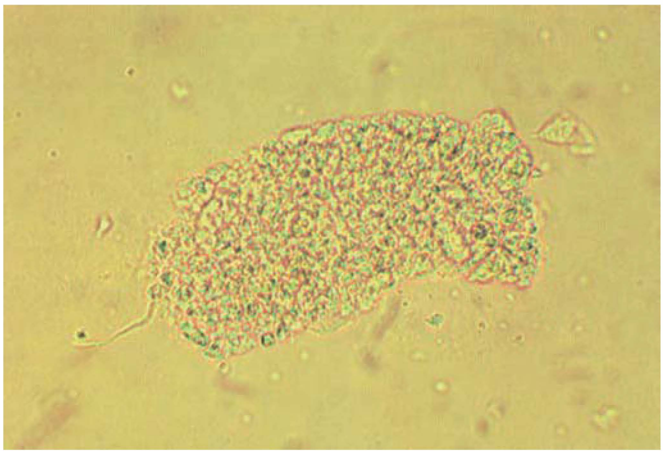


Figure 10-125. Broad granular cast (400×).

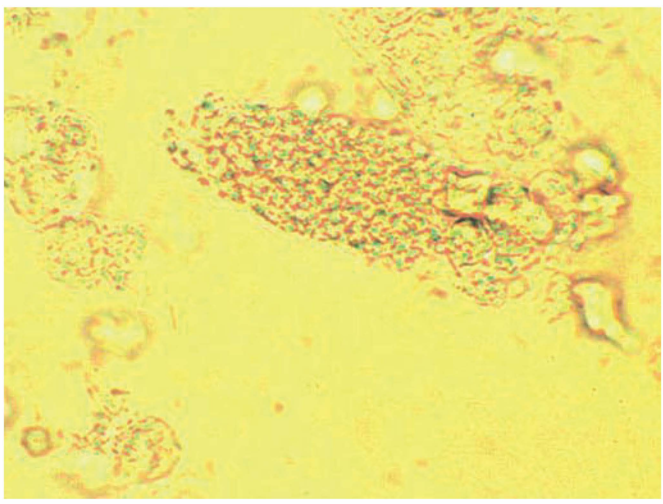


Figure 10-126. Fine granular cast, WBCs, and RBCs (500×).

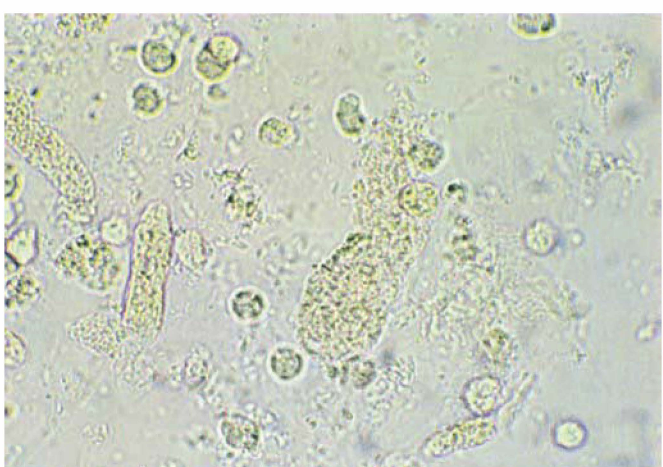


Figure 10-129. Fine granular casts and WBCs (400×).

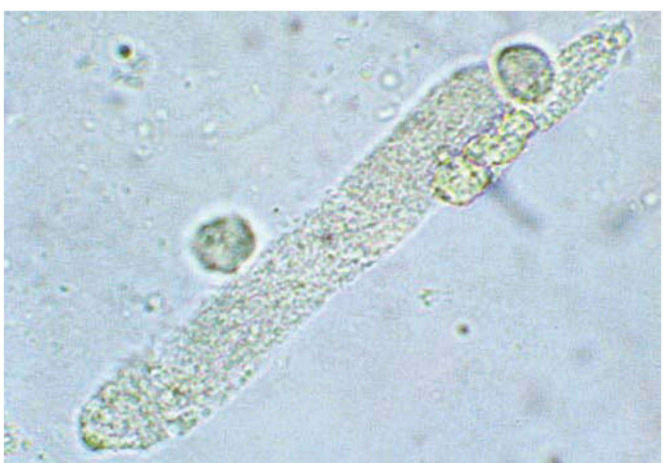


Figure 10-127. Fine granular casts and WBCs. Note the smaller cast (500×).

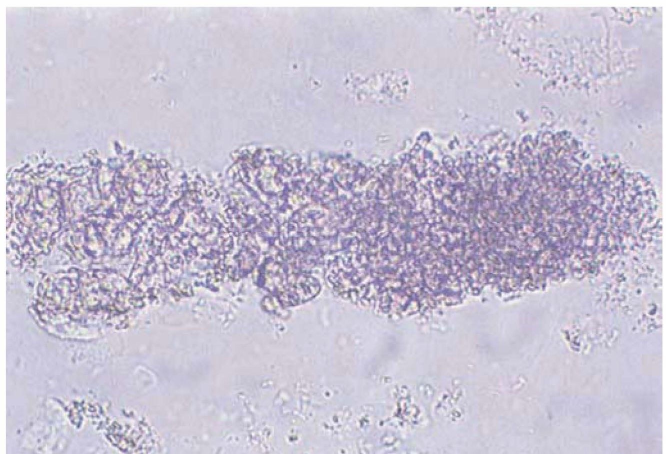


Figure 10-130. Coarse granular cast (500×).

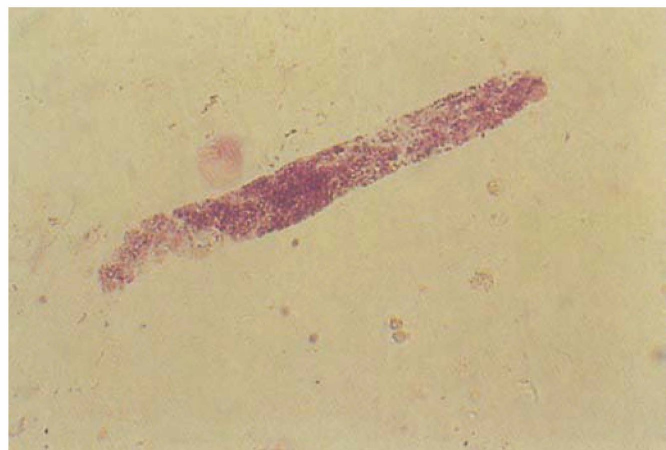


Figure 10-128. SM-stained granular cast (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

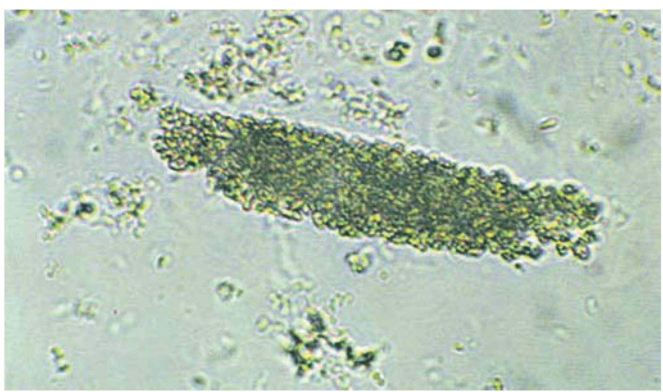


Figure 10-131. Coarse granular cast (400×).

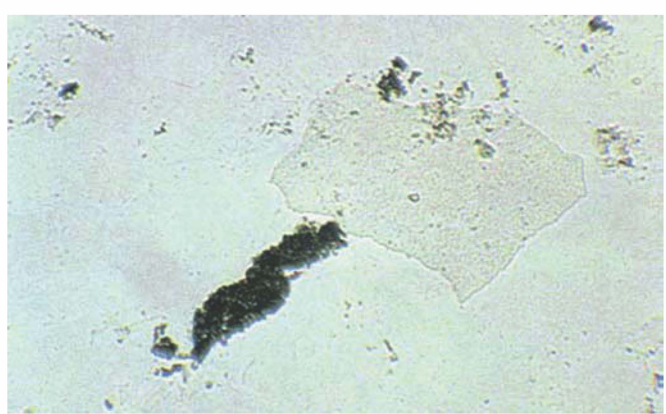


Figure 10-132. Coarse granular cast, calcium phosphate plate, and amorphous phosphates (200×).

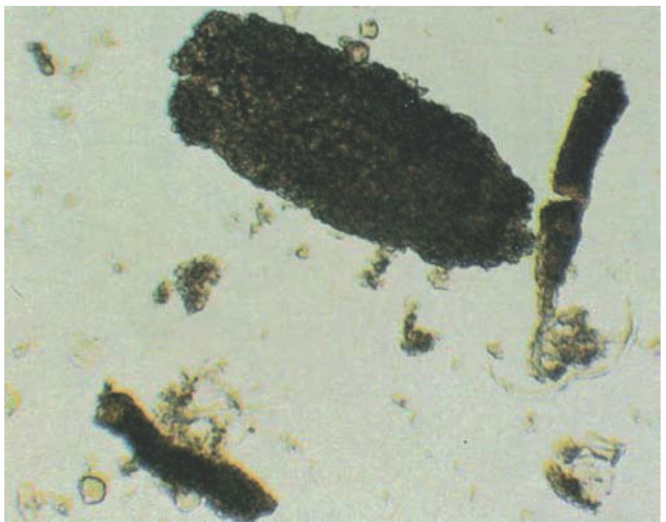


Figure 10-133. Broad and narrow coarse granular casts (200×).

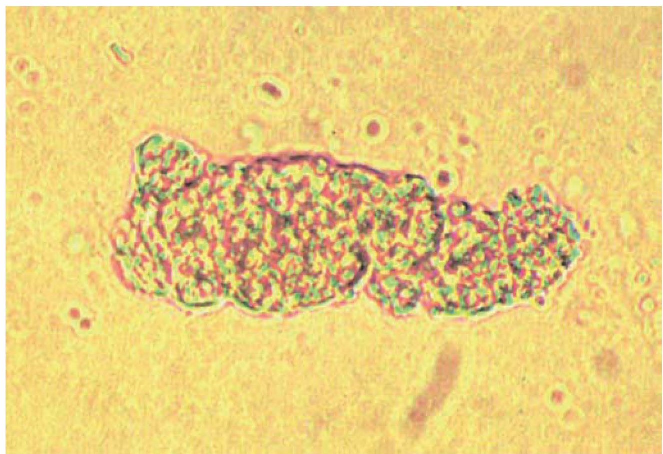


Figure 10-134. Coarse granular cast (400×).

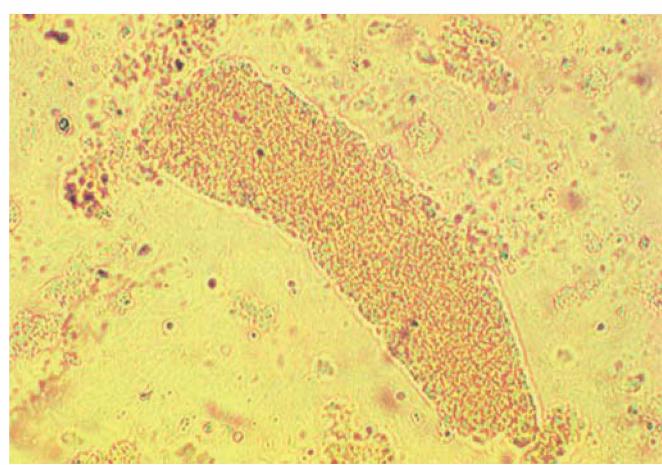


Figure 10-135. Bilirubin-stained granular cast (500×).

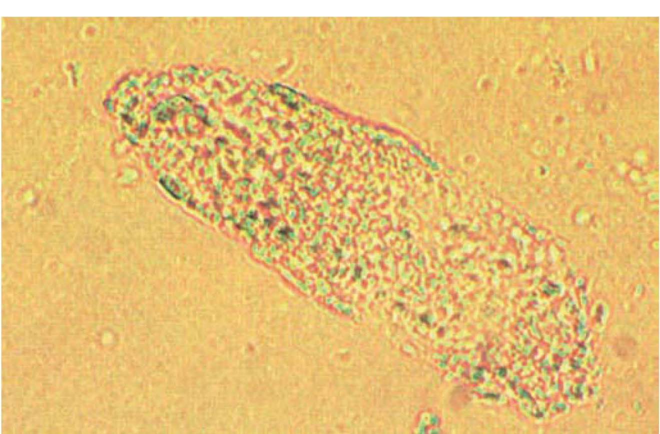


Figure 10-136. Fine granular cast (400×).

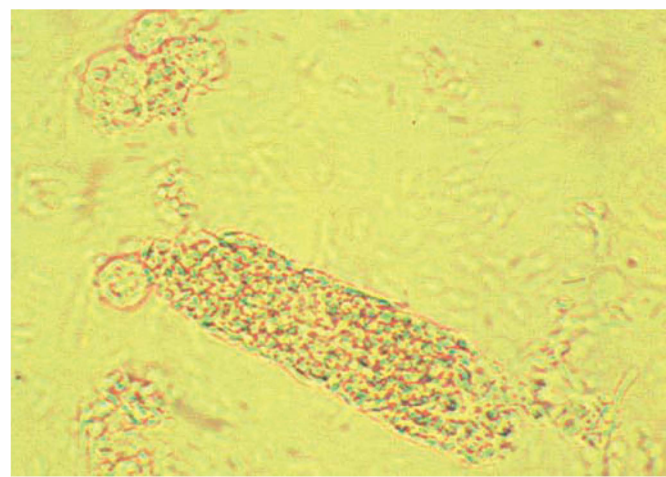


Figure 10-137. Fine granular cast, WBCs, and bacteria (400×).

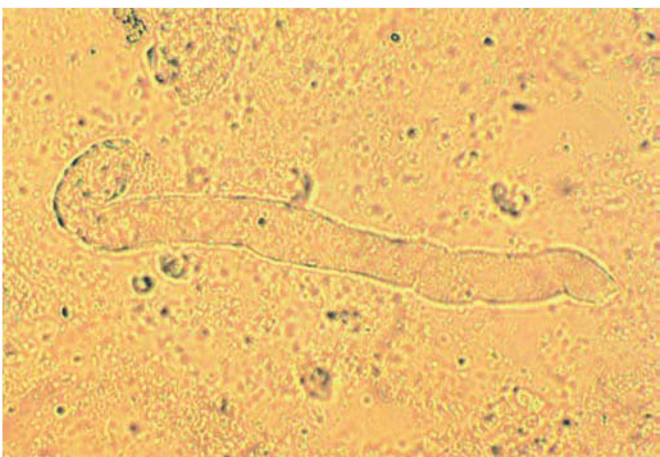


Figure 10-138. Waxy cast and amorphous urates. Note the indentations on the sides of the cast (500×).

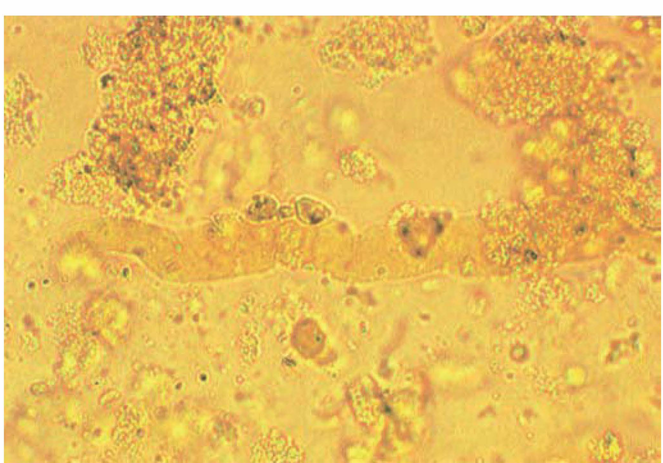


Figure 10-139. Bilirubin-stained waxy cast, granular cast, WBCs, and amorphous sediment. Note the convolutions near the center of the waxy cast (500×).

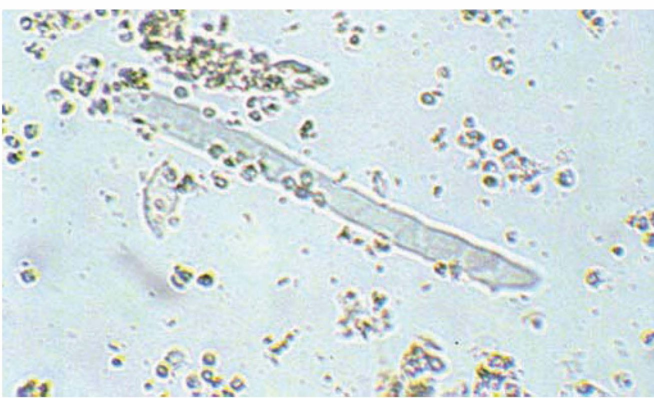


Figure 10-140. Long waxy cast, WBCs, and an epithelial cell. The surface of this cast is more refractile than that of a hyaline cast (200×).

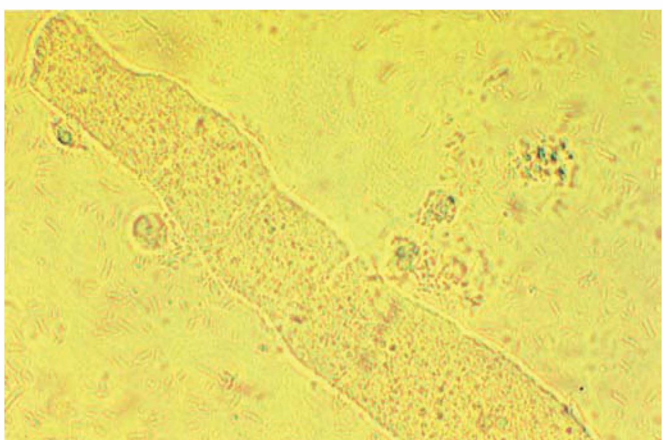


Figure 10-141. Fine granular cast becoming a waxy cast. This cast would best be classified as a waxy cast, because of the typical cracks on the sides of this cast, even though the surface is still granular (500×).

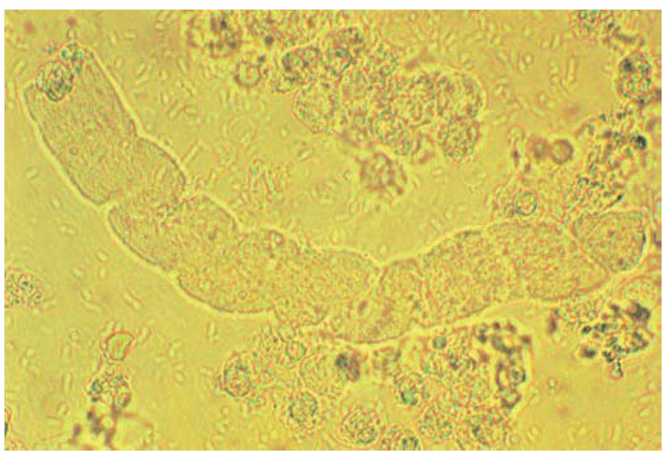


Figure 10-142. Convoluted waxy cast. This field also contains WBCs, rare RBC, and bacteria (500×).

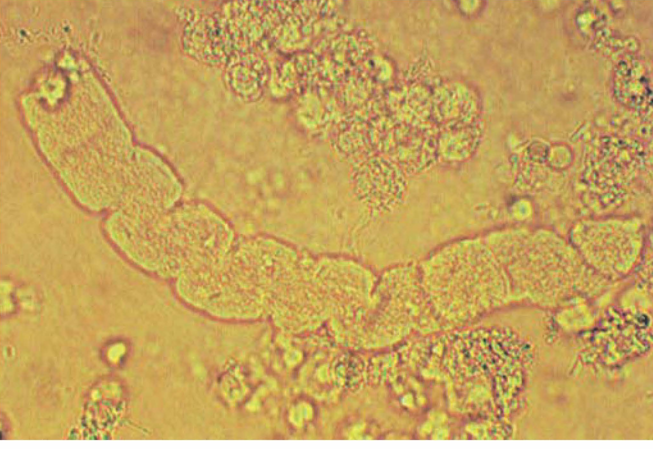


Figure 10-143. Convoluted waxy cast. This is the same image as the previous figure, but when the fine adjustment is turned slightly, the cast seems to develop a dark edge because of the high refractive index of the cast (500×).

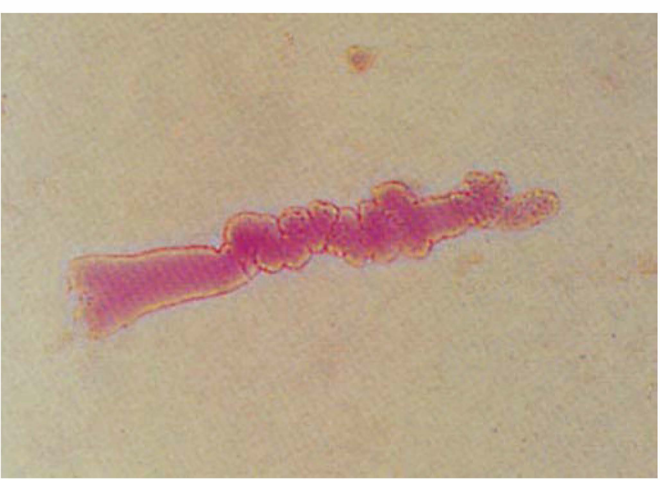


Figure 10-144. SM-stained waxy cast (100×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

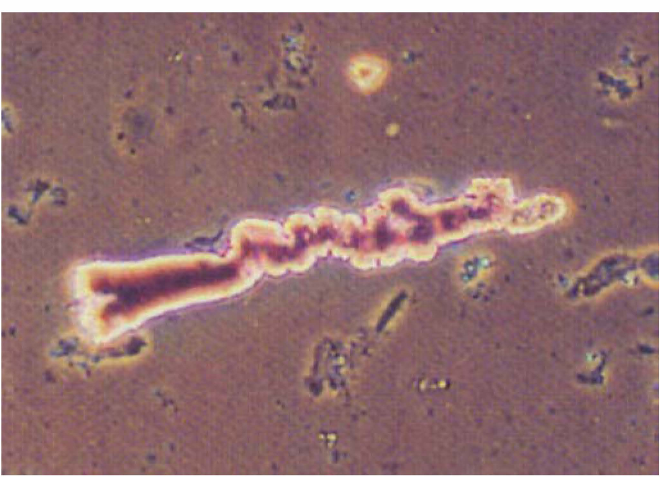


Figure 10-145. Same field as previous figure using phase contrast microscopy (100×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

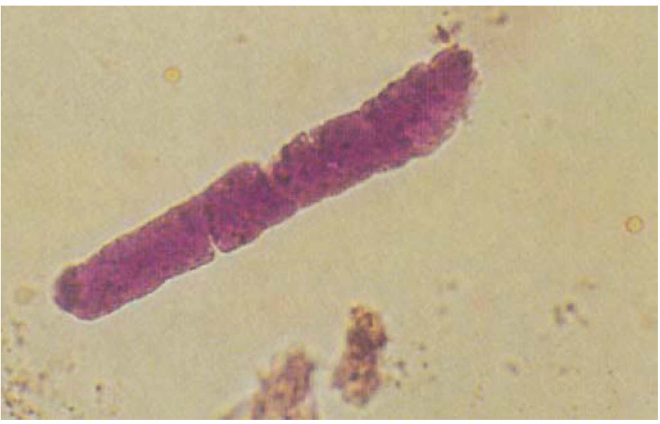


Figure 10-146. SM-stained waxy-granular cast (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

MISCELLANEOUS IMAGES

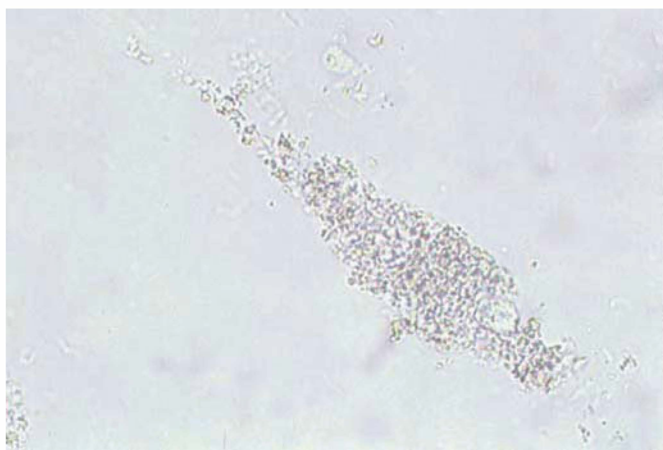


Figure 10-147. Granular cylindroid (500×).

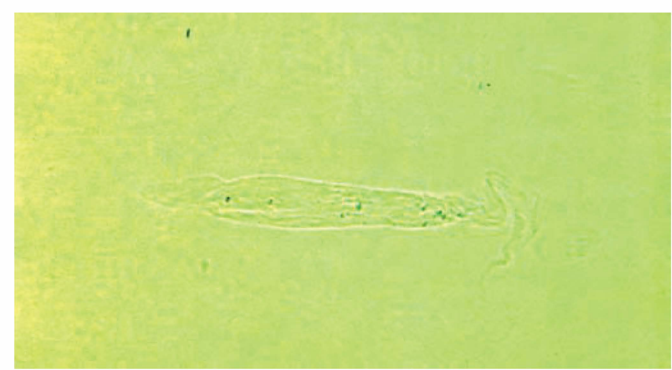


Figure 10-148. Hyaline cylindroid. Note the tapering tail (160×).

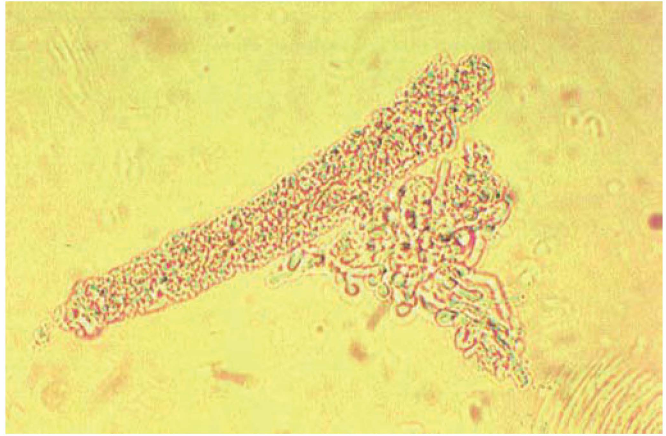


Figure 10-149. Fine granular cast and yeast (400×).

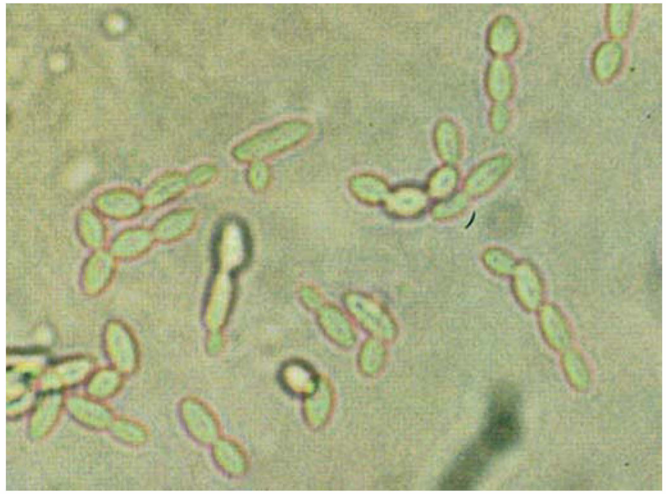


Figure 10-152. Yeast (1,000×).

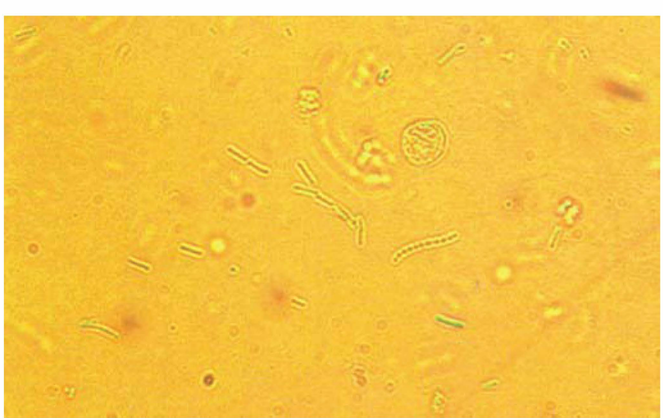


Figure 10-150. Bacteria. This field contains rods, cocci, and chains (500×).

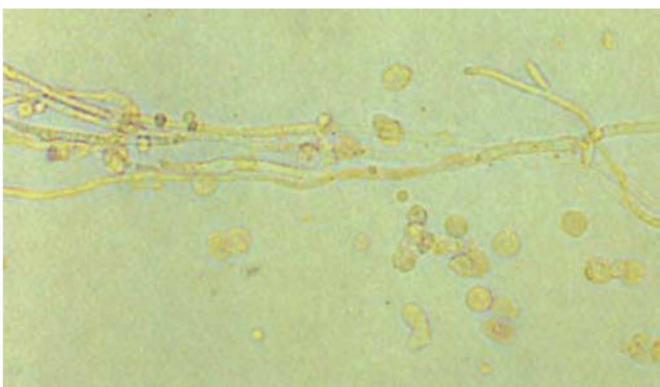


Figure 10-153. SM-stained yeast with pseudohyphae and WBCs (200×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

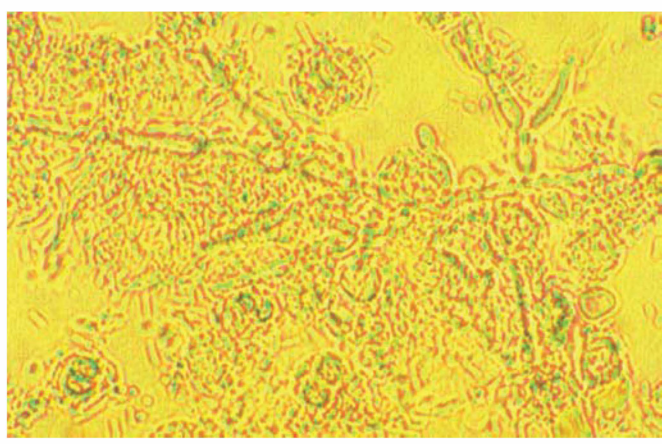


Figure 10-151. Yeast, WBCs, rare RBC, and bacteria (500×).

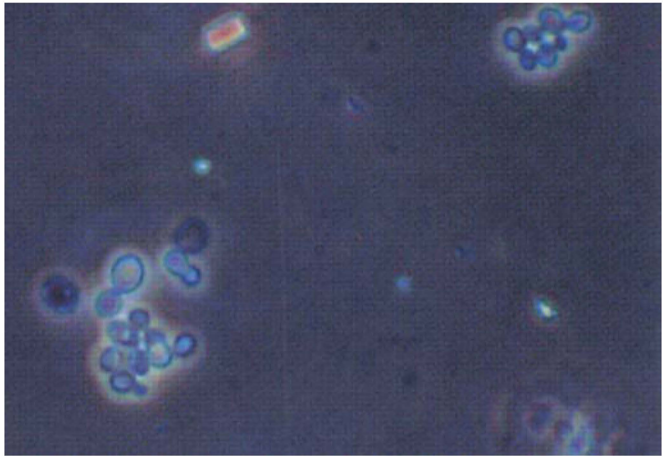


Figure 10-154. Yeast under phase contrast microscopy (400×). (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

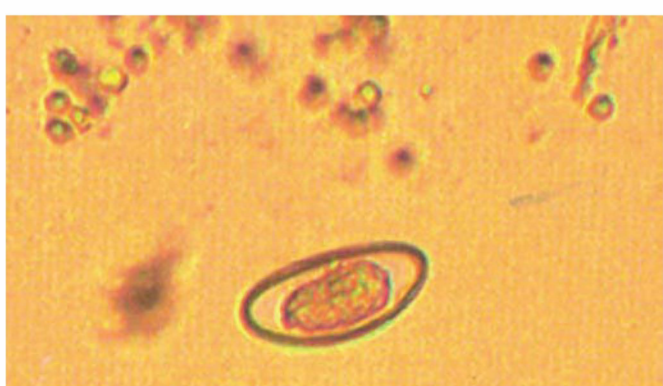


Figure 10-155. Pinworm ovum and WBCs (100×). The characteristics of the pinworm ovum are easily recognized, even under low-power magnification.

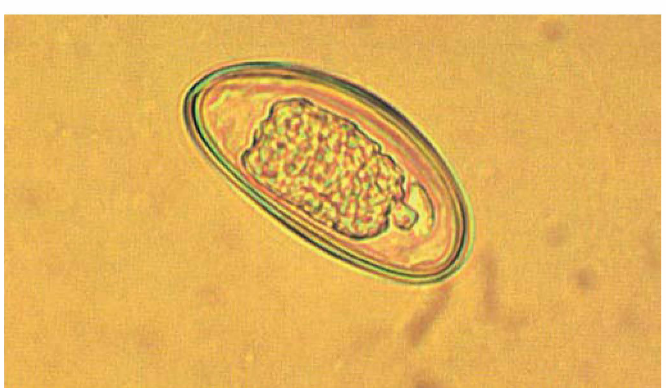


Figure 10-156. *Enterobius vermicularis* or pinworm ovum (400×).

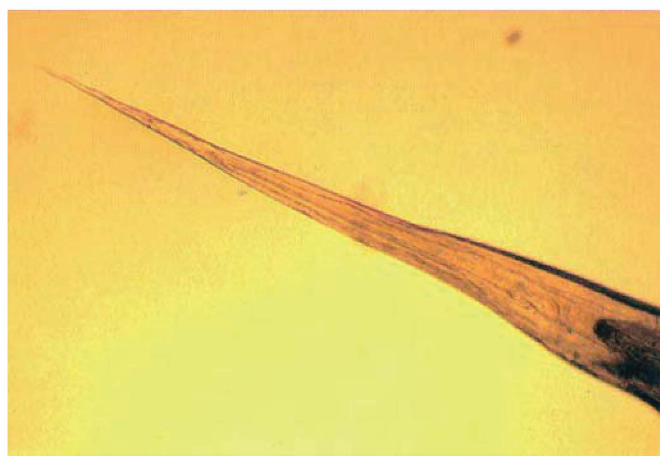


Figure 10-157. Tail of the adult female pinworm. The tail of the female is straight and very pointed, whereas the tail of the male is curved (40×).

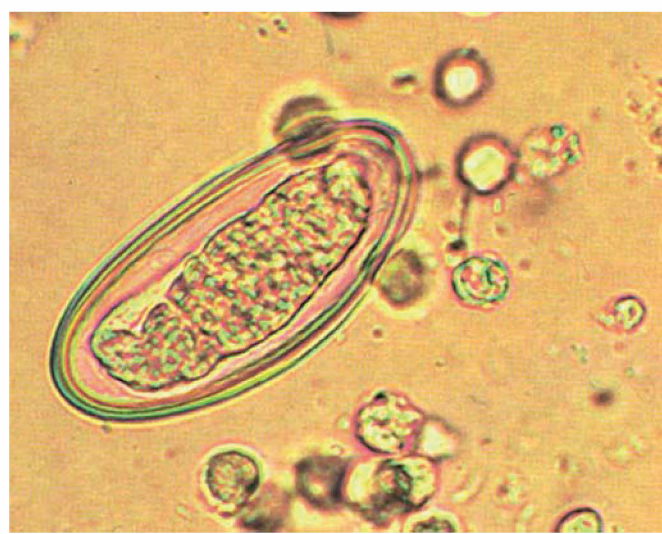


Figure 10-158. Pinworm ovum and WBCs (500×).

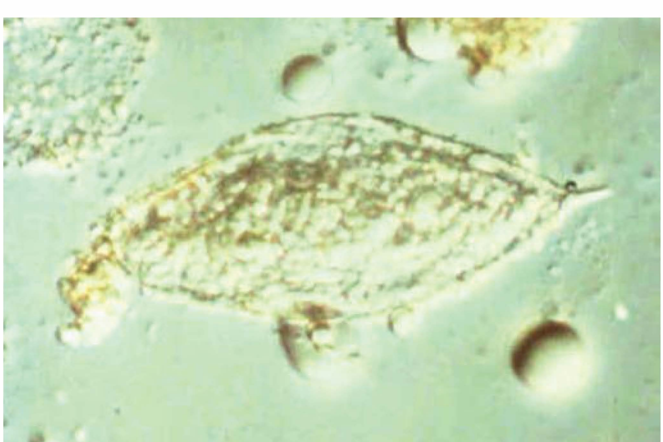


Figure 10-159. *Schistosoma haematobium* ovum under interference contrast microscopy. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

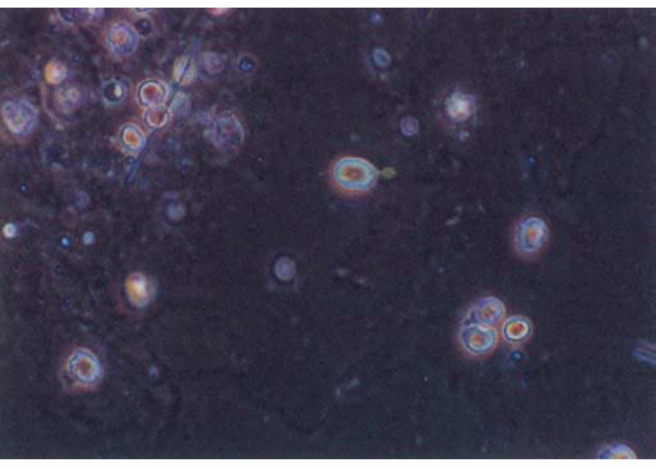


Figure 10-160. *Trichomonas vaginalis* with mixed cellular background viewed under phase contrast microscopy. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia: Lippincott, 1998.)

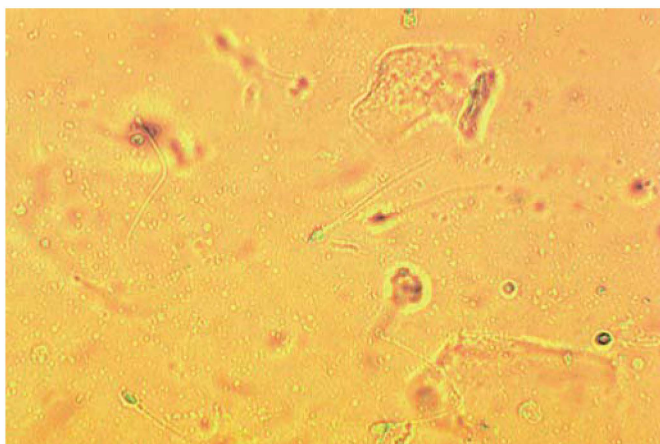


Figure 10-161. Sperm and epithelial cells (500×).

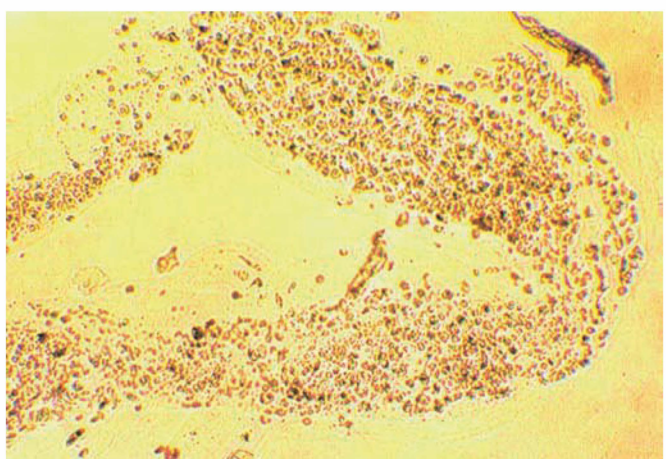


Figure 10-164. Mucus containing WBCs and RBCs (200×).

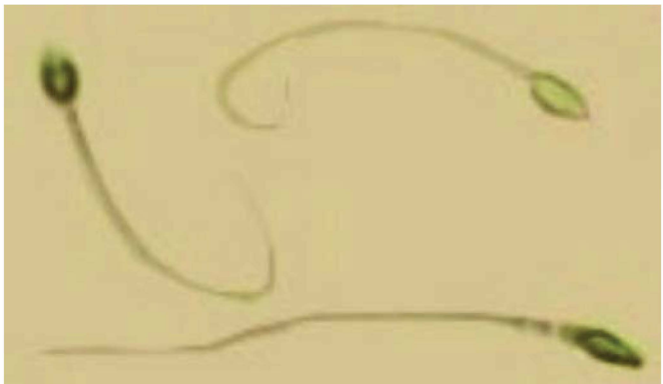


Figure 10-162. Sperm (wet-mount).

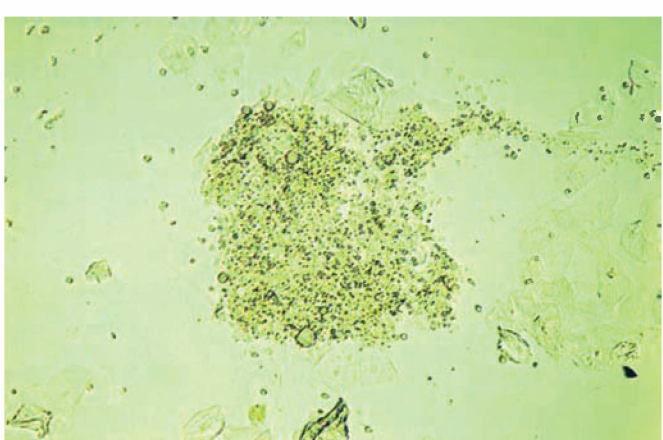


Figure 10-165. Fat droplets and epithelial cells (160×).

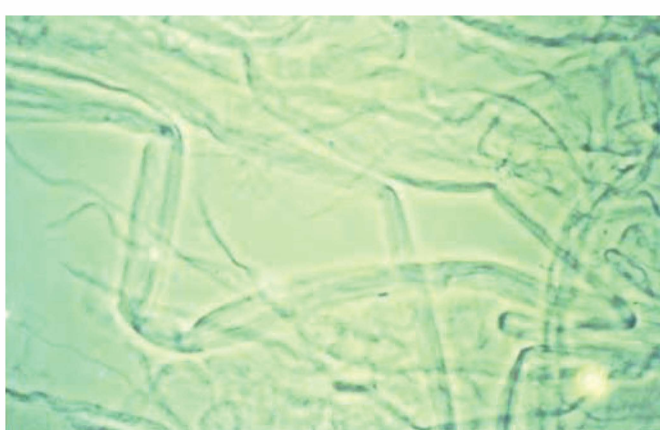


Figure 10-163. Mucus. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

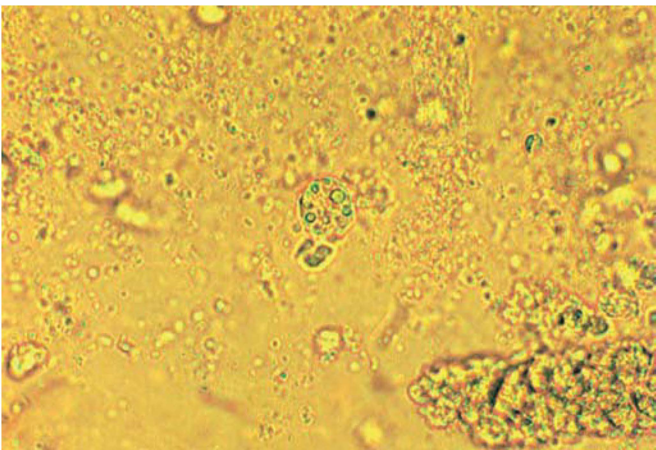


Figure 10-166. Oval fat body, granular cast, and amorphous urates. The oval fat body contains only a few fat droplets, thus, having a smaller size than other fat bodies (500×).

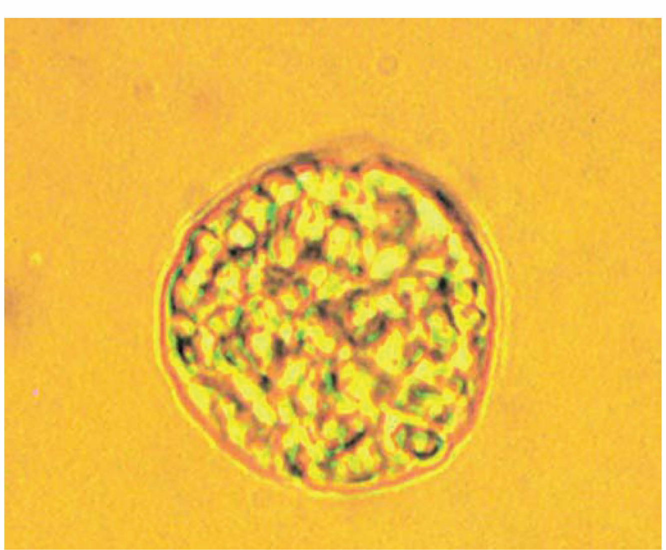


Figure 10-167. Oval fat body (400×).

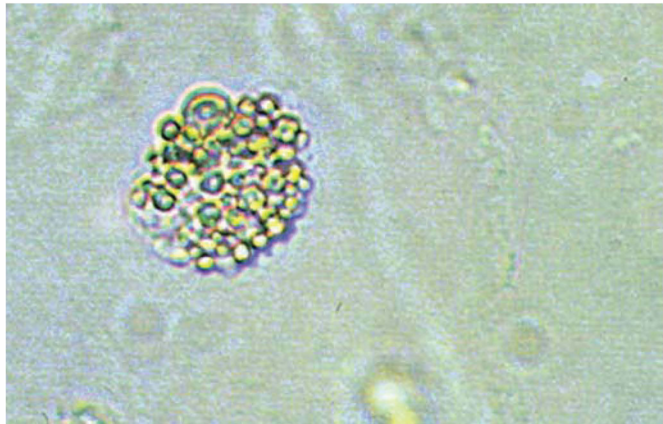


Figure 10-168. Oval fat body. The cell is bulging with fat droplets, so the cell membrane is not visible (500×).

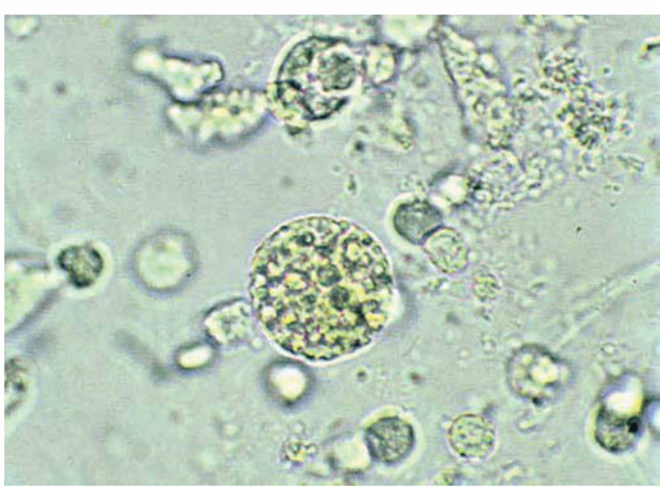


Figure 10-169. Oval fat bodies and WBCs (500×).

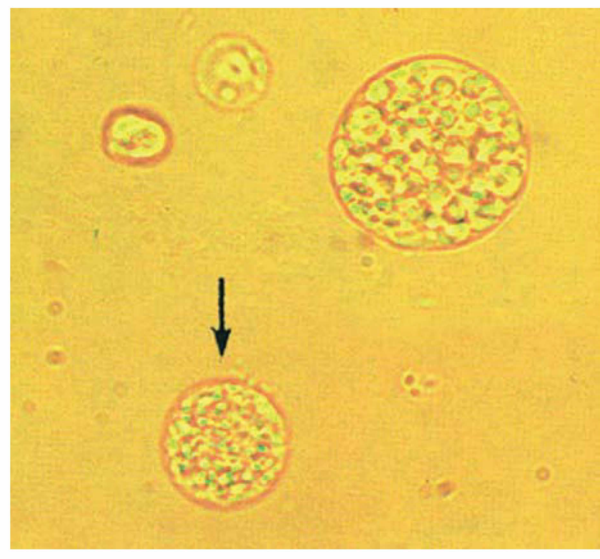


Figure 10-170. Oval fat body. This field also contains a cell with a few small fat droplets in it (*arrow*) (400×).

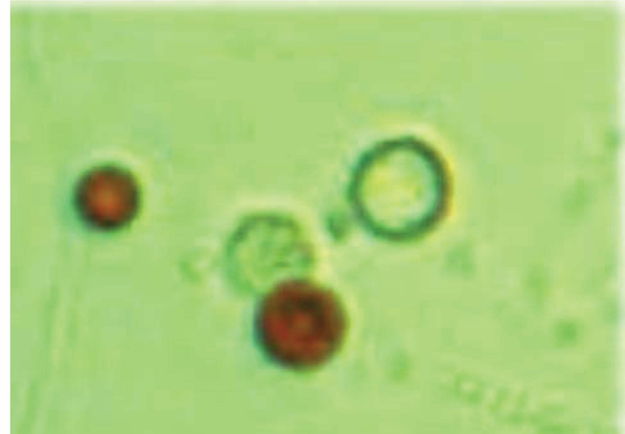


Figure 10-171. Sudan III-stained fat droplets. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

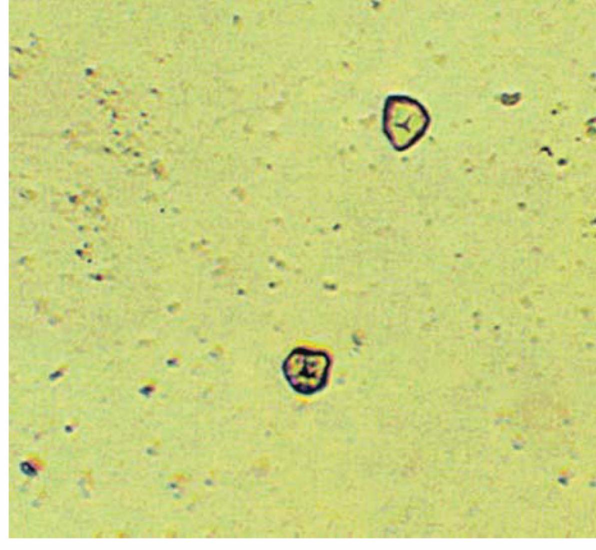


Figure 10-172. Starch granules (200×).

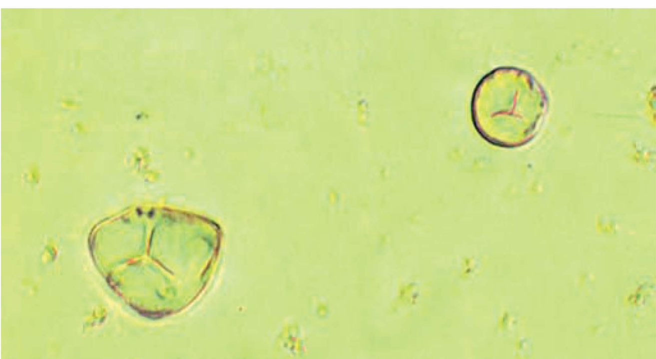


Figure 10-173. Starch crystals. The indentation in the center of each crystal is very distinguishable (500×).

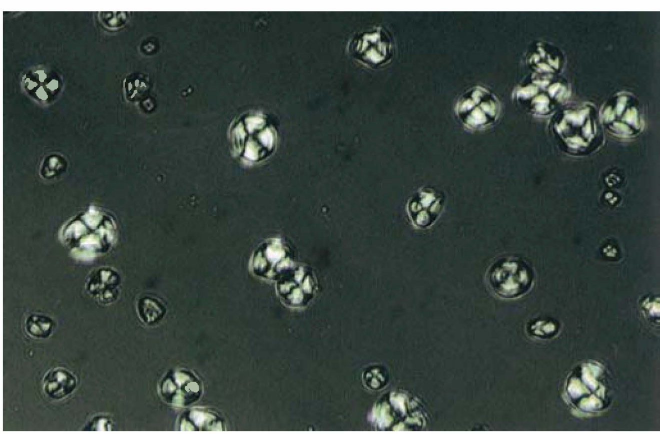


Figure 10-175. Starch crystals under polarized light demonstrating the typical "Maltese-cross" formation (400×).

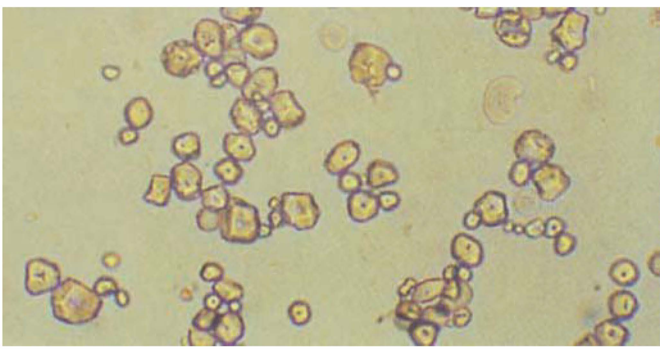


Figure 10-174. Starch granules. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

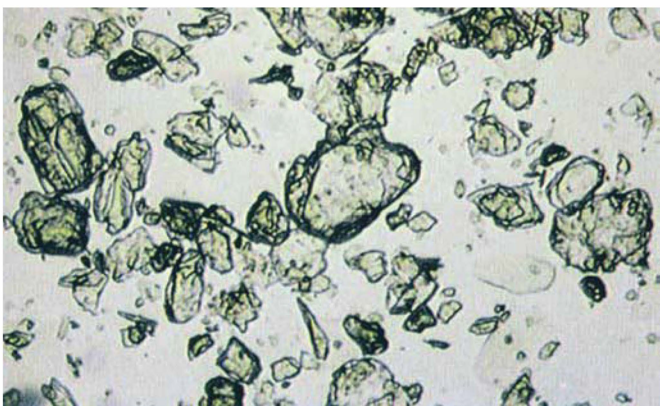


Figure 10-176. Talcum powder particles and a few squamous epithelial cells (160×).

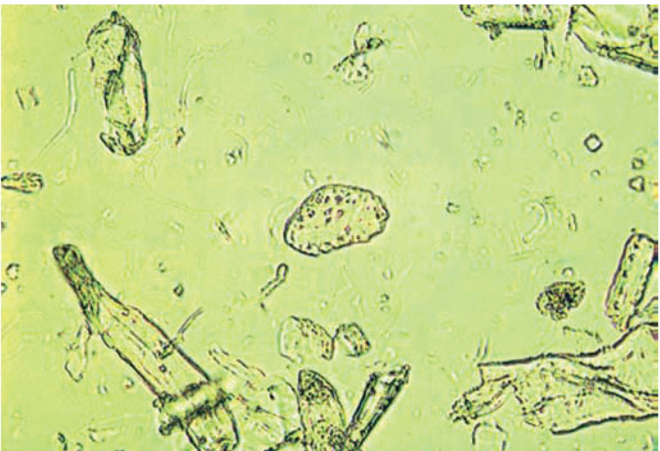
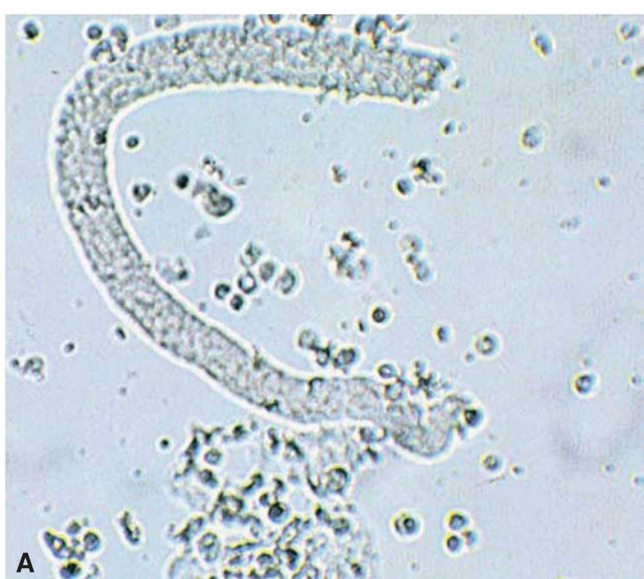


Figure 10-177. Debris from a diaper. The piece of debris in the center of the field is a common contaminant (400×).



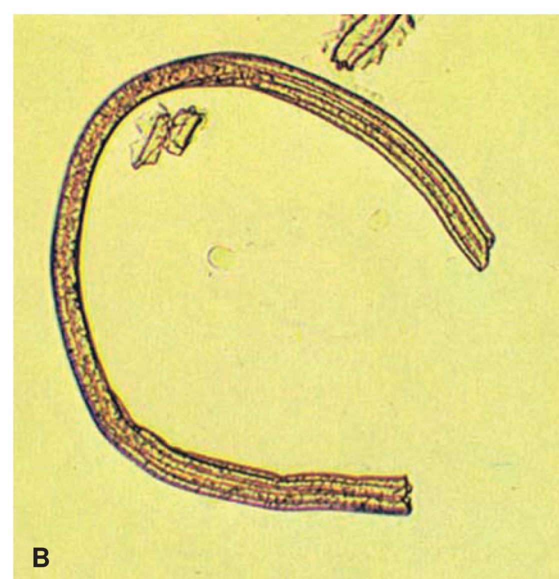


Figure 10-178. **A:** Fine granular cast and WBCs. Note the detail of the cast. **B:** Fiber. Note the dark edges and the difference in texture between this piece of debris and the cast in **(A)** (200×).

Figure 10-179. Fiber. Note the dark edges (400×).

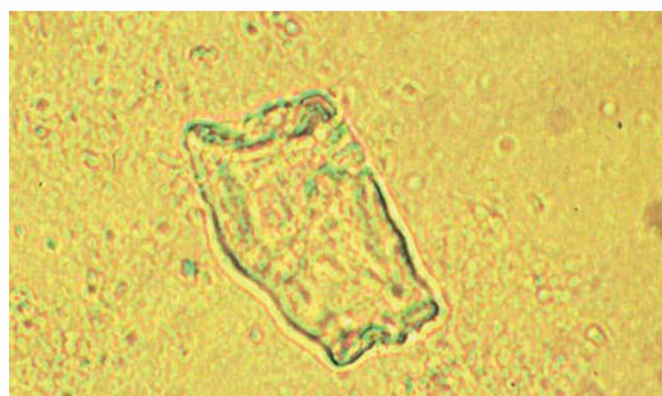


Figure 10-181. Fiber. Note the thick rolled edges of this fiber (400×).

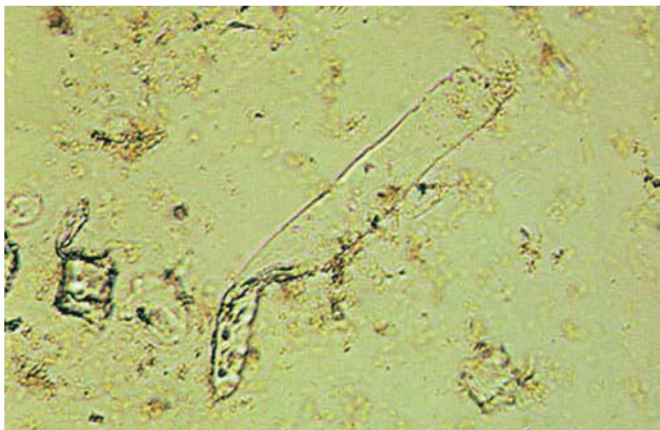


Figure 10-180. Fiber. This fiber could be confused with a waxy cast, but the structure is determined to be flat because of the part of the fiber that is turned on its side (400×).

Figure 10-182. Debris from a diaper. This squeezed-out specimen was worthless for microscopic analysis. Note the various types of fibers present (200×).

Figure 10-183. Fibers. The striations (seen only under low-power magnification) and dark edges are characteristics of these fibers (400×).

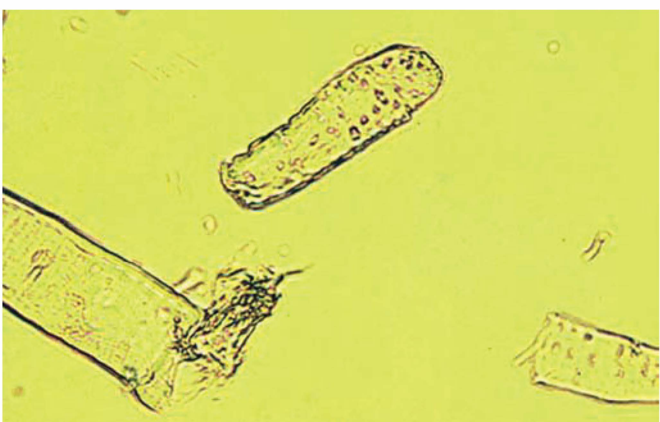


Figure 10-184. Fibers. These are the same fibers as in the previous figure. Note the indentations in the surface of the center fiber (400×).

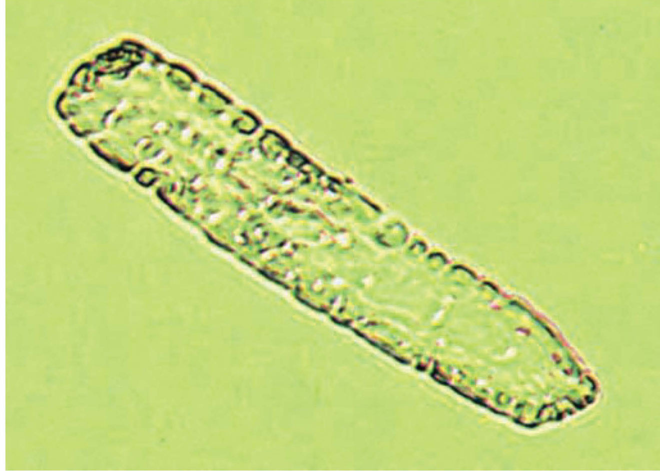


Figure 10-185. Fiber. Note the nodular indentations and nodular end of this very common contaminant (400×).

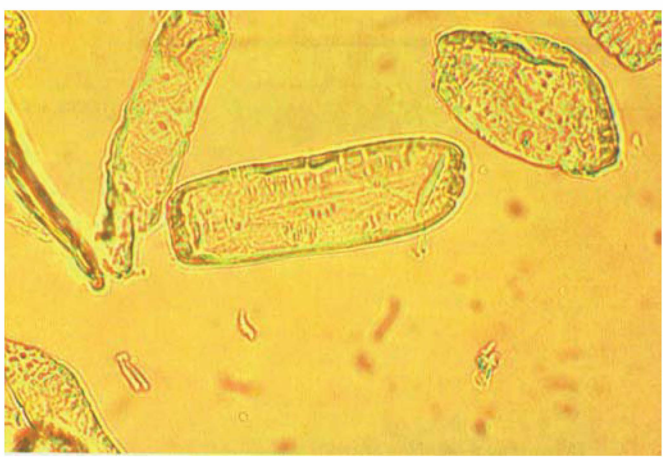


Figure 10-186. Fibers. The center fiber shows a thick nodular border (500×).

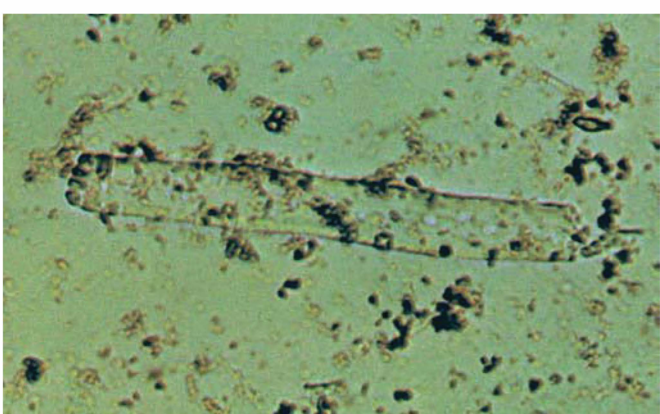


Figure 10-187. Fiber, calcium oxalate crystals, and amorphous urates. Note the nodular ends on the fiber (400×).

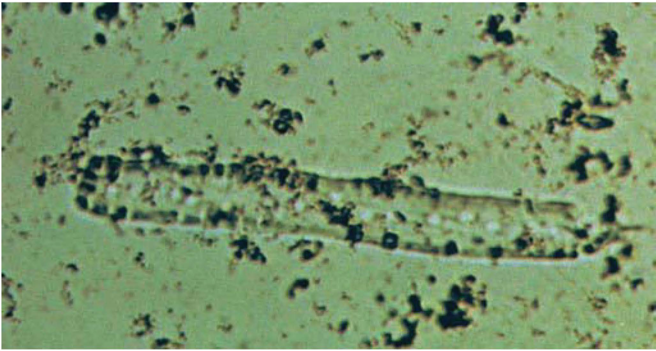


Figure 10-188. Fiber. This is the same field as in the previous figure but on a different focal plane. Changing the focus brings out the nodular indentations on the side of the fiber (400×).

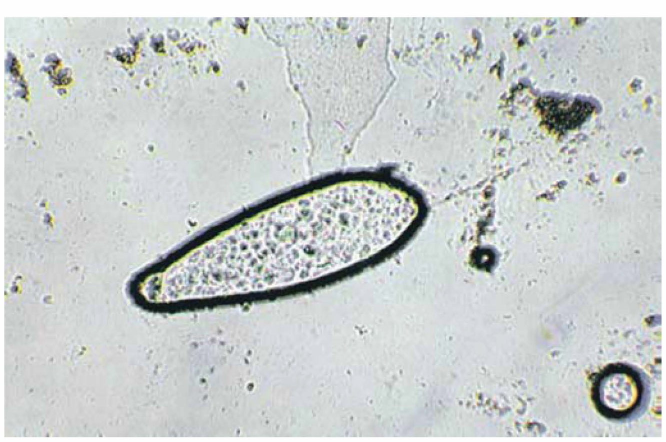


Figure 10-189. Air bubbles, phosphate plate, and amorphous phosphates. Air bubbles can assume a variety of shapes, especially if the coverslip is moved or depressed (200×).

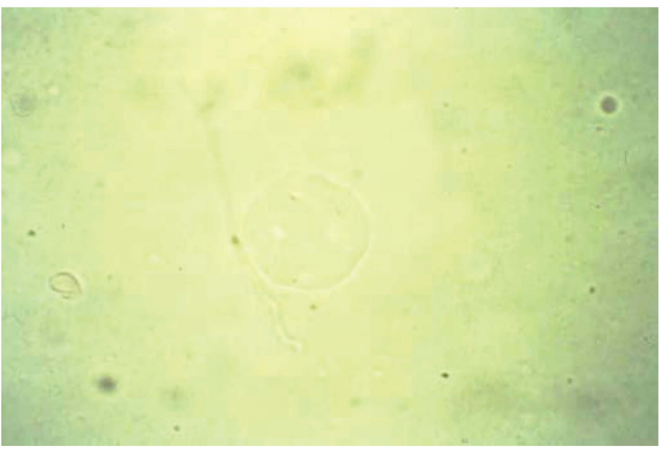


Figure 10-190. Coverslip defects. (Courtesy of Smith B, Foster KA. *The Urine Microscopic*. 5th ed. Educational Material for Health Professionals Inc.; 1999.)

Body Fluids Analysis

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Introduction to Body Fluids

Chapter 11

KEY TERMS

Cytocentrifuge
Effusion
Filtration Pressure
Hemocytometer
Interstitial Fluid Pressure
Lumbar Puncture
Paracardiocentesis
Paracentesis
Thoracentesis

LEARNING OBJECTIVES

1. Describe the composition of body fluids.
2. Describe the main function of body fluids.
3. Explain the process of fluid accumulation in body cavities.
4. Name the procedure used to collect fluid from each body cavity.
5. List the tests that are normally performed on body fluids.
6. List the causes for abnormal appearance of body fluids.
7. Explain the use of a hemocytometer in performing body fluid cell counts.
8. Correlate diluents that may be used during hemocytometer counts with the fluid for which they most likely would be used.
9. Explain the use of a cytocentrifuge in preparing smears.
10. Suggest techniques to minimize cell destruction during smear preparation.

The study of body fluids presents challenges to the laboratory. Analysis involves multiple departments of the laboratory and specialized knowledge of each type of body fluid. Hematology is important in examining the cells and crystals found, chemical analyses are required to assess significant physiologic changes in the patient, microbiology can help detect infectious agents in a nearby body cavity or membrane, and immunological tests and other miscellaneous tests can also provide the physician with critical information. Further consultation with pathology may be required for the identification of tumor cells and other abnormal cells.

Body Fluid Composition

While body fluids vary in composition, they share some elements in common. The critical roles of water and electrolytes are important determinants of any fluid composition and movement in the body. Water and electrolytes play crucial roles in many metabolic processes. Water enters the system through consumption of either water or food and also through cellular metabolic processes. For example, the water of oxidation can yield about 300 mL of water per day.

Fluids of the body can be intracellular or extracellular, with about 55% of the water being intracellular and about 45% being extracellular. Extracellular fluid can be further divided into interstitial fluid, transcellular fluids in various body cavities, and plasma. Fluids typically move around in body because of various forces and body conditions. The electrolyte and enzyme composition of intracellular fluid differs from extracellular fluids and knowledge of these differences can aid in understanding disease processes. For example, potassium levels are higher inside the cell than outside and sodium concentrations also vary between the intracellular fluid and the extracellular fluid. Depending upon the local conditions of various adjacent membranes and tissues, other fluid constituent concentrations can be altered as well. Examining these biochemical differences, along with examination of cellular elements, can assist in diagnosing and monitoring the patient's condition.

Types of Body Fluids

Body fluids are diverse, with variation in physical appearance, properties, cell types, and cell counts. In general, studies of body fluids are most helpful to assess inflammation, infection, malignancy, and hemorrhage. Body fluids can be divided into categories such as cerebrospinal fluid, various serous fluids from cavities lined with serous membranes, synovial fluid, semen, vaginal secretions, respiratory secretions such as from bronchoalveolar lavage, amniotic fluid, and even feces, which is considered in this category, although there are more body fluids in this category not included in this list. Technically, urine is also in this category but is covered in other chapters.

Accumulation of Excess Body Fluids

The amount of serous fluids found in the space between an organ and the membrane sac that encompasses the organ varies according to body site. Normally, only a small amount of fluid is present: 30-mL pleural fluid, 50-mL pericardial fluid, and 100-mL ascites. Body fluids are necessary for lubrication of the body cavity/organ interface during movement. A delicate equilibrium is maintained by the capillaries and the lymphatic vessels. Any obstruction or altered pressure in these vessels can affect the amount of fluid and its constituents.

Several forces, within and outside of the capillaries, work together to maintain fluid equilibrium. The tissue's colloidal osmotic pressure (**interstitial fluid pressure**), along with the capillary's hydrostatic pressure (**filtration pressure**), regulates the outward flow of fluid from the capillary. The colloidal osmotic pressure of the capillary and the tissue's hydrostatic pressure regulate the inward flow of fluid into the capillary from the tissue. Figure 11-1 illustrates the direction of these forces. Normal removal of fluids entering into the interstitial space is handled by the lymphatic system. Figure 11-2 shows the normal flow of fluids among the bloodstream, tissues, and lymphatic vessels. However, an imbalance in pressures causes excess egress of fluid into tissue spaces and can lead to accumulation of fluid in the body cavity. This accumulation of fluid is called an **effusion**. The causes and types of effusions are explained in the Chapter 13.

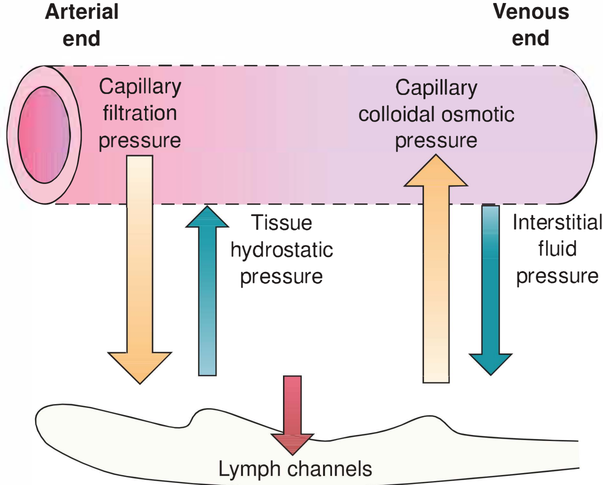


Figure 11-1. Forces governing the exchange of fluid at the capillary level.

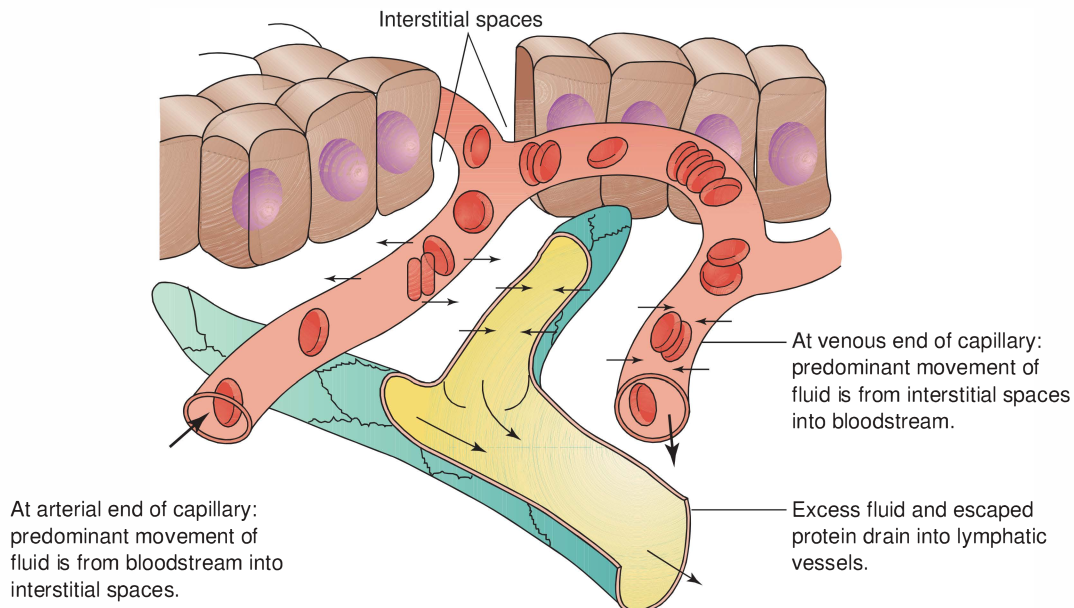


Figure 11-2. Exchanges through capillary membranes in the formation and removal of interstitial fluid.

Body Fluid Collection

The procedure for collecting body fluid specimens involves a minor surgical procedure that is usually named for the site of collection. A pleural fluid collection is termed **thoracentesis**, while cerebral spinal fluid (CSF) is obtained by **lumbar puncture** (spinal tap). Fluid from the abdomen is collected by **paracentesis** and fluid collecting around the heart is collected by **paracardiocentesis**. Table 11-1 lists the body fluids commonly examined and the procedure used to collect each.

BODY FLUID VOLUME

The volume of body fluid also varies greatly depending upon the source of the body fluid. In addition, disease can tremendously alter the amount of body fluid present. This is especially true in serous fluid where the normal amount of fluid is quite small, only the amount between the two adjacent serous membrane layers. In disease, this fluid level can increase from a few milliliters to a few liters of fluid.

Table 11-1 Commonly Analyzed Body Fluids

BODY FLUID	PROCEDURE
Cerebral spinal fluid (CSF)	Lumbar puncture
Pleural fluid	Thoracentesis
Paracardial fluid	Paracardiocentesis
Peritoneal fluid (ascites)	Paracentesis (general term used for puncture of any body cavity)

BODY FLUID APPEARANCE

The normal color and turbidity of body fluids is dependent on the body cavity from which they are obtained. Cerebrospinal fluid and synovial fluid are normally colorless and clear, whereas serous fluids are usually slightly yellow and clear. Terms used to describe the appearance of body fluids are listed in Table 11-2. Abnormal color or turbidity may indicate a disease process's physiological changes in the body cavity from which the fluid is obtained.

Table 11-2 Commonly Used Terms in the Description of Body Fluid Appearance

TURBIDITY	COLOR
Clear	Colorless
Hazy	Pink or red (indicated by the presence of hemoglobin or RBCs)
Cloudy	Serous (resembling serum)
Milky (indicates the presence of fat)	Sanguineous (resembling blood)
Oily (indicates the presence of radiographic dye)	Xanthochromic (indicates the degradation of hemoglobin)
Purulent (indicates the presence of many WBCs)	Yellow-green (indicates sepsis)
Pellicle (only CSF, indicates the presence of excess protein)	
Clotted	

Cell Counts in Body Fluids

Although automated cell counters are continually improving (see Chapter 20), most cell counts are still performed manually using a **hemocytometer** (specialized slide with a grid for counting). The most common hemocytometer used is the Neubauer hemocytometer. As shown in Figure 11-3, this hemocytometer has a platform on each of two sides. Each platform contains an etched grid that is scored with markings for ease of counting. The largest sections on the grid are each 1 mm^2. The grid is laid out as 3 mm by 3 mm for a total of 9 mm^2. Each square millimeter is divided further with varying degrees of detail.

These grids are etched into thick glass plates and have a moat that isolates them. The outer wall of the moat is 0.1 mm taller than the platform on which the grids are etched. A special optically corrected coverslip is placed over the grid area with edges resting on the moat walls. This depth must be considered when calculating cell counts performed on the hemocytometer. Both sides of the hemocytometer are loaded with well-mixed undiluted specimen. Once the specimen settles to the grid lines, both sides are counted and averaged if within 20%. If 20% or better precision is not obtained, the specimen is mixed again, reloaded, and recounted. Refer to Appendix C for counting details.

Normally, the cells in the entire 9 mm^2 are counted and a cell count per square millimeter is calculated. However, if the concentration of cells is high, adjustments can be made to the procedure. Fewer square millimeters may be counted, dilutions may be made, or a combination of both. If many red blood cells (RBCs) are present, counting the entire center square millimeter may be as accurate as counting all nine. If the RBC concentration is extremely high, making a dilution and counting five (four corners and the center) of the medium areas in the center square millimeter may be acceptable. Laboratory professionals must use judgment in establishing criteria for when to employ adjusted cell counting techniques for body fluid cell counts.

If many nucleated cells are present, counting these cells in the four corner square millimeters is often sufficient. A simple formula to remember when performing hemocytometer count calculations is as follows:

Cell count equals $[N \times D \times 10]/A$.
N = number of cells counted.
D = dilution factor.
Multiplying by 10 is necessary to bring the depth up to 1 mm.
A = area in square millimeters counted.

This calculation results in number of cells per cubic millimeter. It is important to note that square millimeters are NOT the same as number of squares counted. For example, if an undiluted body fluid is counted and 35 white blood cells are counted in 9 mm^2, the calculation is: $[35 \times 1 \times 10]/9 = 39$ per cubic millimeter.

If a specimen is rather bloody, it may be diluted and less area may be counted. For example, if a 1:10 dilution is made using saline and 127 RBCs are counted using the center of the middle square millimeter and its four corners (one-fifth of a square millimeter), the calculation is:

$$[127 \times 10 \times 10]/(1/5) \text{ OR } [127 \times 10 \times 10 \times 5]$$

Because to divide by a fraction is to multiply by its reciprocal. Therefore, multiplying by 5 is the same as dividing by 1/5. This example results in 63,500 RBCs per cubic millimeter.

In order to accurately perform these counts, some body fluids require addition of diluents or other substances to the sample to facilitate counting by reducing viscosity or preventing coagulation of the sample. Acetic acid is often used in cell count diluents to lyse RBCs and enhance the nuclei of WBCs. Acetic acid cannot be used for synovial fluid because it precipitates the hyaluronic acid present in synovial fluid. A solution of hyaluronidase may need to be added to perform the synovial fluid count accurately. For CSF and serous fluids, hyptonic saline or 1% ammonium oxylate can lyse RBCs, while keeping WBCs intact for counting. Stains may also be added to differentiate cells, such as methylene blue for differentiating RBCs from WBCs in cell counts.

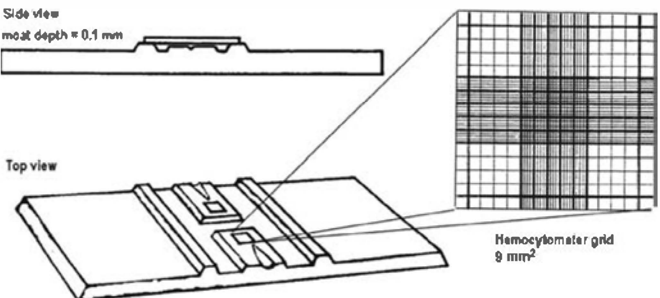
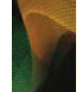


Figure 11-3. Neubauer hemocytometer diagrams. (Modified from Spahn MA, Brackman E. *Manual Cell Counting Techniques*. Dayton, OH: Educational Materials for Health Professional Inc.; 1987)



Cellular Morphologies and Differentials

Morphologies of both abnormal cells and cells that are normal for that body fluid need to be learned to assess body fluids. A cytologist or pathologist can assist in identifying these cells, especially in malignancies. Preparations made using a **cytocentrifuge** are preferred for body fluid cell examination. A cytocentrifuge (Fig. 11-4) is specifically designed to separate cells from fluids and deposit them onto slides. Cytocentrifugation requires relatively little sample, is fast, requires little skill, and provides good cell recovery with much less cell distortion. Cytocentrifuged body fluid preparations are suitable for a variety of staining techniques. A cuvette and slide are assembled and placed in the centrifuge head as shown in Figure 11-5. The cuvette top is removed and

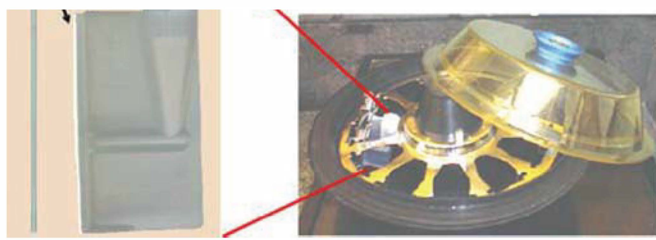


Figure 11-5. Cytocentrifugation method of body fluid concentration. An assembly of a special cuvette, with attached filter paper, and a glass slide are clipped into place in the cytocentrifuge. One to two drops of well-mixed fluid are pipetted into the cuvette. The lids are closed and the sample is centrifuged at 500 rpm for 5 minutes.

Match the hydrodynamic force with its direction of fluid movement.

A. Fluid into the capillary

B. Fluid out of the capillary

1. _________ capillary colloidal osmotic pressure

2. _________ capillary hydrostatic pressure

3. _________ tissue colloidal osmotic pressure

4. _________ tissue hydrostatic pressure

5. Water enters various body systems through:
   a. Consumption of water
   b. Consumption of food
   c. Cellular metabolic processes
   d. All of these
6. The main function of body fluids is:
   a. Cushioning interfaces between body cavities and organs
   b. Creating a barrier between organ systems
   c. Providing hydration for organs
   d. All of these

Match each body fluid with the procedure used to obtain it.

A. Lumbar puncture

B. Paracardiocentesis

C. Paracentesis

D. Thoracentesis

7. ________ ascites fluid
8. ________ cerebral spinal fluid
9. ________ paracardial fluid
10. ________ pleural fluid

Match the appearance description for body fluids with the probable cause for each (may have more than one match).

A. Milky

B. Oily

C. Purulent

D. Sanguineous

E. Xanthochromic

F. Yellow-green

11. ________ high number of red blood cells
12. ________ high number of white blood cells
13. ________ indicates the degradation of hemoglobin
14. ________ high amounts of fat may be present
15. ________ may contain radiographic dye
16. ________ possible sepsis
17. Body fluid cell counts normally need to be performed using which dilution?
    a. 1:1 (undiluted)
    b. 1:2 (1 part fluid to 1 part diluent)
    c. 1:10 (1 part fluid to 9 parts diluent)
    d. 1:20 (1 part fluid to 19 parts diluent)
18. The preferred method of performing differential cell counts on body fluids uses:
    a. An automated cell counter
    b. Concentration of the specimen by sedimentation
    c. Cytocentrifugation of the specimen
    d. Stain added to the hemocytometer count

Match each substance with its appropriate use in body fluid cell counts.

A. Acetic acid

B. Albumin

C. Hyaluronidase

D. Methylene blue

19. _______ breaks down thick synovial fluid
20. _______ lyses RBCs, enhances WBCs
21. _______ preserves cell structure
22. _______ differentiates RBCs from WBCs

CASE STUDY

Case 11-1 A fluid obtained by thoracentesis was received in the hematology department of the clinical laboratory. The undiluted specimen contained 190 red blood cells and 840 nucleated cells in the nine large squares of the hemocytometer. A few drops of the specimen were used to make a smear by cytocentrifugation and then stained with Wright stain.

1. What are the cell counts on this specimen?
2. The cytocentrifuge preparation contained cells that were not recognizable, many of which appeared fragmented. What can be done to obtain a better smear?

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12

Chapter

Cerebrospinal Fluid

KEY TERMS

Arachnoid Villi
Blood–Brain Barrier
Central Nervous System
Cerebral Ventricle
Cerebrospinal Fluid
Choroidal Cells
Choroid Plexus
Dural Sinuses
Dura Mater
Ependymal Cells
Erythrophagocytosis
Hematin Crystals
Meninges
Otorrhea
Pia-Arachnoid Mesothelial (PAM) Cells
Pia Mater
Pleocytosis
Rhinorrhea
Siderophages
Traumatic Tap
Xanthochromic

LEARNING OBJECTIVES

1. Explain the utility of cerebrospinal fluid analysis in evaluating the central nervous system.
2. Explain the function of cerebrospinal fluid.
3. Explain the function of the blood–brain barrier.
4. List the indications and contraindications for performing a cerebrospinal fluid analysis.
5. Describe the anatomy of the central nervous system.
6. Explain the formation, circulation, and reabsorption of cerebrospinal fluid.
7. Name and describe the procedure for collection of cerebrospinal fluid.
8. Compare and contrast the appearance of cerebrospinal fluid in health and disease.
9. List the normal constituents of cerebrospinal fluid and their normal levels.
10. Discuss the mechanism for maintenance of normal cerebrospinal fluid chemical levels.
11. Suggest tests on CSF to diagnose central nervous system disorders (meningitis, hemorrhage, etc.).
12. Differentiate between uncompromised and compromised cerebrospinal fluid results (hemorrhage vs. traumatic tap, side effects created by test procedures and interventions)
13. Use chemistry test results to evaluate the integrity of the blood–brain barrier.
14. Compare and contrast cerebrospinal fluid chemistry results in health and disease.
15. Identify cells normally present in cerebrospinal fluid.
16. Explain the pathophysiology resulting in abnormal cellular constituents in cerebrospinal fluid.
17. Suggest appropriate microbiology procedures for the detection of micro-organisms in cerebrospinal fluid.
18. Explain the use of immunologic procedures in diagnosing central nervous system disorders.
19. Interpret cerebrospinal fluid analysis results.
20. Correlate cerebrospinal fluid analysis results to possible etiologies for central nervous system disorders.

Cerebrospinal fluid (CSF), the fluid surrounding the brain and spinal cord, is produced in the brain and serves several functions. This fluid provides physicians with a tool by which to evaluate the **central nervous system** (CNS). Indications for performing a lumbar puncture and CSF examination include suspicions of encephalitis, meningitis, multiple sclerosis, neurosyphilis, and subarachnoid hemorrhage, among other disorders. This procedure is usually performed on patients with unexplained seizures or on those who have fever of unknown origin. Dementia and acute states of confusion may also prompt the physician to perform a lumbar puncture and CSF examination. Lumbar puncture should not be performed if there is infection or inflammation over the puncture site. Using such a site may induce meningitis.

This chapter contains a brief overview of the anatomy and physiology of CSF. Laboratory testing is detailed, and conditions affecting CSF test results are outlined.

Cerebrospinal Anatomy

The brain is contained within the skull, whereas the spinal cord runs down the center of the vertebrae. The brain and spinal cord are enclosed in three layers of membrane, the **meninges**. The outer most membrane is the **dura mater**, the membrane in the middle is the arachnoid (also referred to as arachnoidea), and the innermost membrane is the **pia mater**. The pia mater adheres to the surface of the neural tissue. The dura mater layer contains sinuses. Located in the **dural sinuses** are the **arachnoid villi**. These villi are herniations of arachnoid membrane into the lumen of the dural sinuses. Epithelial cells that originate from the ectoderm line several structures of the CNS. The structures lined by epithelial cells called **ependymal cells** include the **cerebral ventricle** and the neural canal of the spinal cord. The epithelial cells that line the **choroid plexus** are called **choroidal cells**. Together with the endothelium of capillaries form the **blood–brain barrier**. Epithelial cells that originate from the mesoderm line the pia and the arachnoid. These cells are called **pia-arachnoid mesothelial (PAM) cells**.

Ependymal cells and choroidal cells have similar morphologic characteristics. Both cell types are 25 to 40 microns and contain a single large nucleus. The nucleus is either round or oval and comprises one-third of the cell. The nucleus is usually located eccentrically in the cytoplasm and may exhibit nucleoli. The cytoplasm may contain vacuoles. Both cell types can be seen in clusters of the same type of cell.

PAM cells resemble cells of the monocytic lineage. PAM cells are 15 to 25 microns in size with a nucleus about half the size of the cell. The nucleus in PAM cells is round or oval and has a loose chromatin pattern and may contain nucleoli. The cytoplasm may contain vacuoles. PAM cells can be seen in clusters and have the ability to transform into macrophages.

FUNCTION

CSF serves as a protective fluid, cushioning and lubricating the brain and spinal column. This cushions and helps prevent injury to the brain that could happen as a result of gravitational or inertial forces. CSF circulates in the space between two membranes, the arachnoid and the pia mater. It also bathes the brain and spinal cord and serves as a nutrient and metabolic waste exchange fluid. Another function of the CSF is to adjust its volume in response to changes in cerebral vessel changes.

FORMATION

CSF arises from two sources. The tufts of capillary blood vessels in the cerebral ventricles, known as the ventricular choroid plexuses, produce approximately 70% of the CSF. The process that occurs is a combination of active secretion and plasma ultrafiltration. Approximately 30% is formed by the ependymal lining cells of the ventricles and the cerebral/subarachnoid space. CSF volume is normally from 90 to 150 mL in adults, with a rate of production from 500 mL/day or 20 mL/h. Neonate volume is normally 10 to 60 mL.

CIRCULATION

After its formation in the ventricles, CSF exits from the ventricles through the foramina and circulates over both the hemispheres of the brain, downward over the spinal cord, and to the nerve roots. Circulation of the CSF occurs slowly, allowing the time needed for long contact with cells in the CNS. In the dural sinuses, CSF is reabsorbed by the arachnoid villi. Figure 12-1 illustrates the flow of CSF around the CNS, whereas Figure 12-2 details the structure of the meninges.

COMPOSITION

The secretions and ultrafiltered plasma that comprise the CSF are complex and reflect the concentration of plasma substances. Water and water-soluble substances such as chloride, CO_2, creatinine, glucose, and urea diffuse rapidly across the blood–brain barrier. Some substances, such as creatinine, glucose, and urea require several hours to reach equilibrium. Lipid-soluble substances including drugs and alcohol diffuse from plasma to the CSF in proportion to their solubility properties. A concentration gradient from plasma to CSF controls the diffusion of proteins, with larger molecules taking longer to diffuse. The concentration of substances found in CSF should always be compared with their concentration in the plasma.

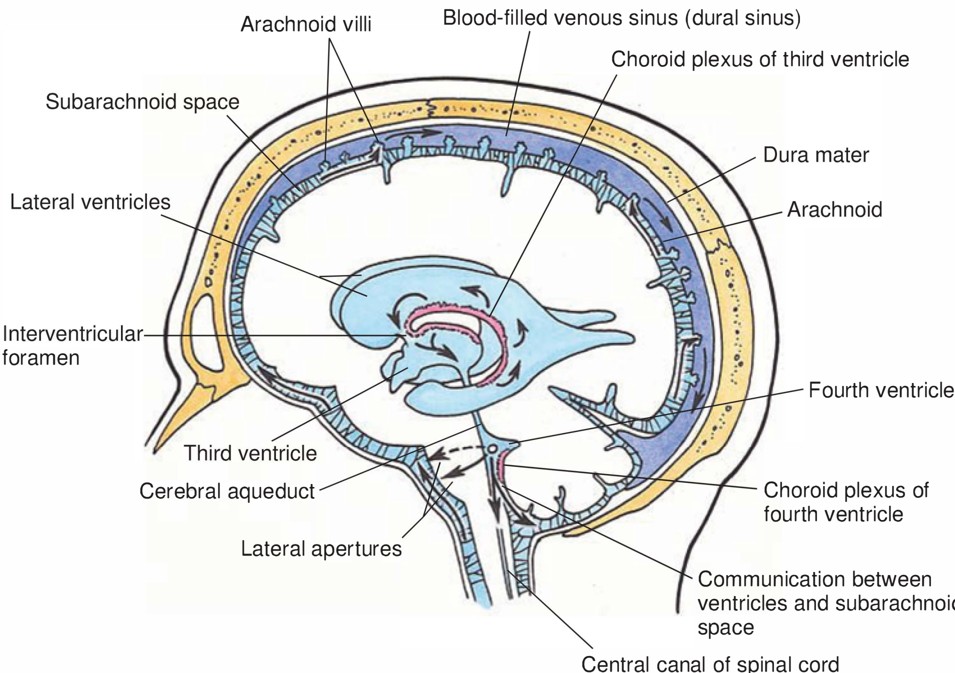

Figure 12-1. Anatomy of the central nervous system. From Snell R. *Clinical Neuroanatomy*. Philadelphia, PA: Lippincott Williams & Wilkins; 2001.

Specimen Collection

Indications for examination of CSF includes CNS malignancy, demyelinating diseases, meningeal infection, and subarachnoid hemorrhage. The procedure for obtaining CSF is known as a lumbar puncture. Contraindication to performing this puncture is the presence of infection at the puncture site. Lumbar puncture through an area of infection may cause the spread of infection into the meninges. However, bacteremia is not a contraindication because CSF examination can confirm or rule out concurrent meningitis.

The most common site used for lumbar puncture is the intervertebral space between lumbar vertebrae L3 and L4. Using this site avoids damage to the spinal cord in adults because the spinal cord does not extend that far down. The spinal cord may extend that far in small children and infants; therefore the intervertebral space between L4 and L5 is used for them. Figure 12-3 shows placement of the needle for CSF collection between L4 and L5.

The lumbar puncture site is thoroughly cleansed and a local anesthetic is applied. The lumbar puncture is made and with the needle seated in the dura mater, CSF pressure is measured using a graduated manometer attached to the syringe. CSF is collected if this pressure is normal and if there is no significant fall in pressure when collection begins.

Typically, 10 to 20 mL of CSF is slowly removed into three or four sterile tubes that are numbered sequentially. What tests are performed on which tubes is dependent upon laboratory protocol or physician's specific request. A common

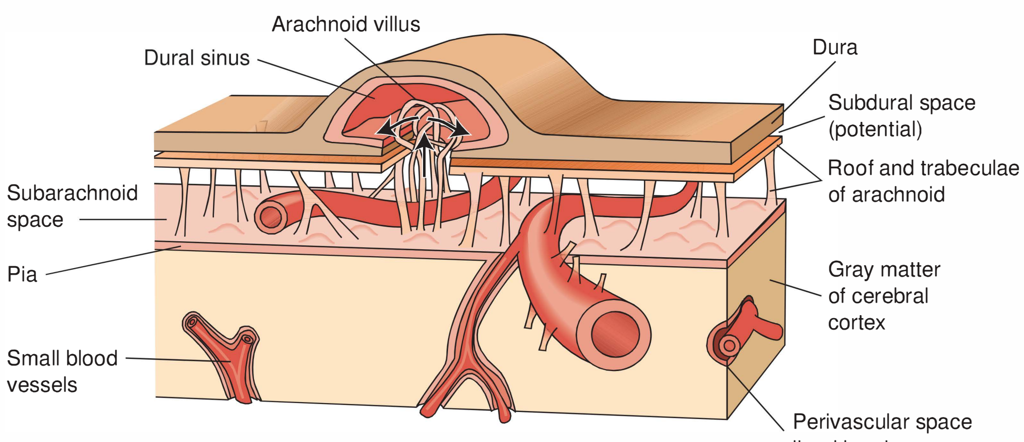

Figure 12-2. Detail of meninges. From Cormack DH. *Ham's Histology*. 9th ed. Philadelphia, PA: J. B. Lippincott; 1987:367.

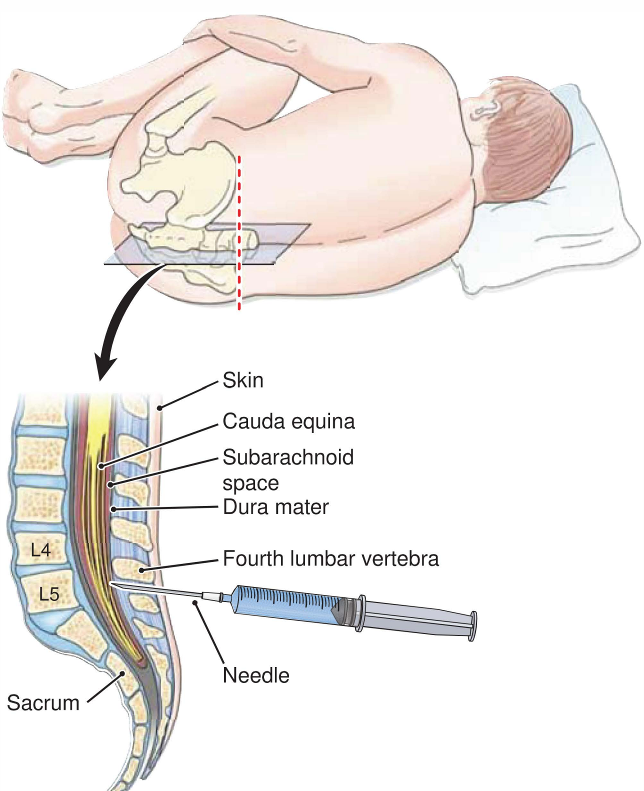

Figure 12-3. Placement of the needle for CSF collection. (Courtesy of Wolters Kluwer, Skokie, IL.)

protocol is the performance of chemical and immunologic analysis on tube number one, microbiological procedures on tube number two, and cells counts on tube number three. Figure 12-4 shows the typical containers into which CSF is collected. Notice that the tubes are prenumbered.

Laboratory Examination

Nearly every section of the laboratory can be involved in the evaluation of CSF. Laboratory tests that may be performed on CSF include, but are not limited to, macroscopic evaluation, microscopic evaluation of cell count and type, chemical analysis, microbiology cultures, and immunologic and molecular analyses. Some of these tests are beyond the scope of this text. However, the more common laboratory procedures are included.

PHYSICAL CHARACTERISTICS

Color and turbidity are noted. If a fourth tube is collected, or when the cell counts are completed, the tube may be refrigerated and observed for pellicle formation.

Normal CSF is clear and colorless and demonstrates a viscosity similar to water. Abnormal turbidity is observed if blood cells, microorganisms, or flecks of protein are present. Varying degrees of CSF cloudiness due to the presence of elevated numbers of white blood cells is termed **pleocytosis**. An oily appearing CSF may contain radiographic contrast media.

Figure 12-4. Specimen containers for cerebral spinal fluid specimens.

Abnormal colors reflect the presence of various substances. Red blood cells can add a red, pink, or smoky color to CSF. If hemoglobin is present, the CSF can appear red or **xanthochromic** (yellowish tinge) if the hemorrhage is older. Other substances that make CSF xanthochromic include bilirubin, carotene, and melanin.

Because blood cells may be introduced into a CSF specimen at the time of lumbar puncture, careful differentiation between **traumatic tap** (a spinal tap in which blood vessels or bone are punctured) and hemorrhage must be made.

If there is a significant difference in the amount of blood present between the first and last tubes collected (later tubes gradually clearing), then the puncture was traumatic. If all tubes collected show the same degree of blood, then a subarachnoid hemorrhage is most likely. Figure 12-5 demonstrates the difference in appearance of normal clear CSF, red CSF in hemorrhage, xanthochromic CSF from an old hemorrhage, and CSF from a traumatic tap.

If lumbar puncture is performed within the first 4 hours after a subarachnoid hemorrhage, the CSF will appear pale pink to red, depending on the degree of hemorrhage. Red blood cells lyse in CSF due to the low level of proteins and lipids as compared to plasma. After hemolysis, the CSF will change from a cloudy or hazy pink-red to a clear pink-red and then progress through various shades of light orange, yellow, and amber, as oxyhemoglobin changes to methemoglobin. After about 12 hours bilirubin is formed. Gradual decrease in CSF color occurs over the first 2 days, clearing in about 2 to 4 weeks.

Clotting of CSF is associated with a traumatic tap rather than hemorrhage, because CSF contains nearly no fibrinogen. Clotting of CSF may be present in cases of neurosyphilis and tubercular meningitis.

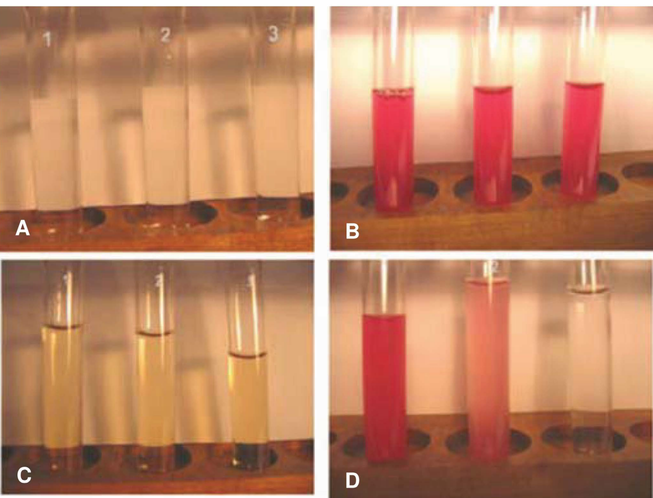

Figure 12-5. Comparison of cerebral spinal fluid appearance between **(A)** normal CSF, **(B)** red CSF from fresh hemorrhage, **(C)** xanthochromic CSF from old hemorrhage, and **(D)** CSF from a traumatic tap.

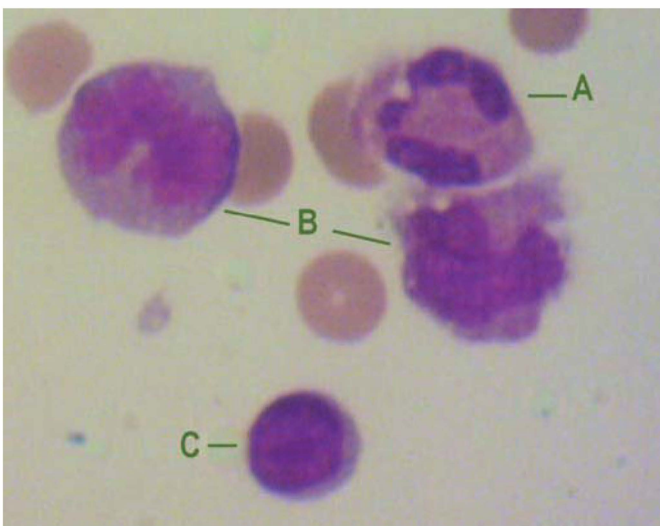

Figure 12-6. Cells that can normally be found in cerebral spinal fluid. **(A)** Neutrophil, **(B)** monocyte, and **(C)** lymphocytes. Red blood cells are not normally present in CSF (Wright stain 1,000×).

MICROSCOPIC EVALUATION

Microscopic examination of CSF includes the counting of cells on a hemocytometer and differentiation of cell types on stained smears. As for all body fluids, cell counts on CSF must be performed as soon as possible, because deterioration of cells in body fluid specimens begins within 2 hours of collection. Cell counts are performed manually rather than using automation because of the low level of cells normally present.

CELL COUNTS

CSF hemocytometer cell counts are performed on well-mixed, undiluted specimens. However, if the CSF is grossly bloody, a dilution with saline may be necessary. Normally, no red blood cells are present in CSF. Red blood cells add little to the diagnostic value but are often reported as they may help identify a traumatic tap. Nucleated cells are counted as described in Chapter 11. A dilution with HCl eliminated erythrocytes and enhances nuclei. Although the nucleated cell count is reported as a leukocyte count, not all nucleated cells found in CSF are white blood cells (WBCs). Occasionally, ependymal cells or choroid plexus cells enter the CSF. Tumor cells may be present. In addition, nucleated red blood cells and other bone marrow cells may be seen in traumatic tap during which a vertebral process was nicked. Normal adult CSF can contain 0 to 5 WBCs per microliter. Children can exhibit higher CSF WBC counts; however, normal ranges for children are poorly documented.

DIFFERENTIAL COUNT

A CSF differential count is usually performed on cytocentrifuged preparations that have been stained with Wright stain. Of the few cells normally present in CSF, lymphocytes and monocytes are predominant. Neutrophils are not a common finding in CSF, and CNS lining cells are only rarely seen. Figure 12-6 pictures cells that can normally be found in CSF. In adults, normal proportions of cells in CSF usually range from 28% to 96% lymphocytes, 16% to 56% monocytes, and 0% to 7% neutrophils. Eosinophils, ependymal cells, and histiocytes are only rarely seen.

CSF normally contains very few WBCs. In pleocytosis, the type of WBC present in CSF correlates with various forms of inflammation, infection, or malignant condition. WBCs that can be present in CSF include neutrophils, lymphocytes, plasma cells, eosinophils, monocytes, and macrophages. Other cells that may be present in CSF include CNS lining cells and malignant cells. Causes for various types of pleocytosis are outlined in Table 12-1.

In addition to cell count and differential, WBCs present in CSF can be studied using flow cytometry methods.

Neutrophils

Neutrophilic pleocytosis is present in cases of bacterial meningitis and in the early stages of other forms of meningitis. Other causes for neutrophilic pleocytosis in CSF include cerebral abscess, subdural empyema, CNS hemorrhage, intrathecal treatments, and postseizure. Figure 12-7 illustrates neutrophilic pleocytosis.

Lymphocytes

Lymphocytic pleocytosis predominates the later stages of meningitis that are viral, tubercular, fungal, or syphilitic in nature. Lymphocytes in CSF undergo the same morphologic changes as in peripheral blood lending to the presence of various lymphocyte forms. Increased numbers of lymphocytes

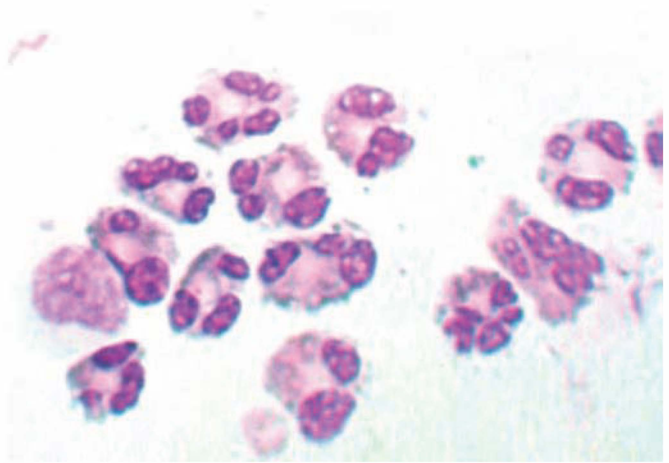

Figure 12-7. Neutrophilic pleocytosis in cerebral spinal fluid (Wright stain 1,000×).

can also be seen in other inflammatory processes and degenerative disorders such as Guillain–Barré syndrome. Figure 12-8 illustrates lymphocytic pleocytosis.

Plasmacytes

Plasmacytes are not normally found in normal CSF. They can appear in the same disorders in which there is lymphocytic pleocytosis. In addition, plasma cells can be seen in multiple sclerosis, where they may be the only abnormality. Figure 12-9 illustrates plasma cells in CSF.

Eosinophils

Eosinophils are a rare finding in normal CSF. If eosinophils comprise greater than 10% of cells in CSF, an eosinophilic pleocytosis is present. Eosinophils can be increased in parasitic and fungal infections of the CSF or allergic reactions to

Table 12-1 Causes for Pleocytosis in Cerebrospinal Fluid (CSF) by Cell Type

CAUSE	NEUTROPHILS	LYMPHOCYTES	EOSINOPHILS	MONOCYTES	PLASMA CELLS	OTHER
Bacterial infections (meningitis and meningoencephalitis)	X	X (syphilitic, leptospiral, and unusual organisms, e.g., Listeria)	X (rare)	X (chronic, leptospiral, syphilitic)	X (syphilitic)	
Viral meningitis	X (early)	X	X (rare)	X		
Fungal infections	X (early)		X	X		
Parasitic infestations	X (amebic)	X	X	X (amebic)		
Tubercular	X (early)		X (rare)		X	
Guillain–Barré	X				X	
Multiple sclerosis		X		X	X	
Sarcoidosis		X				
Polyneuritis		X	X			
Cerebral infarct	X			X (also lipophages)		
CNS hemorrhage	X			X (also macrophages)		
Malignancies	X	X	X (rare)	X		CNS tumor cells; carcinoma metastases
Traumatic tap	X					Bone marrow cells; cartilage cells
Other	Radiographic procedures, intrathecal injections	Sarcoidosis of meninges	Reaction to shunts and drugs		Sarcoidosis, sclerosis	Ependymal/ choroid plexus cells in neonates, trauma, surgery, shunts, and injections

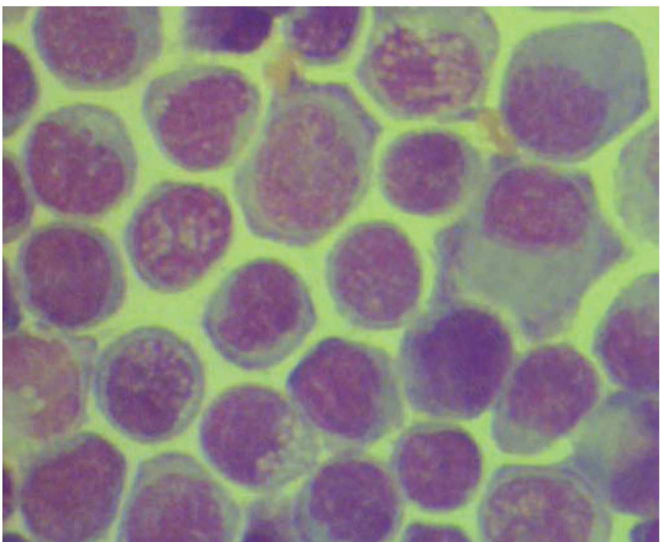

Figure 12-8. Lymphocytic pleocytosis in cerebral spinal fluid (Wright stain 1,000×).

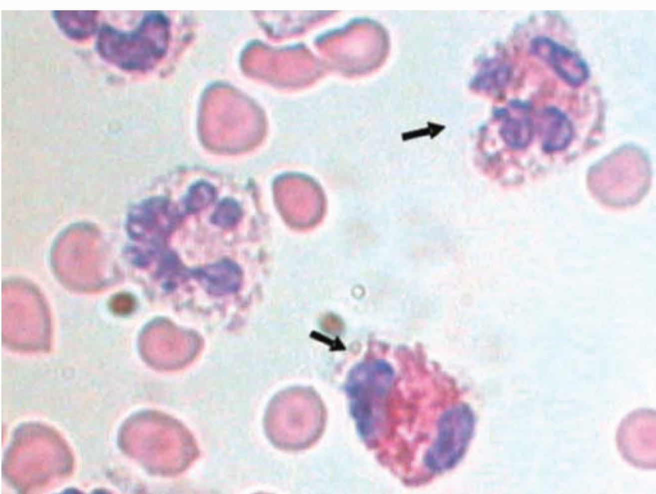

Figure 12-10. Eosinophils in cerebral spinal fluid (Wright stain 1,000×).

malfunctioning intracranial shunts, radiographic contrast media, and drugs. Figure 12-10 illustrates eosinophils cells in CSF.

Monocytes and Macrophages

Monocytic pleocytosis is a rare finding. Although monocytes may be increased in CSF, they usually do not predominate. Increased numbers of monocytes in CSF occur with increased numbers of other cells, mixed pleocytosis. Mixed pleocytosis can be present in chronic bacterial meningitis, meningitis of tubercular or fungal origin, or rupture of a cerebral abscess. Figure 12-11 illustrates a mixed cell pleocytosis in CSF.

Macrophages originate from monocytes and are not a normal finding in CSF. Macrophages are a common finding after CNS hemorrhage and may be seen with phagocytized erythrocytes (**erythrophagocytosis**), digested erythrocytes, and hemosiderin (**siderophages**) or **hematin crystals** following decomposition of large amounts of hemoglobin. Macrophages that are present after CNS hemorrhage can help roughly identify the time at which hemorrhage occurred. Table 12-2 displays the changes that occur in the types of cells present after hemorrhage. Figures 12-12 to 12-16 illustrate macrophages at various stages after a CNS hemorrhage. A lipophage, macrophage with ingested fat, may be seen in CSF following brain infarct (Fig. 12-17).

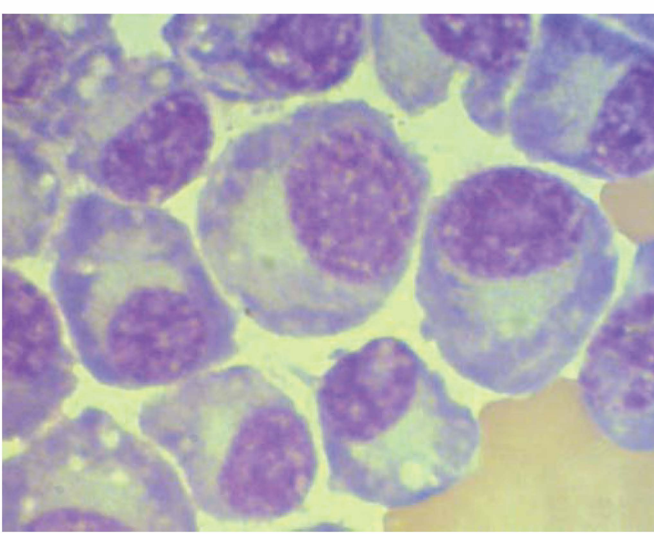

Figure 12-9. Plasma cells in cerebral spinal fluid (Wright stain 1,000×).

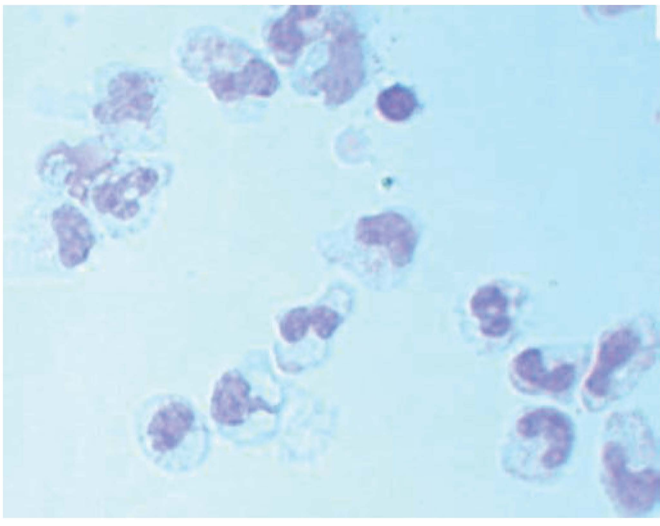

Figure 12-11. Cerebral spinal fluid with mixed pleocytosis (Wright stain 1,000×).

Table 12-2 Changes in Cells Present in Cerebrospinal Fluid (CSF) Following Hemorrhage

HOURS AFTER HEMORRHAGE	CELLS PRESENT
2–24 hours	Erythrocytes, neutrophils, lymphocytes, monocytes, macrophages
12–48 hours	Lymphocytes, macrophages, erythrophagocytosis (macrophages with engulfed RBCs)
2–4 days	Erythrophagocytosis, vacuolated macrophages (digestion of RBCs), siderophages (macrophages with stored iron)
1–8 weeks	Siderophages, macrophages containing hematin crystals

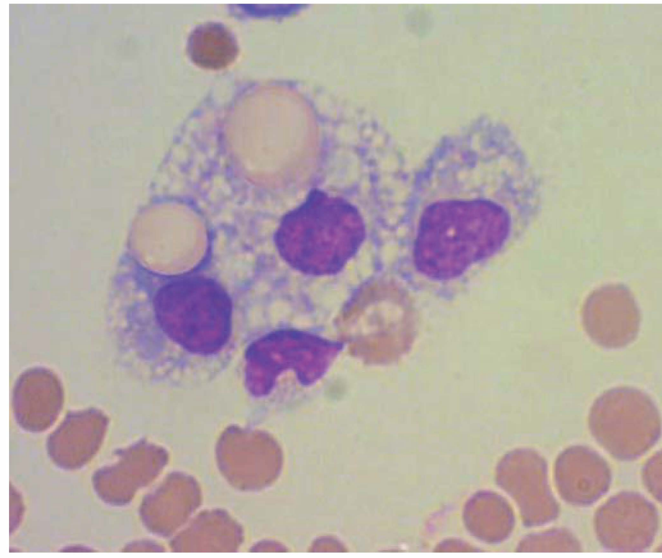

Figure 12-13. Macrophage demonstrating erythrophagocytosis in cerebral spinal fluid can be seen 12 to 48 hours following CNS hemorrhage (Wright stain 1,000×).

Other Cells

Other cells that may occasionally be present in normal CSF include ependymal cells, choroidal cells, and PAM cells. Ependymal cells can be seen in Figure 12-17. The morphology of these cells was discussed earlier in this chapter. Neonates normally can have increased numbers of these cells. Children with hydrocephalus will also have increased numbers of ependymal cells in their CSF. Ependymal cells and choroidal cells may be present in CSF in high numbers after traumatic brain injury, pneumoencephalography, surgery, myelography, ischemic infarction of the brain, ventricular shunts, and intrathecal injections. Ependymal cells, choroidal cells, and PAM cells can occur in clusters (Fig. 12-19), making them difficult to differentiate from clustered malignant cells.

Malignant cells arise from various tumors, either primary CNS tumors or one that has metastasized to the CNS. Tumors that commonly metastasize the CNS include carcinomas of the breast, gastrointestinal tract, lung, and melanoma and leukemia. Figure 12-20 shows cells in CSF from a patient with acute lymphocytic leukemia.

Nucleated red blood cells may also be present in specimens from a traumatic tap in which the vertebrae were nicked. Figure 12-21 shows the appearance of nucleated red blood cells in CSF.

Rarely will a squamous epithelial cell (Figure 12-22) be present in CSF. The contamination of CSF with cells from the skin can occur during the lumbar puncture.

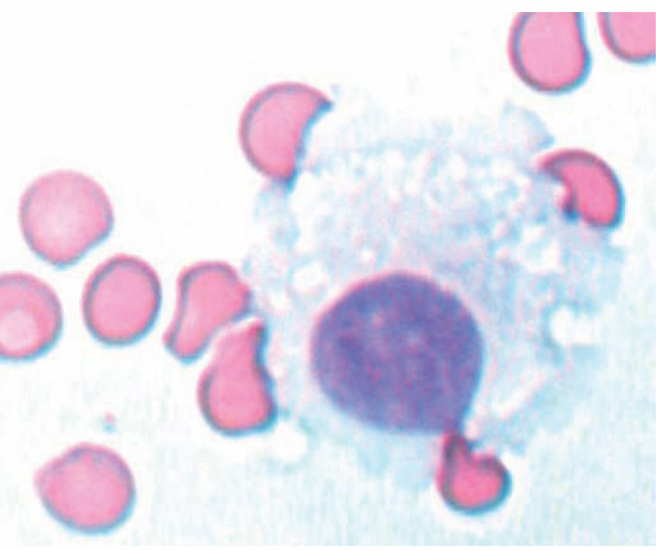

Figure 12-12. Macrophage in cerebral spinal fluid (Wright stain 1,000×).

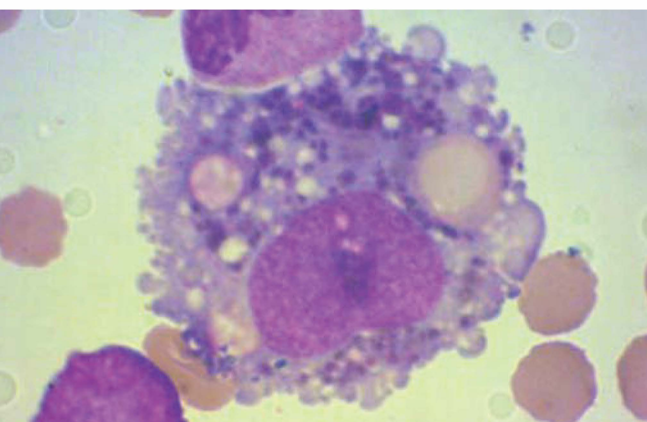

Figure 12-14. Macrophage demonstrating erythrophagocytosis in cerebral spinal fluid can be seen along with siderotic granules 2 to 4 days following CNS hemorrhage (Wright stain 1,000×).

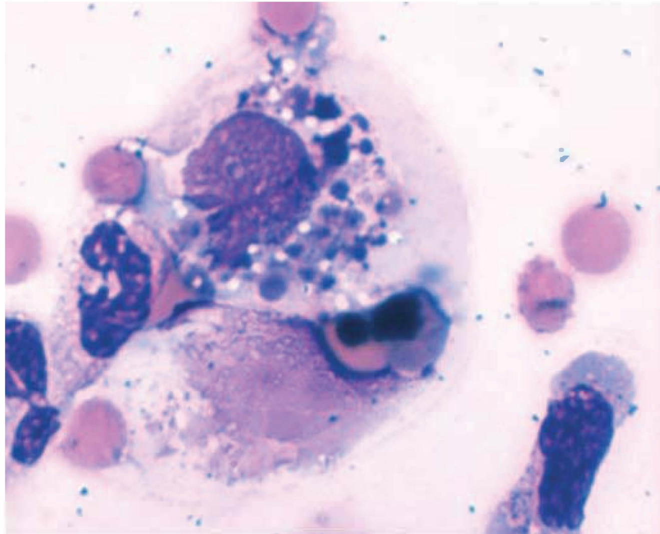

Figure 12-15. Macrophage with iron inclusions (siderophage) in cerebral spinal fluid can be seen from 1 to 8 following CNS hemorrhage (Wright stain 1,000×).

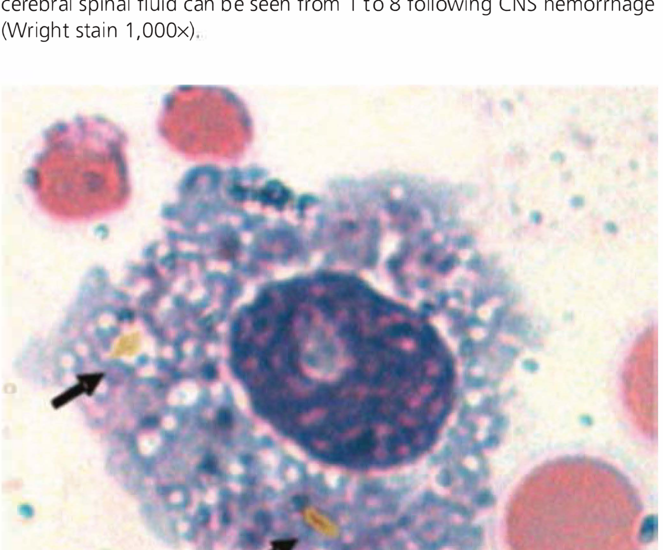

Figure 12-16. Macrophage with hematin inclusions (arrows) in cerebral spinal fluid (Wright stain 1,000×).

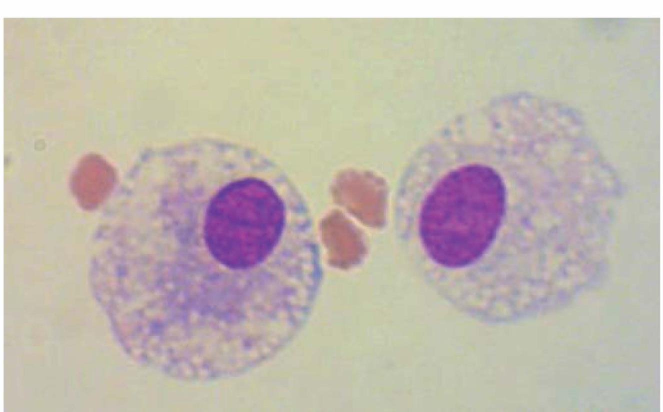

Figure 12-17. Macrophages with possible fat inclusions (lipophage) in cerebral spinal fluid (Wright stain 1,000×).

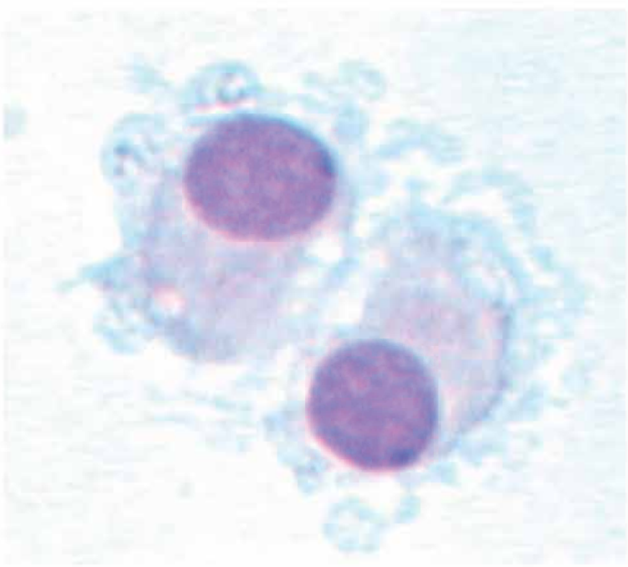

Figure 12-18. Ependymal cells in cerebral spinal fluid (Wright stain 1,000×).

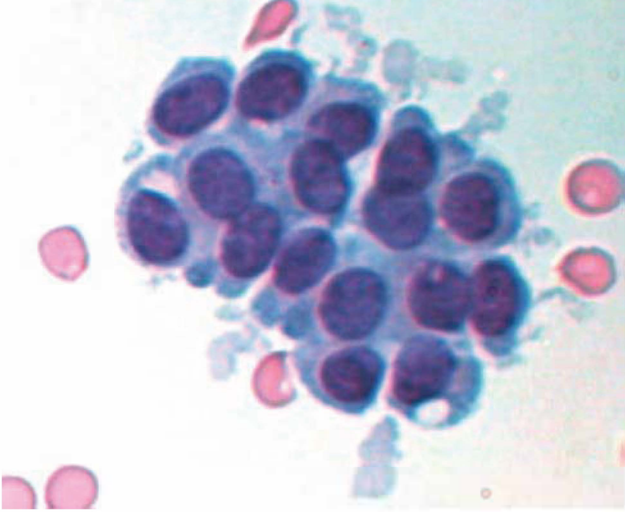

Figure 12-19. Clumped choroid plexus in cerebral spinal fluid (Wright stain 1,000×).

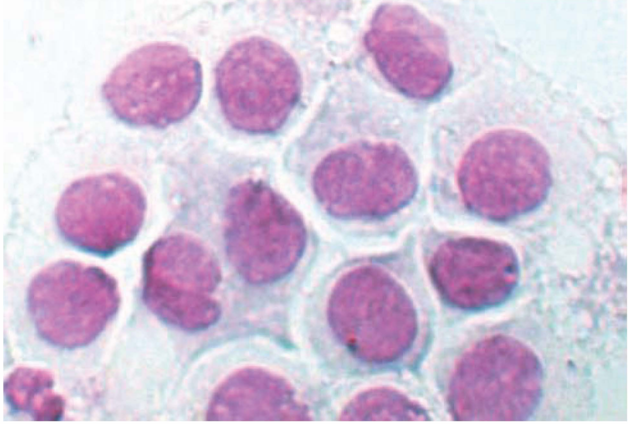

Figure 12-20. Cells of lymphocyte lineage may be seen in CSF from patients with lymphocytic leukemias (Wright stain 1,000×).

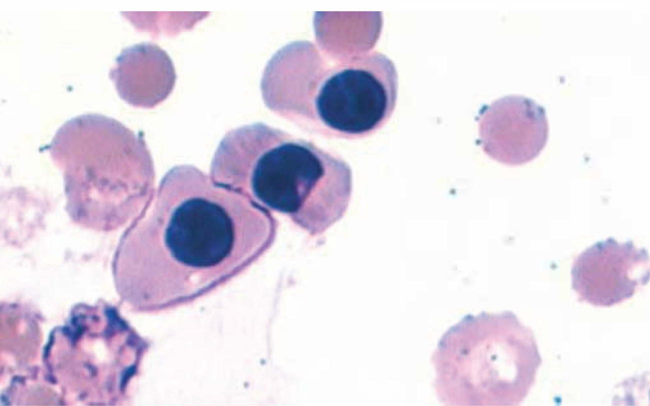

Figure 12-21. Nucleated red blood cells in cerebral spinal fluid are usually due to a traumatic tap (Wright stain 1,000×).

Chemical Analyses

Laboratory tests routinely performed on CSF include glucose, protein, immunoglobulins, and electrophoresis. Other tests commonly ordered on CSF include lactate and ammonia.

PROTEIN

Low–molecular-weight proteins that comprise CSF are derived from plasma proteins that are transported across capillary endothelial cells of the choroid plexus and meninges and from intrathecal synthesis. Generally, CSF total protein ranges from 15 to 45 mg/dL. However, CSF protein varies with age. Neonates and adults older than 40 years of age usually exhibit higher protein concentrations. In addition, CSF protein levels are dependent upon site of collection. Protein levels are higher in CSF collected by lumbar puncture, whereas protein levels on specimens obtained from the ventricles are lower.

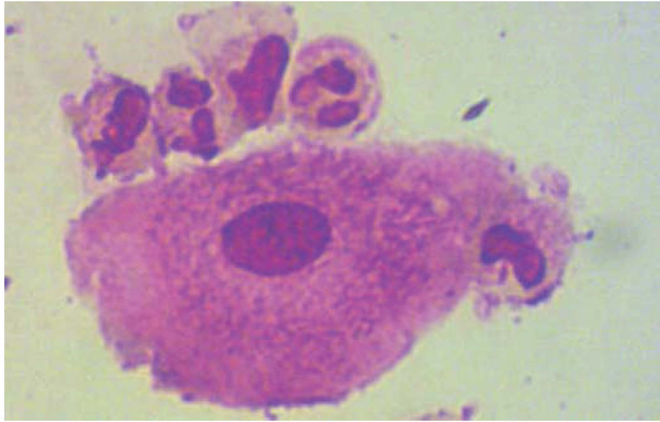

Figure 12-22. Squamous epithelial cells are rarely found in CSF and are contaminants introduced during the lumbar puncture procedure. This field of view also contains five neutrophils (Wright stain 1000×).

Standard methods are used for determining total protein in CSF. These methods include dye-binding, immunochemistry, and turbidimetric methods. These methods are usually performed in most chemistry sections of clinical laboratories.

Conditions associated with increased total protein in CSF include endocrine disorders, hemorrhage, infections, obstruction, traumatic tap, and toxic conditions. Decreased levels of CSF protein are associated with loss of fluid from a dural tear, a sudden loss of CSF volume as during performance of a pneumoencephalography, increased absorption by arachnoid villi caused by increased cranial pressure, and hyperthyroidism.

One of the specific proteins evaluated in CSF is albumin. CSF albumin is derived from transport across the blood–brain barrier, because the CNS does not synthesize albumin. Evaluation of the blood–brain barrier is often performed by calculating a CSF albumin: serum albumin ratio or a CSF/serum albumin index. CSF/serum albumin ratio or CSF/serum albumin index as shown here.

CSF/serum albumin ratio/index = CSF albumin (g/dL)/ serum albumin (g/dL)

CSF/serum albumin index = CSF albumin (mg/dL)/ serum albumin (g/dL)

The normal CSF/serum albumin ratio is about 1:230 while the normal CSF/serum albumin index is less than 9. Index values of 9 to 14 correlate with slight impairment, 15 to 30 with moderate impairment, and 30 with severe impairment. A complete breakdown of the blood–brain barrier is indicated by a CSF/serum albumin index in excess of 100. The CSF normal contains small amounts of the immunoglobulin, IgG. The CNS can produce increased amounts of IgG in pathologic conditions, or IgG can be increased due to increased plasma protein transport.

The CSF IgG ratio and CSF IgG index can be calculated with a similar formula as albumin.

CSF/serum IgG ratio = CSF IgG (g/dL)/serum IgG (g/dL)

CSF/serum IgG index = CSF IgG (mg/dL)/serum IgG (g/dL)

Normally, the CSF/serum IgG ratio is 1:369, while the CSF IgG index ranges from 3 to 8. Conditions that are specific to increased intrathecal synthesis of IgG can be identified by using albumin values as a reference to calculate a CSF IgG index by the following formula.

CSF IgG index = [CSF IgG (mg/dL)/serum IgG (g/dL)] [serum albumin (g/dL)/CSF albumin (mg/dL)]

The CSF IgG index normally ranges from 0.30 to 0.70.

An increased CSF IgG index is associated with multiple sclerosis, inflammatory neurologic disorders, and increased intrathecal production. A decreased CSF IgG index is seen when the blood–brain barrier is compromised.

Protein Electrophoresis

Protein electrophoresis is performed on concentrated CSF to identify the type and relative amounts of proteins that may be present in the CSF. The protein bands that are present are

similar to those found in serum protein electrophoresis, but in different amounts and proportions. Transthyretin (prealbumin) and albumin make up the greatest portion of CSF protein, while beta is about double that seen in serum, and gamma is normally one-half that seen in serum. Other protein bands that are present in CSF include transferrin and small amounts of alpha-1 antitrypsin. One of the transferrins, tau transferrin, is made primarily by the CNS. Discovering this protein band on protein electrophoresis of fluid from the ear or nose confirms diagnoses of **otorrhea** (discharge from the ear) and **rhinorrhea** (discharge from the nasal cavity).

More commonly, CSF protein electrophoresis is performed to detect oligoclonal bands in the gamma region. Finding these bands in CSF and not in serum may help establish a diagnosis of multiple sclerosis. Other CNS disorders in which oligoclonal bands are present in the CSF include subacute sclerosing panencephalitis, neurosyphilis, cryptococcal meningitis, bacterial and viral meningitis, acute necrotizing encephalitis, human immunodeficiency virus type I infections, and Guillain–Barré syndrome.

Myelin Basic Protein

The myelin sheath surrounding the axons provides for proper nerve function. It consists of about 70% lipid and 30% proteins. One of these proteins, myelin basic protein (MBP), can be present in CSF in demyelinating diseases such as multiple sclerosis. Normal levels of MBP in CSF are less than 4 ng/mL. During acute exacerbations of multiple sclerosis, MBP levels can be in excess of 8 ng/mL. Other conditions that may increase CSF MBP levels include trauma to the head, hypoxia, myelopathy, and intrathecal administration of chemotherapy.

GLUCOSE

Glucose is present in CSF at a level 60% to 70% of plasma in normal adults. It is maintained at equilibrium with plasma glucose and is both actively transported by endothelial cells and also moves across a concentration gradient by simple diffusion.

The normal range for CSF glucose is 50 to 80 mg/dL, with a normal CSF glucose to serum glucose ratio of 0.6. CSF glucose levels may be increased in hyperglycemia. A traumatic tap may produce an elevated CSF glucose level because the contaminating blood will contain higher levels of glucose.

Decreased CSF glucose levels are usually observed in CNS infections because both leukocytes and microorganisms will consume glucose. Other conditions that decrease CSF glucose include hypoglycemia, impaired glucose transport, increased CNS glycolytic activity, and metastatic carcinoma.

LACTATE

Lactate is present in CSF due to CNS anaerobic metabolism. Its level is independent from plasma lactate levels. Normal lactate concentration ranges from 11 to 22 mg/dL. Increased CSF lactate levels usually reflect CNS tissue hypoxia and is associated with cerebral infarct, trauma to the brain, cerebral ischemia/arteriosclerosis, intracranial hemorrhage, cerebral edema, hypotension, meningitis, decreased arterial partial pressure of oxygen, and hydrocephalus.

In addition, CSF lactate level can help differentiate viral meningitis (rarely elevating CSF lactate beyond 30 mg/dL) from other forms such as bacterial, fungal, and tubercular (often producing CSF lactate levels greater than 35 mg/dL).

D-DIMERS

D-dimers are degradation products of fibrin and can help to differentiate between traumatic tap and CNS hemorrhage. If present in CSF, D-dimers indicate that a clot has formed at the hemorrhage site. If D-dimers are not present, then the appearance of RBCs in the CSF is probably due to a traumatic tap. D-dimers may also be present in the CSF of patient with lymphoma or carcinoma.

Microbiology Procedures

Among the most serious diagnoses made on CSF is meningitis. Detection of meningitis involves several microbiologic procedures. Of the three to four tubes of CSF normally collected, the most sterile is used for microbiology procedures. The tube least likely to be contaminated by the puncture site includes the second tube collected or any subsequent tube. If the volume of CSF collected is inadequate to fill more than one tube, microbiology procedures should be performed first and then any remaining specimen may be used for cell counts and chemistries.

STAINS

Several stains may be used on concentrated CSF. Specimens are concentrated using standard centrifugation or cytocentrifugation. Cytocentrifugation results in a higher yield of microorganisms. Initially, a Gram stain is performed, which demonstrates 60% to 90% sensitivity. Figure 12-23 compares a Wright stain of CSF with microorganisms with that of a Gram stain on the same CSF. Additional stains may be required for the detection of some microorganisms. Ziehl–Neelsen stain and fluorescent rhodamine stain are used to stain *Mycobacteria tuberculosis. Cryptococcus neoformans* is best detected by India ink (sensitivity of 25% to 50%) or nigrosin stain. Figure 12-24 illustrates a positive India ink stain.

CULTURES

Culture and sensitivity follow the staining procedures. Sediment of centrifuged CSF is inoculated into thioglycolate broth as well as plates of blood agar, chocolate, and MacConkey

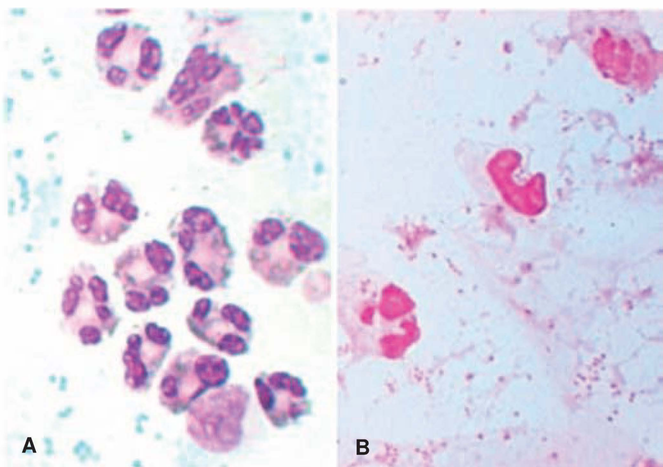

Figure 12-23. Comparison of Wright-stained and Gram-stained bacteria in cerebral spinal fluid. **A:** Wright stain (notice bacteria stain blue). **B:** Gram stain reveals that the bacteria are gram-negative (*pink*).

agar. Strips of X–V may be applied to the blood agar plate if Haemophilus is suspected. If fungal meningitis is suspected, Sabouraud dextrose agar should be inoculated. Inoculate Middlebrook broth and agar if Mycobacteria is suspected.

Microorganisms most commonly responsible for causing meningitis include *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae,* and Klebsiella species. Meningitis can be caused less frequently by staphylococci, other streptococci, *Listeria monocytogenes,* coliform bacteria, *M. tuberculosis, C. neoformans,* other fungi, leptospira, anaerobic bacteria, amebae, and parasites.

It is important to note that if antibiotic therapy was administered prior to the collection of CSF for culture, the recovery of microorganisms may be significantly reduced.

IMMUNOLOGIC TESTS

Immunologic tests for microorganisms provide a rapid method for detection of meningitis-causing agents. However, sensitivity and specificity vary among assays, and the possibility of false positives and false negatives creates complications in interpretation. Immunologic tests do not replace microbiologic stains and cultures as standard procedures.

Various methods are used in immunologic testing. These methods include coagulation, counterimmunoelectrophoresis, enzyme-linked immunosorbence, fluorescent treponemal antibody test, latex agglutination, radioimmunoassay, and Venereal disease research laboratory (VDRL) test. In addition, molecular techniques such as polymerized chain reaction (PCR) are beginning to be used for identification of infectious agents in body fluids. Detail of each of these procedures is not within the scope of this text.

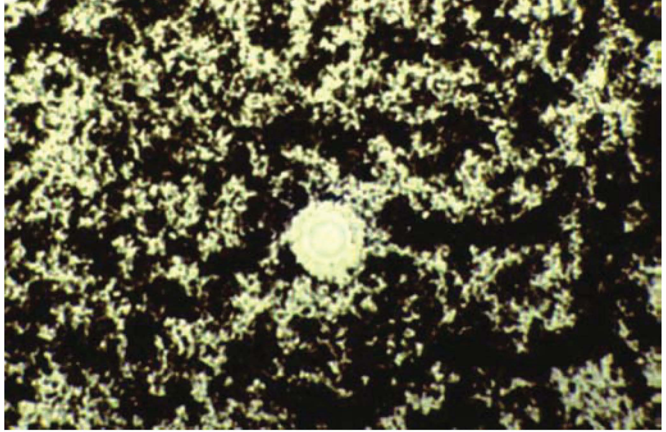

Figure 12-24. Positive India ink stain. (Courtesy of Wolters Kluwer.)

CLINICAL CORRELATIONS

The importance of performing CSF analysis rapidly and correctly cannot be stressed enough. If the diagnosis of meningitis is overlooked, the results are often fatal. Meningitis is categorized as one of four types: bacterial, fungal, tubercular, and viral. Parasites also cause meningitis, but to a lesser extent.

Along with the presence of bacteria, bacterial meningitis is accompanied by elevated CSF protein levels and decreased CSF glucose. Increased numbers of leukocytes (pleocytosis) is present with neutrophils comprising the greatest percentage. Sometimes microorganisms can be seen on Wright stain, either intracellularly or extracellularly. These should be confirmed by Gram stain. Within a week of antibiotic therapy, the CSF leukocyte count decreases and the differential count shifts back to lymphocytes and monocytes. In addition, protein and glucose levels gradually return to normal. CSF results in meningitis caused by *M. tuberculosis* exhibit similar findings as bacterial meningitis.

Viral meningitis usually exhibits a lymphocytosis containing a variety of reactive lymphocyte morphology. However, atypical forms of lymphocytes in CSF must be differentiated from those that may be seen in leukemia. The later stages of viral meningitis exhibit more monocytes and macrophages than lymphocytes.

CSF glucose levels may remain normal in some forms of viral meningitis, although CSF protein is elevated. Normal CSF glucose levels may also be seen in fungal meningitis, although CSF glucose can also be decreased. CSF protein is usually elevated in fungal meningitis. Leukocytes are increased in CSF during fungal meningitis with lymphocytes predominating. Meningitis may also be caused by parasites such as Acanthamoeba and Naegleria species.

Abnormal CSF results should be interpreted with consideration of side effects created by test procedures and interventions. The CSF leukocyte count and differential may show a neutrophilic pleocytosis if a lumbar puncture is repeated within 12 hours of a previous lumbar puncture. Erythrophagocytosis may also be present. Similar findings of increased numbers of lymphocytes, neutrophils, monocytes, macrophages, and eosinophils can be seen up to several weeks after either a pneumoencephalogram or a myelogram. Intracranial

1. A lumbar puncture should NOT be performed if the patient has:
   a. Dementia or acute states of acute confusion
   b. Infection such as encephalitis or meningitis
   c. Inflammation over or near the puncture site
   d. Unexplained seizures or multiple sclerosis
2. Which of the following is NOT a membrane surrounding the brain?
   a. Arachnoidea
   b. Dura mater
   c. Pia mater
   d. Subarachnoid
3. Central nervous system epithelial cells include (select all that apply):
   a. Choroidal cells
   b. Endothelial cells
   c. Ependymal cells
   d. Pia-arachnoid mesothelial cells
4. A small amount of CSF is formed by the:
   a. Cerebral ventricles capillaries
   b. Dura mater and pia mater
   c. Ependymal lining cells
   d. Ventricular choroid plexuses
5. Water-soluble substances that rapidly diffuse across the blood–brain barrier include (select all that apply):
   a. Alcohol
   b. Chloride
   c. Glucose
   d. Protein
6. Pellicle formation in CSF requires:
   a. Centrifugation
   b. Culture
   c. Refrigeration
   d. Staining
7. CSF may appear oily if the patient has:
   a. A hemorrhage
   b. Had a myelogram
   c. Multiple sclerosis
   d. Normal CSF
8. Typically, the protocol for the performance of CSF analysis when three tubes are collected is which order for cell counts, chemistries, microbiology?
   a. 1, 2, 3
   b. 2, 1, 3
   c. 3, 1, 2
   d. 3, 2, 1
9. A cranial hemorrhage is indicated by CSF that is colored:
   a. Pink
   b. Red
   c. Xanthochromic
   d. All of these
10. The concentration of proteins found in CSF is:
    a. Equal to that of serum
    b. Greater than that of serum
    c. Less than that of serum
11. Cells that are normally seen in CSF include (select all that apply):
    a. Erythrocytes
    b. Lymphocyte
    c. Monocytes
    d. Neutrophils
12. Cells that may be seen in clusters resembling tumor cells may be:
    a. CNS lining cells
    b. Macrophages
    c. Nucleated RBCs
    d. None of these
13. The presence of siderophages in CSF indicates:
    a. A normal finding
    b. Meningitis
    c. Old hemorrhage
    d. Traumatic tap
14. The presence of nucleated red blood cells in CSF indicates:
    a. A normal finding
    b. Meningitis
    c. Old hemorrhage
    d. Traumatic tap
15. The presence of which cell may be the only abnormality in multiple sclerosis?
    a. Choroidal cells
    b. Eosinophils
    c. Macrophages
    d. Plasma cells

16. Malignant cells seen in CSF:
- **a.** Can be of CNS origin
- **b.** Can be leukemia cells
- **c.** Metastasize from other sites
- **d.** May be all of these

17. CSF protein levels are:
- **a.** Higher than serum levels
- **b.** Independent of puncture site
- **c.** Decreased in hyperthyroidism
- **d.** Increased in young adults

18. A myelin basic protein level of 2 ng/mL indicates
- **a.** Brain hypoxia
- **b.** Head trauma
- **c.** Myelopathy
- **d.** Normal results

19. Meningitis results in:
- **a.** Decreased CSF glucose and decreased CSF protein
- **b.** Decreased CSF glucose and increased CSF protein
- **c.** Increased CSF glucose and decreased CSF protein
- **d.** Increased CSF glucose and increased CSF protein

20. If only a small amount of CSF is obtained, which is the most important procedure to perform first?
- **a.** Cell count
- **b.** Chemistries
- **c.** Immunology
- **d.** Microbiology

CASE STUDY

Case 12-1 A clear colorless CSF was obtained by lumbar puncture from a 2-year-old child who is lethargic and has fever. A cell count on the undiluted specimen revealed 860 WBCs and 60 RBCs in 9 mm^2 of the hemocytometer. Figure 12-25 represents the Wright-stained cytocentrifuged preparation of this CSF.

1. What are the cell counts?

2. Approximate the differential count.

3. What else can be observed on the Wright stain?

4. Which organism is most likely the causative agent, *Neisseria meningitidis* or *Streptococcus pneumoniae*? Why?

Case 12-2 An elderly man fell in his home and was brought to the emergency department when discovered by a caregiver. How long ago he had fallen is unclear. Three tubes of CSF were collected and are displayed in Figure 12-26. A Wright-stained cytocentrifuged preparation of this CSF is shown in Figure 12-27.

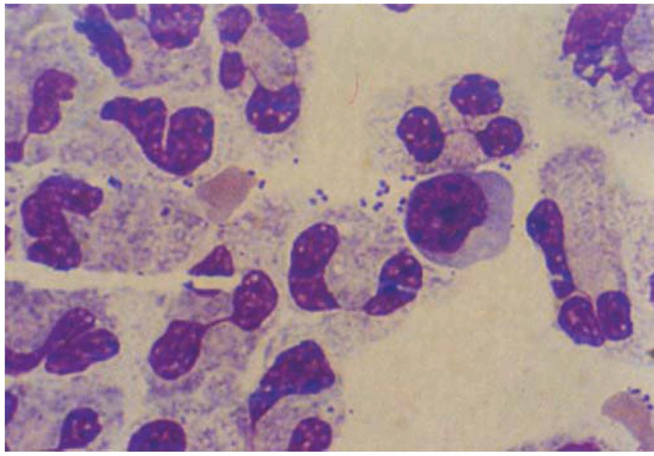

Figure 12-25. Case 12-1 Wright-stained cytocentrifuged CSF.

1. Explain how these results indicate a traumatic tap, fresh hemorrhage, or old hemorrhage.

2. What chemical analysis helps differentiate between CNS hemorrhage and traumatic tap?

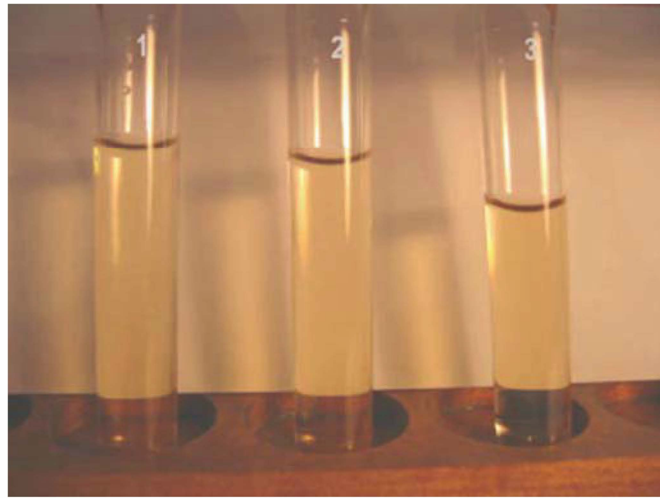

Figure 12-26. Case 12-2 CSF specimen tubes one, two, and three.

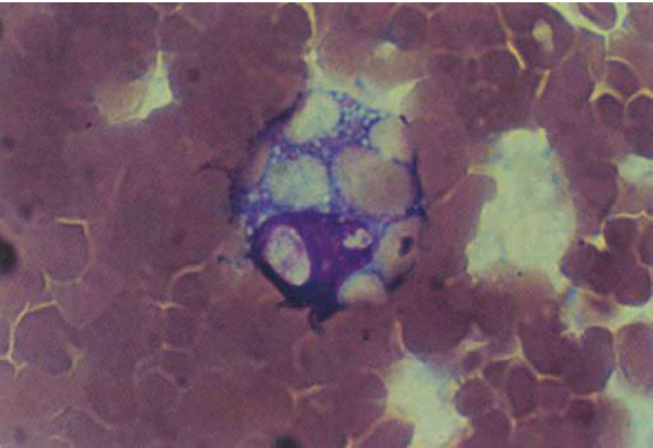

Figure 12-27. Case 12-3 Wright-stained cytocentrifuged CSF.

Case 12-3 Observe the images from this case (Figs. 12-28 and 12-29).

1. Suggest a possible cause for the observations made.

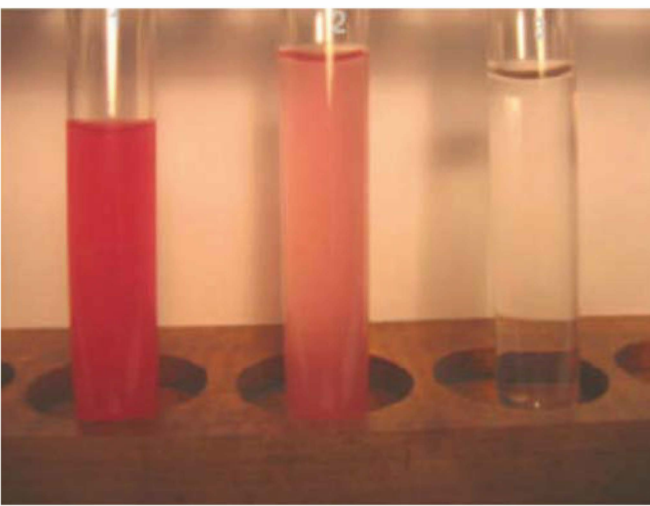

Figure 12-28. Case 12-3 CSF specimen tubes 1, 2, and 3.

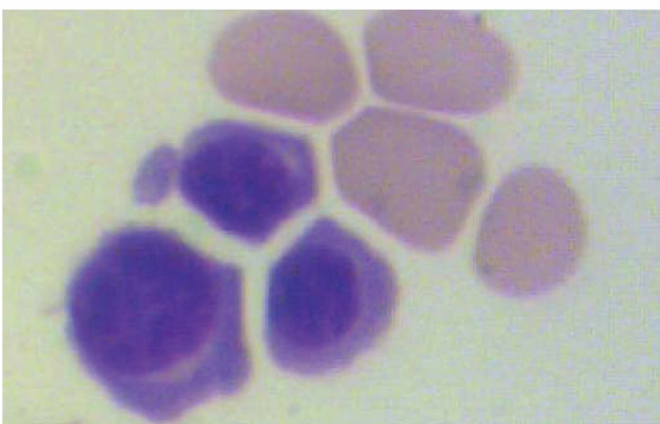

Figure 12-29. Case 12-3 Wright-stained cytocentrifuged CSF.

Case 12-4 A patient comes into the emergency department with otorrhea.

1. Explain how to determine if the fluid is CSF.

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Chapter 13

Serous Body Fluids

KEY TERMS

Ascites
Chyle
Chylous
Edema
Effusion
Exudates
Paracentesis
Pericardial
Pericardiocentesis
Peritoneal
Peritoneal Lavage
Pleural
Pseudochylous
Serous
Thoracentesis
Transudates

LEARNING OBJECTIVES

1. Define serous body fluids.
2. List the various serous body fluids and correlate to body cavity from which they are obtained.
3. Explain the formation of effusions.
4. Describe body fluid collection procedures and the risk involved during the procedure.
5. Differentiate between transudates and exudates using laboratory results.
6. Differentiate between chylous and pseudochylous effusions.
7. List causes for various effusions.
8. Select appropriate laboratory tests for the body fluid being tested.
9. Describe the appearance of normal and abnormal body fluids.
10. Compare and contrast the morphology of cells seen in body fluids.
11. Interpret laboratory results in the analysis of body fluids.

Serous body cavities are those which surround various organs (heart, lungs, abdominal) and are lined with serous membranes. The serous membrane that covers the organ is the visceral portion of the membrane, whereas the serous membrane that lines the body wall is the parietal portion of the membrane. Serous fluid fills the space between the visceral portion and the parietal portion and functions as a lubricant between the membranes of the body wall and organs. Figure 13-1 shows the mesothelial lining of serous body cavities. This chapter contains information regarding the analysis of these fluids (pleural, pericardial, and peritoneal).

Serous Fluid Physiology

The name serous is given to this fluid because of its serum-like composition. Serous fluid is an ultrafiltrate of plasma and is maintained by the pressure forces (tissue colloidal osmotic pressure, capillary hydrostatic pressure, capillary colloidal osmotic pressure, and tissue hydrostatic pressure) and by the absorption of fluid into the lymphatic system. The accumulation of serous fluid is called an **effusion**. Effusions may result from a disruption in the balance of these pressures (**transudates**) or in response to infection and inflammatory processes (**exudates**). Correctly classifying effusions assists physicians to determine a diagnosis. These classifications are based on results from various laboratory tests.

Laboratory Testing of Serous Fluids

Nearly every section of the laboratory can be involved in the evaluation of serous body fluids. Laboratory tests that may be performed on serous body fluids include, but are not limited to, macroscopic evaluation of fluid appearance, microscopic evaluation of cell count and type, chemical analysis, microbiology cultures, and immunologic and molecular analyses. Some of these tests are beyond the scope of this text. However, the more common laboratory procedures are included.

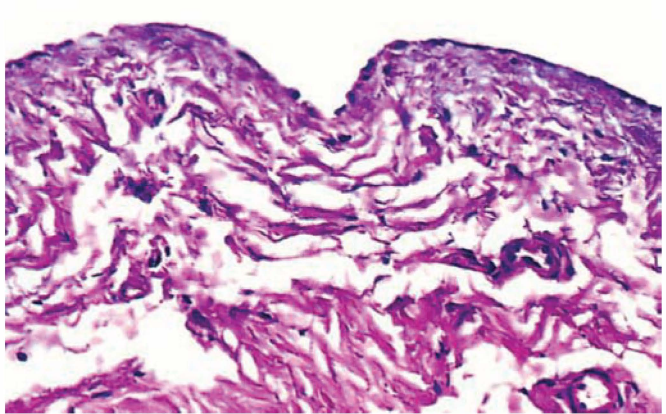

Figure 13-1. Mesothelial lining of serous body cavities. (Image courtesy of Wolters Kluwer, Skokie, IL.)

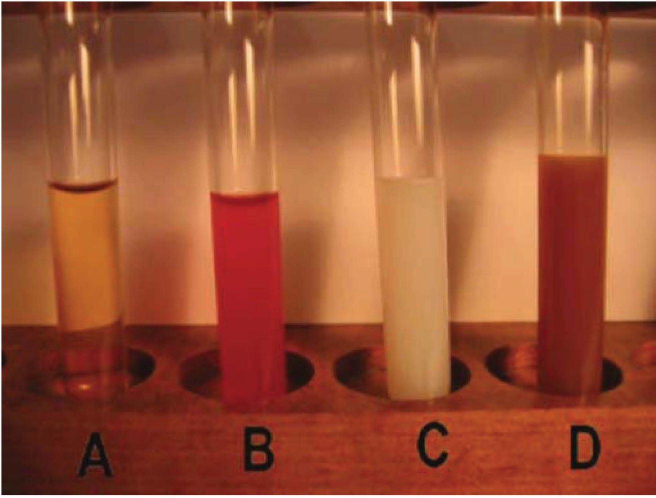

Figure 13-2. Colors of serous fluids: **(A)** normal; **(B)** red/bloody; **(C)** milky/chylous; **(D)** greenish-brown.

MACROSCOPIC EXAMINATION

Serous body fluids normally resemble serum, clear and pale yellow. Chapter 11 includes an overview of terminology used to describe abnormal body fluid color and clarity. Sanguineous specimens may indicate a hemorrhage but also appear similar in traumatic taps. Purulent specimens indicate the presence of white blood cells (WBCs), which correlates with inflammation or infection. Milky fluids may contain **chyle** or be **pseudochylous** (explained later in the chapter). Cholesterol crystals, if present in a fluid, will contribute a golden-green iridescence often termed shimmery or shimmering. A brownish serous fluid may contain a mixture of red and white blood cells along with lining cells or malignant cells. Clotted specimens can be reported as clotted or fibrinous. Figure 13-2 shows some serous fluid specimens of various colors.

CHEMICAL TESTING

Standard chemical tests that are performed on serous fluids include glucose, lactate dehydrogenase (LD), and protein. These are the most common tests used to categorize effusions as transudates or exudates (explained later in this chapter). Tests less commonly performed on body fluids include alkaline phosphatase, ammonia, amylase, bilirubin, chloride, lipids, and pH.

Peritoneal fluid alkaline phosphatase will be increased when the small intestine is perforated. Peritoneal fluid ammonia levels are higher than serum levels in cases of bowel strangulation,

perforated peptic ulcer, ruptured appendix, and ruptured bladder. Ammonia and amylase levels are increased in bowel necrosis. Amylase is also increased in esophageal perforation, metastatic adenocarcinoma, pancreatitis, and bowel necrosis. Esophageal ruptures will cause fluids to become more acidic than their normal pH of 7.3 or higher. The chloride levels of body fluids will be less than that of serum when a bacterial infection is present, due to the presence of both bacteria and WBCs. Testing lipid levels in serous fluids assists in the differentiation between chylous and pseudochylous effusions. Triglycerides are higher in chylous effusions, whereas cholesterol is higher in pseudochylous effusions.

MICROSCOPIC EXAMINATION

Chapter 11 includes an explanation of microscopic examination of body fluids in general. Normally, cells counts and differential are performed. In addition, the presence of crystals is noted.

Red blood cells (RBCs) are not normally seen in body fluids. When present, RBCs may indicate hemorrhage or traumatic specimen collection procedure. WBCs are normally present in low numbers with mononuclear cells predominating. The presence of increased numbers of WBCs correlates with various pathologies and is reflected by their distribution. Types of blood cells that can be seen in serous body fluids include neutrophils, eosinophils, basophils, lymphocytes, plasmacytes, monocytes, histiocytes, and macrophages.

Mesothelial cells that line the serous cavities may also be present in body fluids due to normal sloughing of cells. Mesothelial cells may exhibit reactive morphology that can be confused with plasmacytes, histiocytes, or tumor cells. Mesothelial cells are large with dark blue cytoplasm. Histiocytes (tissue monocytes) may be of similar size to mesothelial cells but have a lighter-colored cytoplasm.

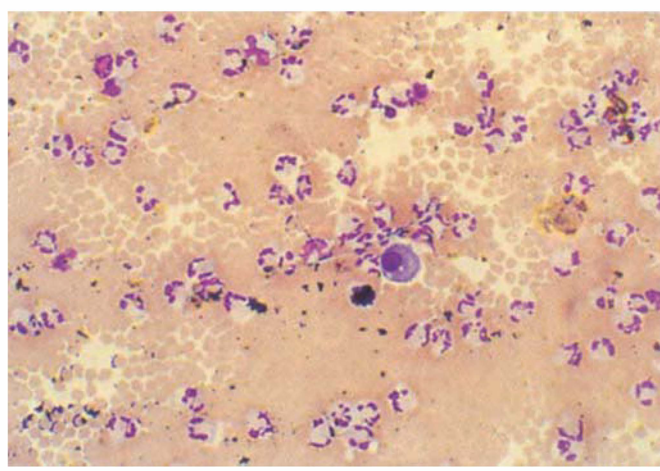

Figure 13-4. Cytospin preparation of pleural fluid containing RBCs, neutrophils, and a mesothelial cell in bacterial infection. Wright stain (200×). (Image courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

Effusions from patients with neoplasms may contain malignant cells. Malignant cells commonly occur in clumps. A pathologist's consult in the identification of malignant cells is a must when suspect cells are seen. The WBC count that is performed usually includes mesothelial and malignant cells, because all nucleated cells are counted for the WBC count. Therefore, the differential count of ten includes mesothelial cells and tumor cells. Cytologic examination should be performed when malignancies are suspected, or to assist in the differentiation between tumor cells and reactive mesothelial cells. Flow cytometry procedures may further establish clonality of lymphocytic populations. Figures 13-3 to 13-12 illustrate the cells that may be seen in various body fluid effusions.

Sometimes, microorganisms may be seen on Wright stained smears while performing differential counts. Though

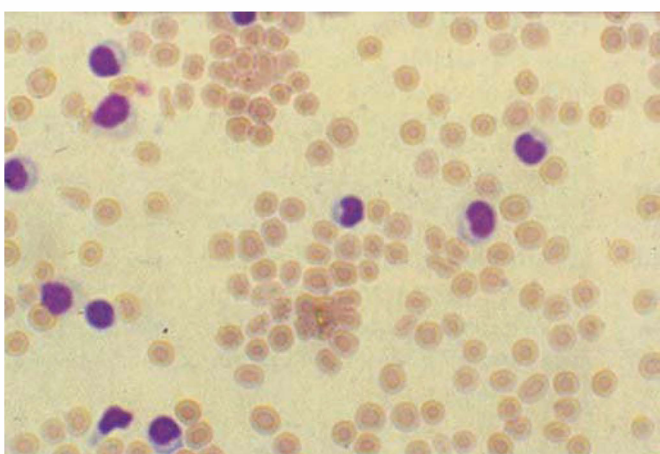

Figure 13-3. Cytospin preparation of pleural fluid containing RBCs and lymphocytes in acute inflammation. Wright stain (400×). (Image courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

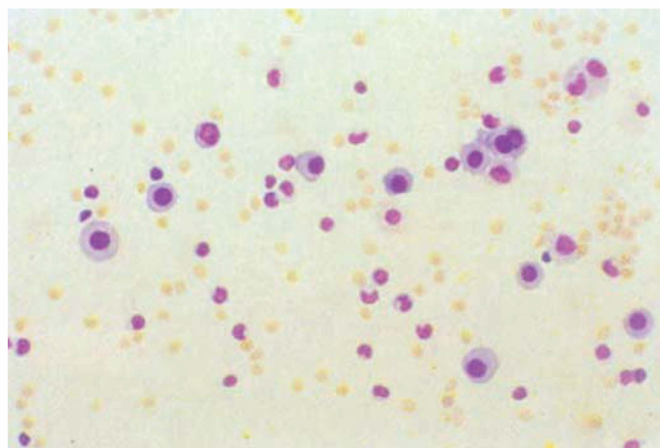

Figure 13-5. Cytospin preparation of peritoneal fluid containing RBCs, lymphocytes, monocytes, and mesothelial cells. Wright stain (200×). (Image courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

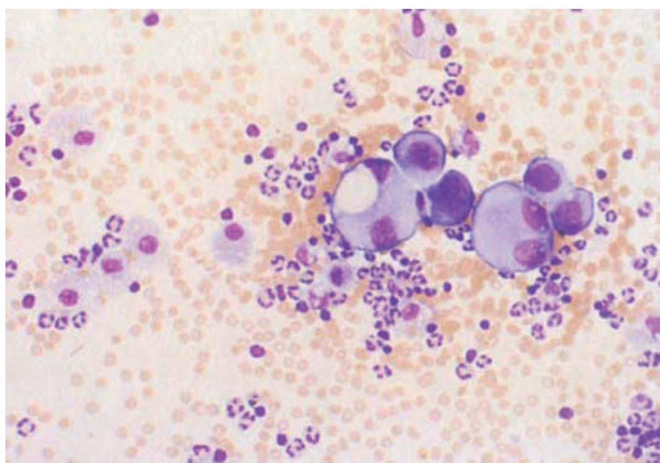

Figure 13-6. Cytospin preparation of pericardial fluid containing RBCs, WBCs, and cells resembling adenocarcinoma. Wright stain (200×). (Image courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

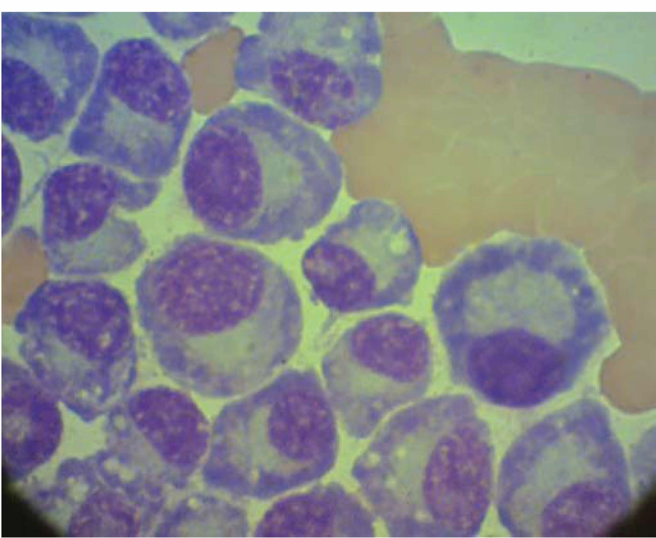

Figure 13-9. Cytospin preparation of ascites fluid containing RBCs, plasmacells from a patient with multiple myeloma. Wright stain (800×).

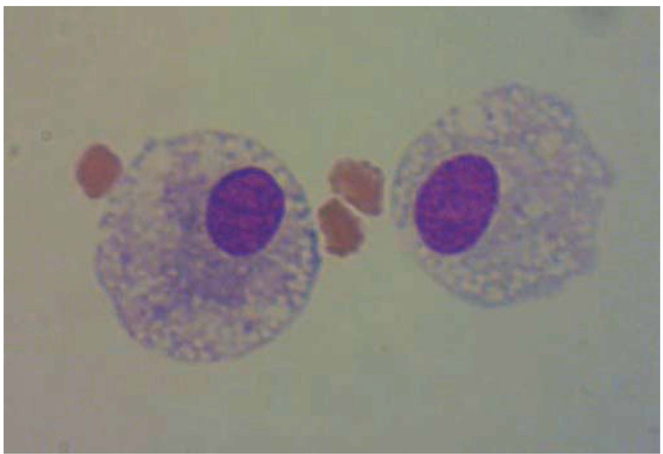

Figure 13-7. Cytospin preparation of pleural fluid containing RBCs, macrophages. Wright stain (800×).

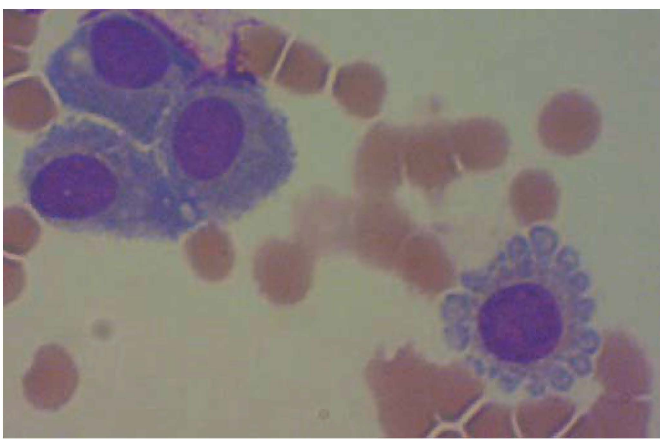

Figure 13-10. Cytospin preparation of ascites fluid containing RBCs, mesothelial cells. Wright stain (800×).

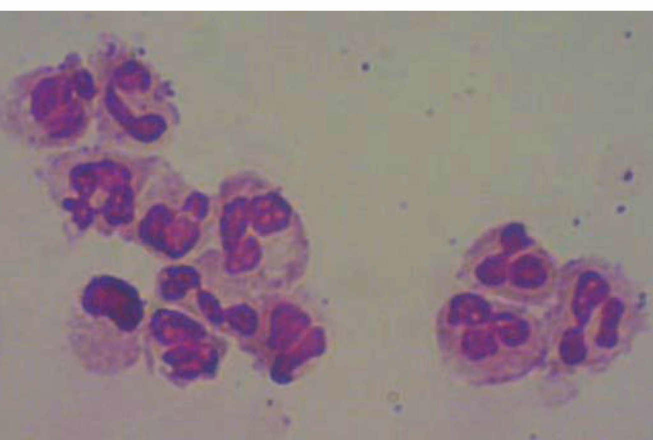

Figure 13-8. Cytospin preparation of pleural fluid containing neutrophils. Wright stain (800×).

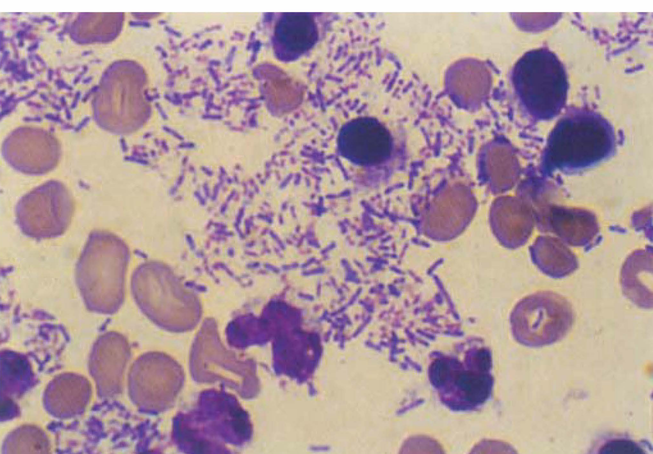

Figure 13-11. Cytospin preparation of peritoneal fluid containing RBCs, WBCs, and many bacteria. Identified by culture as *Escherichia coli.* Wright stain (1,000×). (Image courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott; 1998.)

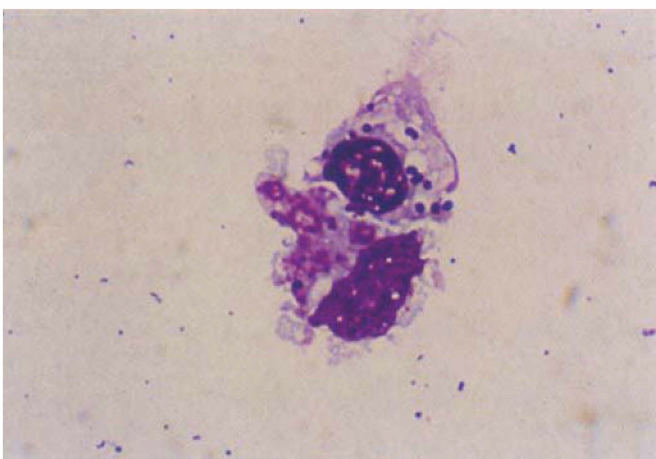

Figure 13-12. Cytospin preparation of peritoneal fluid containing WBCs and few bacteria. Identified by culture as Staphylococcus. Wright stain (1,000×). (Image courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott; 1998.)

these cells can easily be detected, their identification must be made using microbiology procedures.

MICROBIOLOGICAL EXAMINATION

Gram stain and both aerobic and anaerobic cultures should be set up on body fluid specimens to increase the rate of microbial recovery. Pleural fluids should routinely have an acid-fast stain performed for the identification of tuberculosis. In addition, fungal stains and cultures may be set up if a yeast infection is suspected. Table 13-1 summarizes common bacteria observed in effusions.

Categorization of Effusions

Effusions are the accumulation of fluids in the tissue spaces and result from an imbalance in pressures between the tissues and the capillaries. The laboratory tests described earlier assist in the classification of effusions as transudates or exudates.

Table 13-1 Bacteria Commonly Seen in Effusions

EFFUSION	MOST COMMON BACTERIA
Pleural	*Staphylococcus aureus* *Mycoplasma tuberculosis*
Pericardial	*Hemophilus influenzae* *M. tuberculosis*
Peritoneal	*Escherichia coli* *Pneumococci*

TRANSUDATE VERSUS EXUDATE

Transudate effusions occur during various systemic disorders that disrupt fluid filtration, fluid reabsorption, or both. Examples of systemic disorders that may result in the formation of transudates include congestive heart failure, hepatic cirrhosis, or nephrotic syndrome.

Exudate effusions occur during inflammatory processes that result in damage to blood vessel walls, body cavity membrane damage, or decreased reabsorption by the lymphatic system. Examples of these pathologic processes include infections, inflammations, hemorrhages and malignancies. Each of these processes can damage tissues, body cavity membranes, and alter lymphatic function.

Various laboratory tests are used to differentiate between transudates and exudates including fluid appearance, specific gravity, amylase, glucose, LD, and proteins. Table 13-2 outlines this differentiation. Additional tests such as ammonia, lipids, and pH may be useful in confirming the cause of an effusion for specific body sites.

CHYLOUS EFFUSIONS

A **chylous** effusion is an effusion that contains an emulsion of lymph and chylomicrons. Obstruction or damage of lymphatic vessels contributes to the development of a chylous effusion. Chylous effusions appear milky and may appear shimmery (resembling milk mixed with honey) if cholesterol crystals are present. Sheets of cholesterol crystals may be present in serous fluids when a lymphatic vessel located near a cavity is damaged. Figures 13-13 and 13-14

Figure 13-13. Cholesterol crystals in pleural fluid. Bright light (400×).

Table 13-2 Differentiation Between Transudates and Exudates

CHARACTERISTIC	TRANSUDATE	EXUDATE
Color	Pale yellow (serous)	Any abnormal color (brown, cream, green, milky, pink, red, or yellow)
Clarity	Clear	Bloody (sanguinous), cloudy, purulent, turbid
Specific gravity	1.015	1.015
Cell counts (total)	300/L	1,000/L
Fluid: serum amylase	2.0	2.0
Fluid: serum bilirubin ratio	0.6	0.6
Glucose	Equal to serum level	30 mg or more serum level
Fibrinogen (clotting)	Absent (no clotting)	Usually present (usually contains clots)
Lactate dehydrogenase (LD)	60% of serum	60% of serum
Fluid: serum LD ratio	0.6	0.6
Protein	3.0 g/dL	3.0 g/dL
Fluid: serum protein ratio	0.5	0.5

show a pleural fluid with cholesterol crystals from a lymphatic vessel rupture.

Chronic effusions present in disorders, such as rheumatoid arthritis and tuberculosis, may resemble chylous effusions because of the high amount of cellular debris and cholesterol present. These effusions are termed pseudochylous and can be differentiated from chylous effusions using various laboratory tests such as pH and lipid analysis. Table 13-3 lists laboratory test differences between chylous and pseudochylous effusions.

Types of Serous Fluids

Serous body fluids are found in the cavities surrounding the vital organs. This fluid is normally clear and slightly yellow in appearance, resembling serum. Serous cavities include the pericardium, pleura, and peritoneum.

PERICARDIAL FLUID

Pericardial effusions are an accumulation of fluid around the heart. Figure 13-15 illustrates the pericardium surrounding the heart. Normally, the pericardium contains less than 50 mL of fluid. The procedure for removing excess pericardial fluid, **pericardiocentesis**, is dangerous and therefore rarely performed. See Figure 13-16 for an illustration of how this procedure is performed. However, this procedure is necessary to obtain a sample if cultures are needed to investigate an infection or if cytology is needed for suspected malignancy.

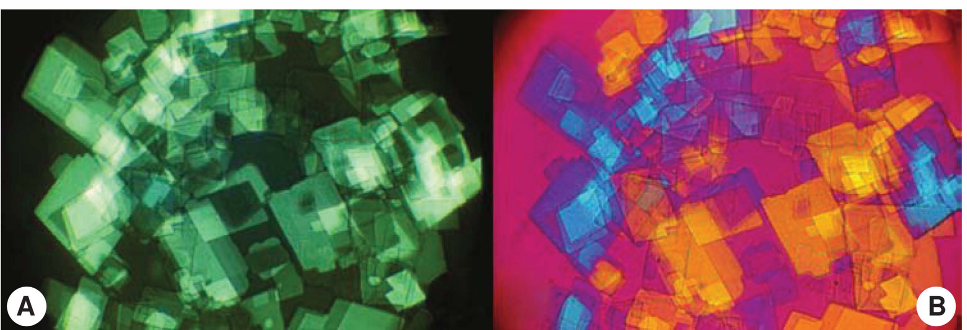

Figure 13-14. Cholesterol crystals in pleural fluid. **A:** Polarized light. **B:** Polarized, compensated light (400×).

Table 13-3 Laboratory Differentiation of Chylous and Pseudochylous Effusions

LABORATORY TEST	CHYLOUS EFFUSION (LYMPHATIC OBSTRUCTION OR DAMAGE)	PSEUDOCHYLOUS EFFUSION (CHRONIC DISORDERS)
Appearance	Milky/creamy (clears after extraction with ether and acidification with HCl)	Milky
Cell counts	Primarily lymphocytes	Mixture of cells
Cholesterol	Lower than serum level	Usually higher than serum level
Triglycerides	Higher than serum level	Lower than serum level
Lipoprotein electrophoresis	Marked elevation of chylomicrons	Chylomicron portion is low or may be absent
pH	Alkaline	Variable

Normal pericardial fluid is pale yellow and clear. Sanguineous (bloody) effusions may be present in pericardial fluid due to a number of causes. Pericardial effusions are all caused by damage to the mesothelium and not by mechanical factors. Therefore, pericardial effusions are usually always exudates. Table 13-4 outlines causes for pericardial exudates as well as causes for other effusions.

PLEURAL FLUID

Pleural effusions occur when fluid accumulates around the lungs. Figure 13-17 illustrates the pleural cavity and its lining. The pleural cavity normally contains less than 30 mL of fluid. Abnormal accumulation of pleural fluid usually begins at the base of the lungs. Factors that contribute to the formation and the removal of pleural fluid include the draining function of the lymphatic system and the exchange of fluids in the capillaries. Fluids enter the pleural sac when there is an increase in capillary hydrostatic pressure and/or a decrease in plasma osmotic pressure.

A **thoracentesis** is performed to remove excess fluid (over 30 mL) from the pleural sac. Removal of pleural fluid not only provides a specimen for laboratory examination but also helps improve patient symptoms and allows for better visualization

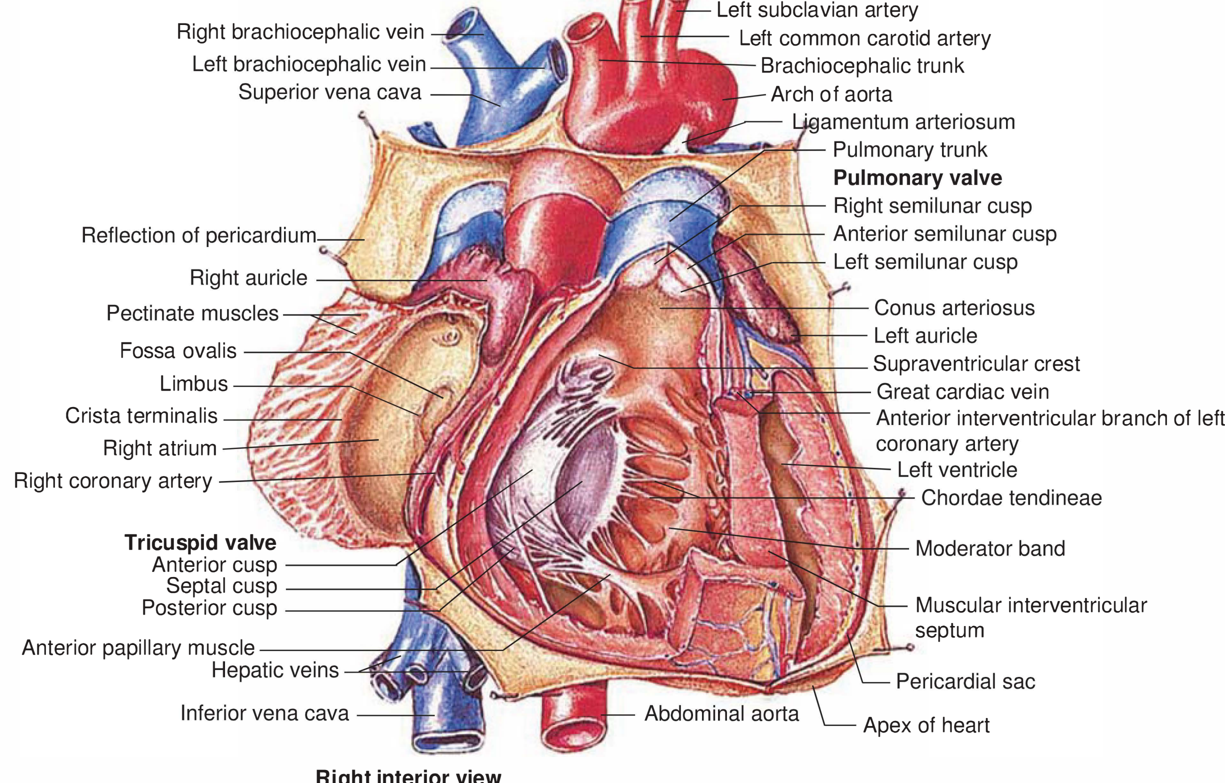

Figure 13-15. The heart and pericardium. (Asset provided by Anatomical Chart Co, Skokie, IL.)

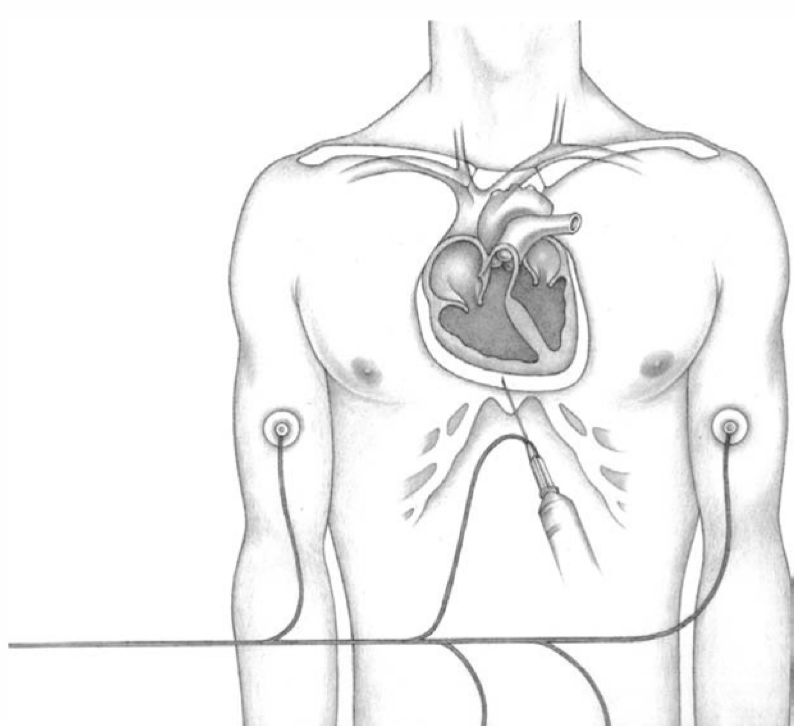

Figure 13-16. Aspirating pericardial fluid. In pericardiocentesis, a needle and syringe are inserted through the chest wall into the pericardial sac (as shown below). Electrocardiographic monitoring, with a lead wire attached to the needle and electrodes placed on the limbs (right arm, left arm, and left leg), helps ensure proper needle placement and avoids damage to the heart. (From *Nursing Procedures.* 4th ed. Ambler, PA: Lippincott Williams & Wilkins; 2004.)

of the lungs and pleural cavity during radiological procedures. An illustration of a thoracentesis is seen in Figure 13-18.

Pleural effusions may be primary or can be secondary to accumulation of peritoneal fluid (ascites). Secondary accumulation occurs because the lymphatic system drains the abdomen toward the right side passing through the diaphragm.

Normal pleural fluid is pale yellow and clear. Abnormal colors and turbidity of pleural fluid indicate various

Table 13-4 Causes for Various Body Cavity Effusions

EFFUSION SITE	CAUSES FOR TRANSUDATE	CAUSES FOR EXUDATE
Pericardial effusion		Cardiovascular disease Coagulation disorders Collagen vascular disorders Infections Metabolic diseases Neoplasms Trauma
Pleural effusion	Acute atelectasis Congestive heart failure Cirrhosis with ascites Hypoproteinemia Peritoneal dialysis Postoperative Postpartum Venous obstruction Congestive heart failure	Collagen vascular disorders Gastrointestinal diseases Infections primary and secondary Neoplasms Postmyocardial infarct Pulmonary emboli or infarct Trauma Bile peritonitis
Peritoneal effusion	Cirrhosis Hypoproteinemia	Infections Neoplasms Pancreatitis Trauma

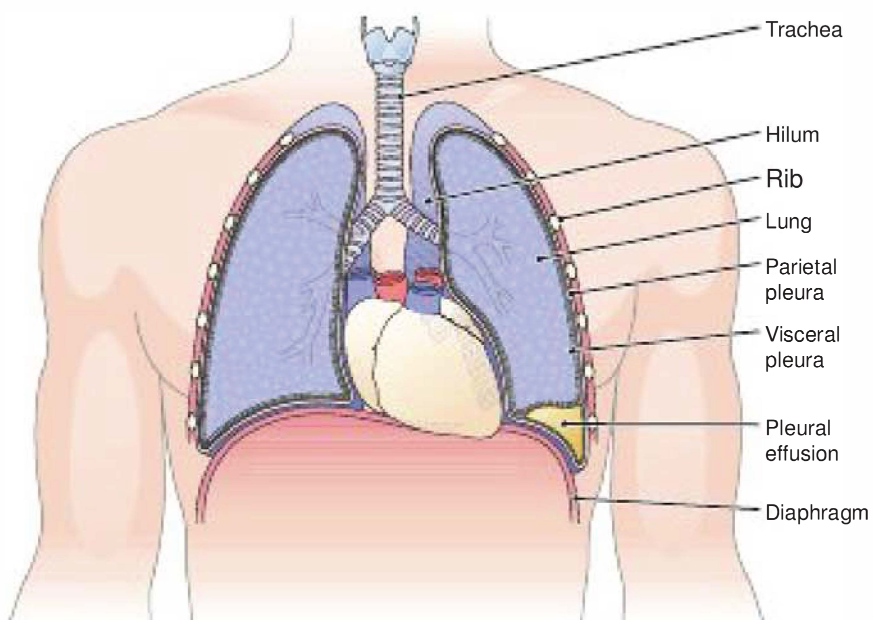

Figure 13-17. The pleural cavity with effusion. (From Cohen BJ. *Medical Terminology*. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2003.)

Figure 13-18. Thoracentesis. A needle is inserted into the pleural space to withdraw fluid. (From Cohen BJ. *Medical Terminology*. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.)

pathologic processes. Among the various colors abnormal pleural fluid may exhibit are sanguineous (if not a traumatic tap), milky, and shimmery. Table 13-4 outlines causes for pleural transudates and exudates as well as causes for other effusions.

PERITONEAL FLUID

A **peritoneal** effusion is the accumulation of peritoneal fluid, also called **ascites**, in the abdominal cavity. Figure 13-19 illustrates the organs that are contained within the peritoneal cavity. Fluid may accumulate in the abdomen as a result of a specific clinical disorder or because of generalized **edema** (accumulation of fluid in tissues).

Ascites is removed by abdominal **paracentesis**, as illustrated in Figure 13-20. The fluid that accumulates during chronic liver disease is a result of a decrease in the plasma colloidal pressure because of the liver's impaired ability to synthesize proteins.

Removal of more than 1,000 mL of ascites can cause hypovolemia and shock. Another procedure used to collect peritoneal fluid is **peritoneal lavage**. Peritoneal lavage is used when the patient has had a blunt or penetrating abdominal trauma.

Normal peritoneal fluid is pale yellow. Abnormal appearances of peritoneal fluid indicate various pathologic processes. Abnormal colors that peritoneal fluid can show include sanguineous (if not a traumatic tap), brown, green, and milky. Table 13-4 outlines causes for peritoneal transudates and exudates as well as causes for other effusions.

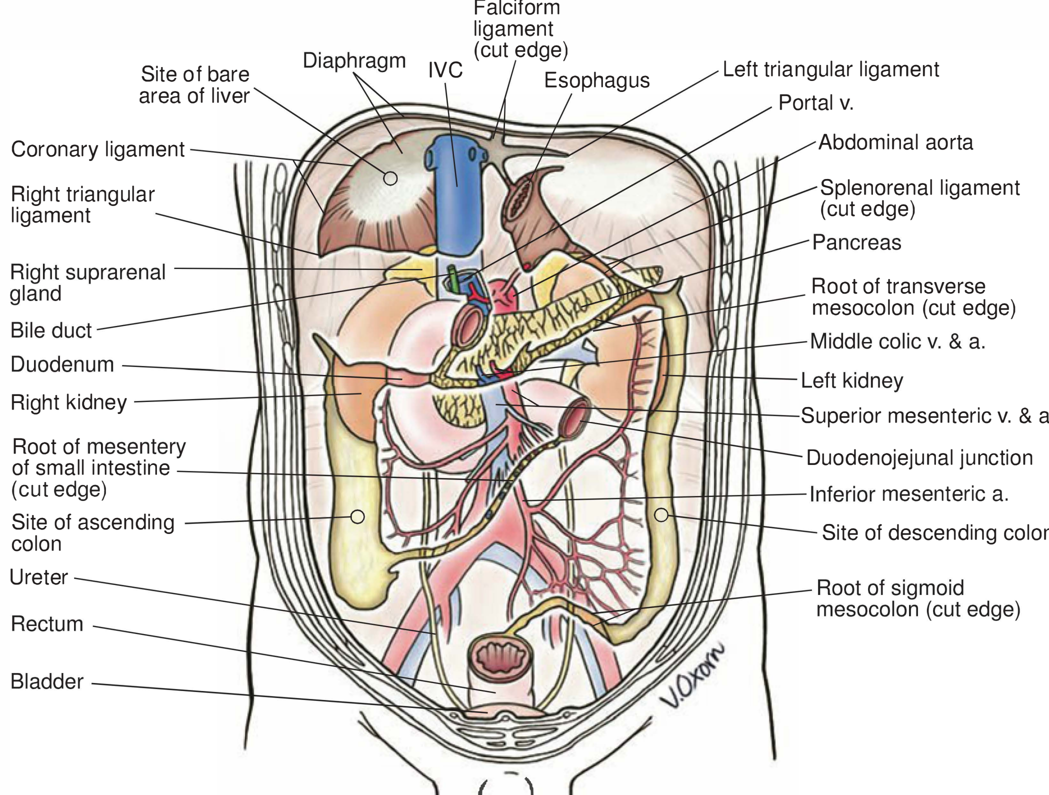

Figure 13-19. The organs of the abdomen. IVC, inferior vena cava. (From Moore KL, Agur A. *Essential Clinical Anatomy*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

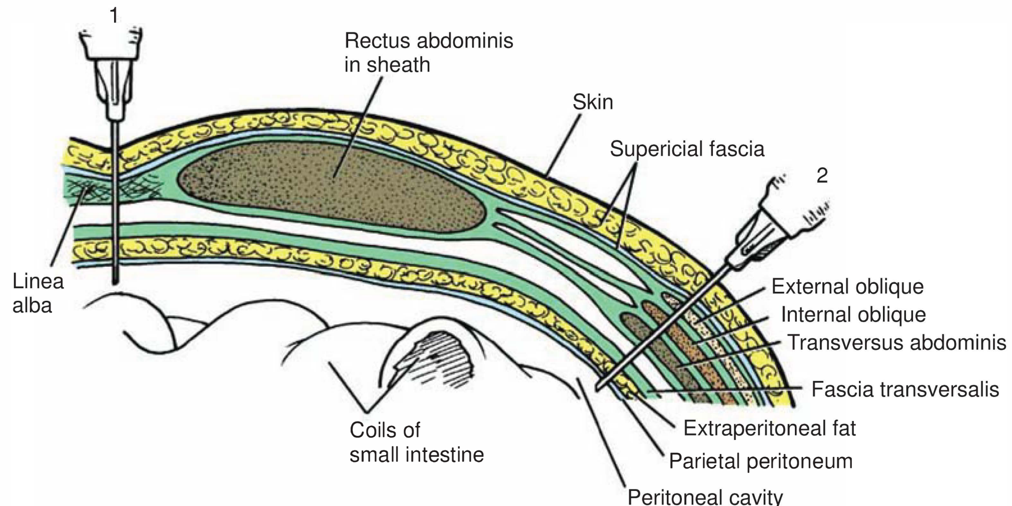

Figure 13-20. Paracentesis of the abdominal cavity in midline. (From Snell RS. *Clinical Anatomy*. 7th ed. Lippincott Williams & Wilkins; 2003.)

Match laboratory findings with type of effusion.

A. Exudate

B. Transudate

1. ______ Clear fluid

2. ______ Purulent fluid

3. _____ Specific gravity 1.015

4. _____ Fluid: serum protein ratio 0.5

5. ______ Protein 3.06/dL

6. _____ High fibrinogen

7. ______ LDH 200 IU

8. _____ Cell count 1,000/cc

9. Serous body cavities are lined with cells derived from the:
a. Endothelium
b. Epithelium
c. Mesothelium

10. Intestinal perforation can be diagnosed by testing the level(s) of _____ in ascites.
a. Alkaline phosphatase
b. Amylase and lipase
c. Blood urea nitrogen
d. Glucose and protein

11. Chronic liver disease can cause fluid to accumulate in the:
a. Pericardium
b. Peritoneum
c. Pleural cavity

12. Removal of more than 1,000 mL of ascites can cause:
a. Edematous extremities
b. Great relief for the patient
c. Hypovolemia and shock
d. Increased lymphatic absorption

13. Which procedure is rarely performed because of the risk involved?
a. Paracardiocentesis
b. Paracentesis
c. Peritoneal lavage
d. Thoracentesis

14. In a pleural effusion caused by bacterial infection, the glucose level is:
a. Equal to that of the serum
b. 30 mg/dL or more higher than serum
c. 30 mg/dL or more less than serum

15. A chylous effusion has a:
a. Cholesterol level lower than serum
b. Triglyceride level higher than serum
c. Milky appearance
d. pH that is alkaline

CASE STUDY

Case 13-1 Ascites fluid was obtained on a patient who had a fever and painful abdominal distention. The fluid appeared cloudy and amber. The cell count was RBCs 20,000/L and WBCs 5,000/L. The differential was neutrophils 70%, lymphocytes 25%, monocytes 5%, and an occasional mesothelial cell. Figure 13-21 represents the smear from this specimen.

1. From which body cavity was this fluid obtained?

2. Classify this effusion as transudate or exudate. Explain the basis for the classification.

3. Which microorganism is most likely present in this fluid?

4. What tests should be done to determine whether there has been an intestinal perforation?

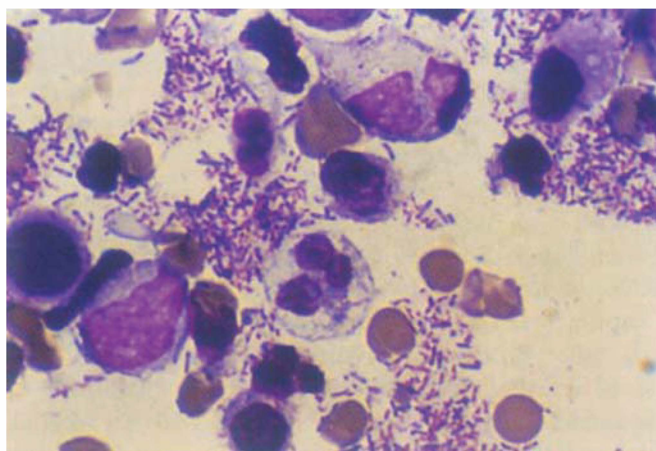

Figure 13-21. Wright stain of ascites fluid. (Image courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

Case 13-2 A pleural fluid was obtained on a patient in the emergency department. It appeared milky and shimmery. The cell count showed no RBCs and WBCs 50/L with nearly 100% lymphocytes. The cell count was difficult to perform because of other objects present on the hemocytometer. Figure 13-22 shows these objects.

1. What are the objects that made the WBC count difficult to perform?

2. Explain the correlation between the macroscopic appearance of this fluid and the microscopic findings.

3. What tests can be performed to determine whether this effusion is due to trauma or is a chronic condition?

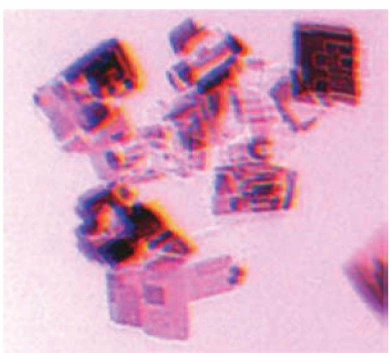

Figure 13-22. Pleural fluid.

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Synovial Fluid

14
Chapter

KEY TERMS

Antinuclear Antibody
Arthrocentesis
Bulge Test
Crystal-induced Arthritis
Diarthrotic
Hyaluronate
Lipophage
Mucin
Ochronotic Shards
Ragocytes
Rheumatoid Arthritis (RA)
Rheumatoid Factor (RF)
Rice Bodies
Rope Test
Septic Arthritis
Synovial
Systemic Lupus Erythematosus (SLE)
Viscosity

LEARNING OBJECTIVES

1. Define synovial.
2. Describe the formation and function of synovial fluid.
3. Explain the collection and handling of synovial fluid.
4. Describe the appearance of normal and abnormal synovial fluids.
5. Correlate the appearance of synovial fluid with possible cause.
6. Describe tests for synovial fluid analysis.
7. Describe cells seen in synovial fluid.
8. Interpret laboratory tests on synovial fluid.
9. Suggest further testing for synovial fluid, based on preliminary results.
10. Classify joint diseases into their recognized categories.

Joint fluid is called **synovial** fluid because of its resemblance to egg white. It is a viscous, mucinous substance that lubricates most joints. Analysis of synovial fluid is important in the diagnosis of joint disease. Aspiration of joint fluid is indicated for any patient with a joint effusion or inflamed joints. Aspiration of asymptomatic joints is beneficial for patients with gout and pseudogout as these fluids may still contain crystals. Evaluation of physical, chemical, and microscopic characteristics of synovial fluid comprise routine analysis. This chapter includes an overview of the composition and function of synovial fluid, and laboratory procedures and their interpretations.

Physiology and Composition

Diarthrotic (movable, articulating) joints are lined with a tissue called synovium. Synovium produces synovia, also called synovial fluid. This fluid capsule cushions diarthrotic joints allowing the bones to freely articulate. A dense connective tissue layer of collagen surrounds the synovial capsule. Figure 14-1 illustrates an articulated joint. Figure 14-2 shows the synovial lining of the synovial capsule.

Synovial fluid is an ultrafiltrate or dialysate of plasma and contains levels of glucose and uric acid that are equivalent to plasma. Synovial fluid protein, however, is at a lower level (about one-third) than that of plasma. Plasma constituents that enter joint fluid must cross a double-barrier membrane.

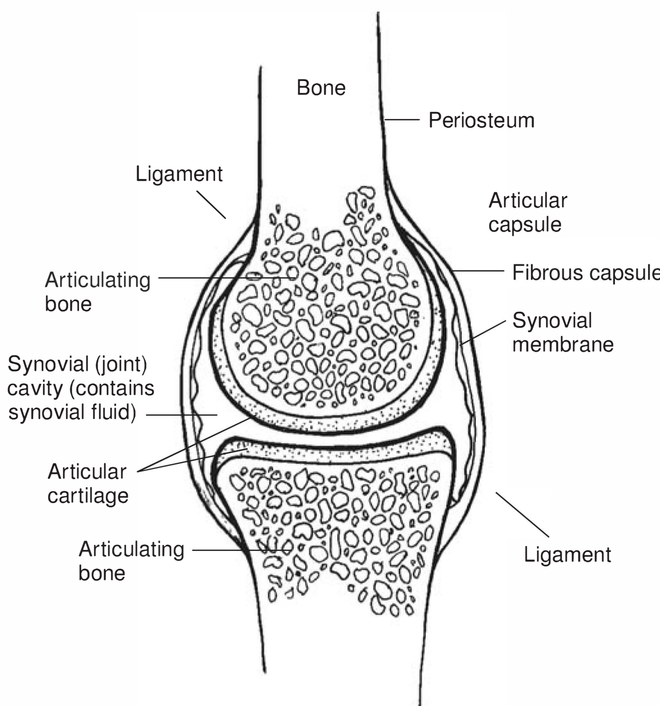

Figure 14-1. Articulated joint. (From Oatis CA. *Kinesiology: The Mechanics and Pathomechanics of Human Movement.* Baltimore, MD: Lippincott Williams & Wilkins; 2003.)

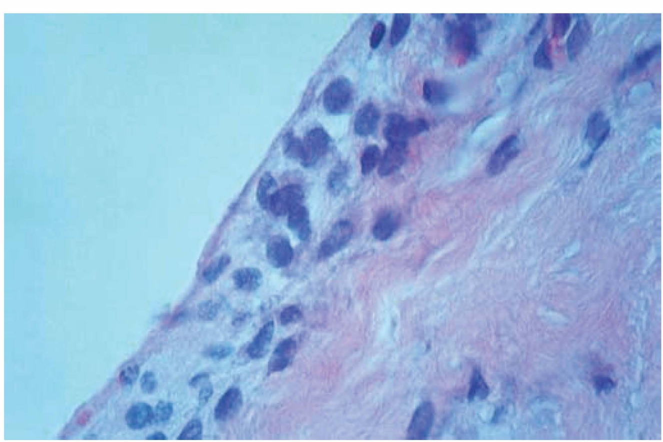

Figure 14-2. Synovial membrane from a normal knee joint shows joint space, synovial membrane composed of synovial cells embedded in a loose connective tissue stroma overlying dense collagen (hematoxylin and eosin). (From McClatchey KD. *Clinical Laboratory Medicine.* 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

First, the endothelial lining of the capillaries is traversed followed by movement through a matrix that surrounds synovial cells. This ultrafiltrate is combined with a mucopolysaccharide (**hyaluronate**) synthesized by the synovium.

Specimen Collection

After finding positive results with a "**bulge test**" (Fig. 14-3), the physician will perform an **arthrocentesis** and aspirate the effected joint. An appropriate gauge needle is attached to a syringe and the entry site is cleansed. A two-step process is employed for arthrocentesis in which the first puncture is made through the skin followed by a second thrust into the synovial capsule. Figure 14-4 illustrates needle placement in arthrocentesis of elbow and knee joints.

After fluid is aspirated and the needle withdrawn from the joint, the needle is removed and an end cap placed on the tip of the syringe. The syringe is properly labeled and sent to the laboratory for testing. Some laboratories require that synovial fluid specimens be placed in specimen containers appropriate

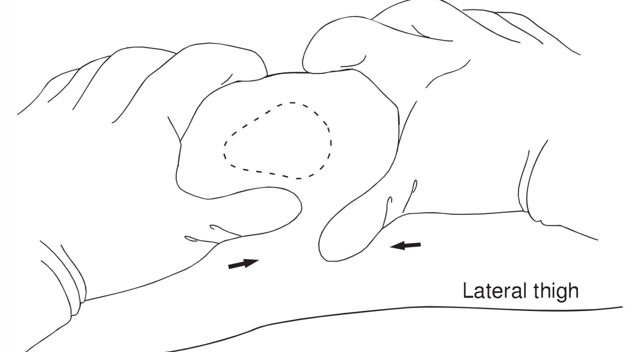

Figure 14-3. Bulge test of joint for the detection of synovial effusion.

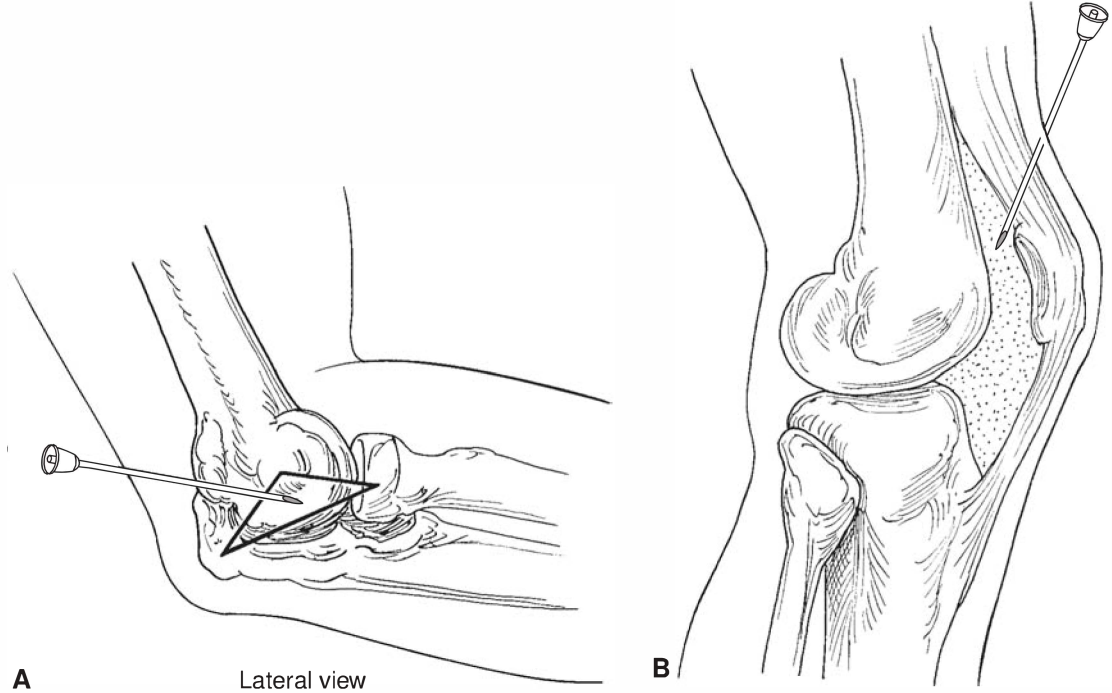

Figure 14-4. Placement of needle in arthrocentesis of **(A)** elbow and **(B)** knee joints.

for the tests ordered. A heparinized tube is preferable to ethylenediaminetetraacetic acid (EDTA) or other anticoagulants for cell counts, sterile containers for microbiology testing, and plain tubes are normally used for chemistry and immunology testing of synovial fluid.

Synovial fluid specimens should be handled like STAT specimens and delivered immediately to the laboratory for testing to avoid alteration of chemical constituents, cell lysis, and problems in microorganism detection and identification. If a glucose test is to be performed, the patient should be fasting for at least 6 hours prior to collection of joint fluid. A 6-hour fast is necessary to establish an equilibrium between plasma and joint glucose levels.

Laboratory Testing

PHYSICAL EXAMINATION

Volume

The amount of fluid contained in joints is usually small. The knee joint normally contains up to 4 mL of fluid. The volume of the aspirate is usually recorded at bedside, but some laboratories may include volume in their reports as well.

Color and Clarity

Normal synovial fluid is colorless and clear. Other appearances may indicate various disease states. Yellow/clear synovial fluids are typical in noninflammatory effusions, whereas yellow, cloudy fluids usually involve an inflammatory process. A white, cloudy synovial fluid may contain crystals; and synovial fluid that is red, brown, or xanthochromic indicates hemorrhage into the joint. In addition, synovial fluid may contain various types of inclusions. Free-floating aggregates of tissue appear as **rice bodies**. Rice bodies are seen in **rheumatoid arthritis (RA)** and result from degenerated synovium enriched with fibrin.

Ochronotic shards are debris from metal and plastic joint prosthesis. These shards look like ground pepper. Figure 14-5 compares normal and blood synovial fluids, whereas Figure 14-6 demonstrates the appearance of synovial fluid inclusions.

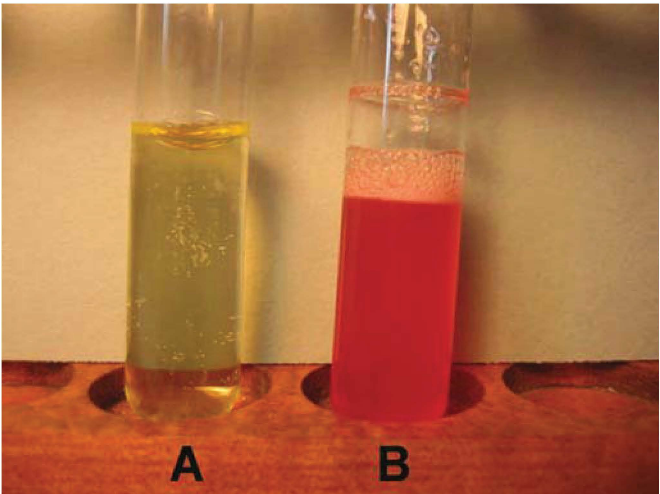

Figure 14-5. Synovial fluid. **A:** Normal. **B:** Bloody.

Figure 14-6. Synovial fluid inclusions. **A:** "Ground pepper" ochronotic shards. **B:** "Rice bodies" fibrin-enriched synovium fragments.

Viscosity

Synovial fluid is very viscous due to its high concentration of polymerized hyaluronate. A string test can be used to evaluate the level of synovial fluid **viscosity** (thickness). After removing the needle or cap from the syringe, synovial fluid is expressed into a test tube one drop at a time. Normal synovial fluid will form a "string" approximately 5 cm long before breaking. In addition, the fluid may cling to the side of the test tube rather than running down to the bottom. Synovial fluids with poor viscosity will form shorter stings (<3 cm) or run out of the syringe and down the side of the test tube like water. Low viscosity of synovial fluid indicates the presence of an inflammatory process. Figure 14-7 illustrates the performance of the string test for synovial fluid viscosity.

Clotting

Clotting of synovial fluid can result when fibrinogen is present. Fibrinogen may have entered into the synovial capsule during damage to the synovial membrane or as a result of a traumatic tap. Clots in specimens interfere with performance of cell counts. Depositing part of the specimen into a tube containing heparin may help avoid clotting of synovial fluid.

Mucin Clot

The **mucin** clot test, also known as **Rope test**, is an estimation of the integrity of the hyaluronic acid–protein complex (mucin). Normal synovial fluid forms a tight ropy clot upon the addition of acetic acid.

Figure 14-7. String test showing normal synovial fluid viscosity.

The procedure for mucin clot varies among laboratories as evidenced by differing fluid to acid ratios appearing in various texts. Clinical laboratory professionals should use the procedure adopted by their laboratories. Table 14-1 demonstrates this variability. In all cases, the interpretation of clot formation is the same. A good mucin clot indicates good integrity of the hyaluronate. A poor mucin clot, one that breaks up easily, is associated with destruction or dilution of hyaluronate. Figure 14-8 illustrates the tight clot of normal synovial fluid.

CHEMICAL EXAMINATION

Protein

Synovial fluid contains all proteins found in plasma, except various high–molecular weight proteins. These high–molecular weight proteins include fibrinogen, beta-2 macroglobulin, and alpha-2 macroglobulin, and can be absent or present in very low amounts. Most commonly used serum protein procedures

Table 14-1 Mucin Clot Procedure According to Referenced Texts

AUTHOR	VOLUME OF SYNOVIAL FLUID	VOLUME AND STRENGTH OF ACETIC ACID
Brunzel	One part	Four parts, 2%
Ross and Neely	One part	Four parts, 2%
McBride	Two parts	One part, 3%
Strasinger and DiLorenzo	Not specified	
Sunheimer, Graves, and Stockwin	Not specified	

Figure 14-8. Mucin clot test of normal synovial fluid.

can be used to measure synovial fluid protein. The normal range for synovial fluid protein is 1 to 3 g/dL. Increased synovial fluid protein levels are seen in ankylosing spondylitis, arthritis, arthropathies that accompany Crohn disease, gout, psoriasis, Reiter syndrome, and ulcerative colitis.

Glucose

Synovial fluid glucose levels should be interpreted using serum glucose levels. A fasting specimen should be used or at least one collected 6 to 8 hours postprandially. Normally, synovial fluid glucose levels are less than 10 mg/dL lower than serum levels. Joint disorders that are classified as infectious demonstrate large decreases in synovial fluid glucose and can be as much as 20 to 100 mg/dL less than serum levels. Other groups of joint disorders demonstrate a less of a decrease in synovial fluid glucose, 0 to 20 mg/dL.

Uric Acid

Synovial fluid uric acid normally ranges from 6 to 8 mg/dL. The presence of uric acid in synovial fluid is helpful in diagnosis gout. Usually, crystal identification is used for this de termination, but synovial fluid uric acid levels may be performed in laboratories that do not a have light polarizing microscope.

Lactic Acid

Lactic acid is rarely measured in synovial fluid but can be helpful in diagnosing septic arthritis. Normally, synovial fluid lactate is less than 25 mg/dL but can be as high as 1,000 mg/dL in septic arthritis.

Lactate Dehydrogenase

Lactate dehydrogenase (LD) can be elevated in synovial fluid, while serum levels remain normal. Synovial fluid LD levels are usually increased in RA, infectious arthritis, and gout. The neutrophils that are increased during the acute phase of these disorders contribute to increased LD levels.

Rheumatoid Factor

Rheumatoid factor (RF) is an antibody to immunoglobulins. RF is present in the serum of most patients with RA, whereas just more than half of these patients will demonstrate RF in synovial fluid. However, if RF is only being produced by joint tissue, synovial fluid RF may be positive while the serum RF is negative. False-positive RF can result from other chronic inflammatory diseases.

MICROSCOPIC EXAMINATION

Cell Counts

Synovial fluid cell counts, as all body fluid cell counts, should be performed within 1 hour of collection. Hemocytometer counts and manual differentials are normally performed on synovial fluid. Saline may be used as a diluent for synovial fluids with a high number of cells. Hypotonic saline, a weak acid, or commercially available white blood cell (WBC) diluent reservoirs may be used when many RBCs are present. Instruments are available to automate these counts (see chapter on "Automation"). Cytocentrifugation of the specimen provides good smears for Wright staining and observation.

Differential

Normal synovial fluid contains small numbers of lymphocytes and only a few neutrophils (Fig. 14-9). The WBC count on normal synovial fluid ranges from 0 to 150 cells per microliter. The mean distribution of these nucleated cells is neutrophils 7%, lymphocytes 24%, monocytes 48%, macrophages 10%, and synovial lining cells 4%. The presence of synovial lining cells is of no significant diagnostic concern. Neutrophils may be vacuolated or contain bacteria or crystals. In addition, cells may exhibit pyknotic nuclei or karyorrhexis. Other cells that may be seen in synovial fluid include plasma cells, eosinophils, and lupus erythematosus (LE) cells. The presence of these cells or abnormal numbers of cells normally seen in synovial fluid indicate various disease processes occurring in joints. An eosinophil count of greater than 2% has been associated with allergic disease with arthritis, hemorrhagic joint effusions, Lyme disease, parasitic arthritis, rheumatoid diseases, and tubercular arthritis.

Septic arthritis exhibits a high number of neutrophils (Fig. 14-10). A predominance of lymphocytes may be seen in the early stages of RA. Neutrophils present in later stages of RA may exhibit inclusions that contain immune complexes

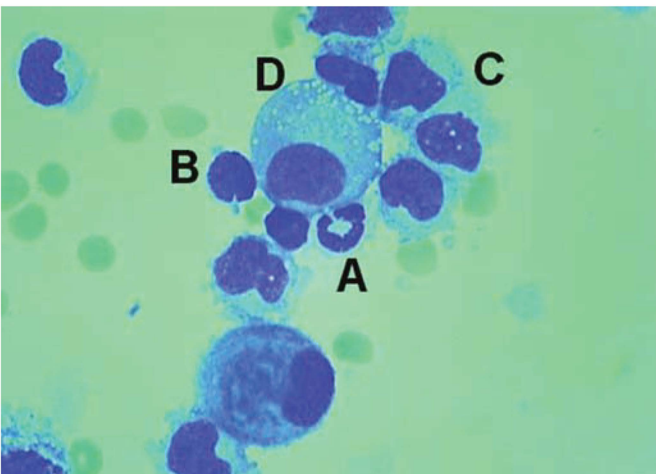

Figure 14-9. Normal cellular elements found in synovial fluid include **(A)** neutrophils, **(B)** lymphocytes, **(C)** monocytes/histiocytes, and **(D)** synovial lining cells. A few red blood cells are almost always present in joint effusions (Wright–Giemsa). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

such as IgG, IgM, complement, and RF. These neutrophils will appear to have dark cytoplasmic granules and are sometimes called RA cells or **ragocytes**. A high number of monocytes may be found in arthritis associated with serum sickness, viral infections, and **crystal-induced arthritis**.

LE cells are seen in synovial fluid in about 10% of patients with **systemic lupus erythematosus (SLE)** and in some patients with RA (Fig. 14-11). LE cells are neutrophils that have engulfed a nucleus of a lymphocyte that has been altered by **antinuclear antibody**. Tart cells, monocytes that have engulfed nuclear material (Fig. 14-12), may be confused with LE cells. Although not specific for Reiter syndrome, Reiter cells may be present in synovial fluid. Figure 14-13 shows a Reiter cell

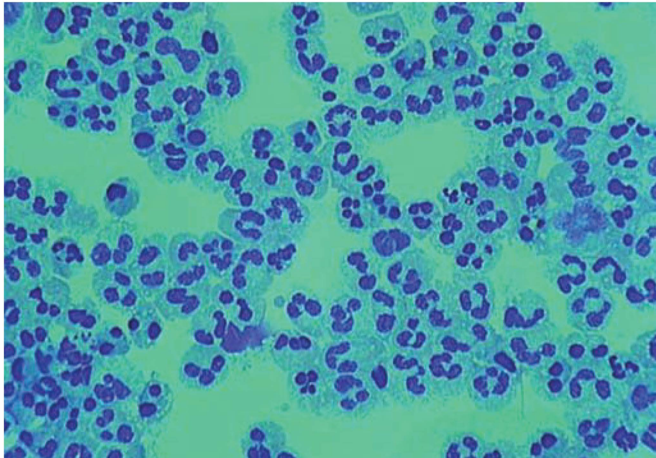

Figure 14-10. Synovial fluid with acute inflammation demonstrating neutrophilic pleocytosis (Wright–Giemsa). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2002.)

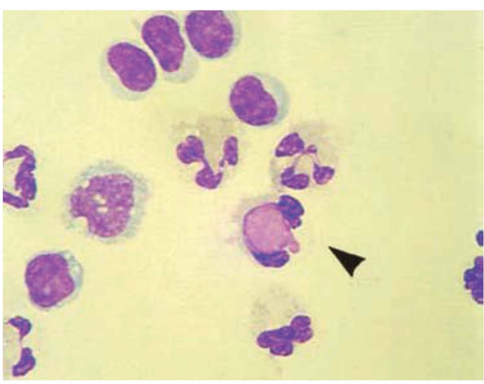

Figure 14-11. LE cell (*arrow*) is a neutrophil containing a phagocytized homogeneous nucleus (Wright–Giemsa). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

(neutrophil-laded macrophage). Lipids may be released from bone marrow after injury to the bone. As a result, **lipophages** (macrophages containing fat) as seen in Figure 14-14 may be present in synovial fluid.

Crystals

Examination of synovial fluid for crystals is a routine test in most laboratories. Crystal analysis is most commonly used to diagnose gout by the presence of monosodium urate (MSU) crystals. Chapter 5 contains an explanation of polarization and compensation of light in the analysis of crystals. MSU crystals that appear in synovial fluid are usually thin, needle-like crystals. MSU crystals polarize light and are negatively birefringent (crystals aligned with the compensator filter are yellow, whereas those lying perpendicular are blue). Figure 14-15 shows MSU crystals under polarized light, whereas Figure 14-16 demonstrates these crystals under compensated, polarized light. MSU crystals are yellow when aligned with the compensator filter and blue when lying perpendicular to the compensator filter.

Other crystals that may be present in synovial fluid include calcium pyrophosphate dehydrate (CPPD) crystals. CPPD

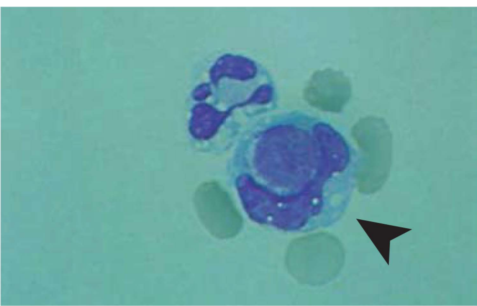

Figure 14-12. Tart cell: a macrophage containing a phagocytized nucleus that retains some nuclear detail (Wright–Giemsa). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

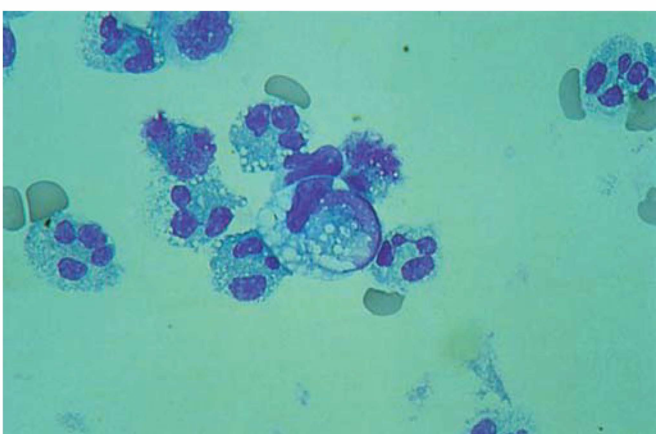

Figure 14-13. Reiter cell (center) is a macrophage that has phagocytosed one or more neutrophils. This finding is not specific for Reiter syndrome. (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

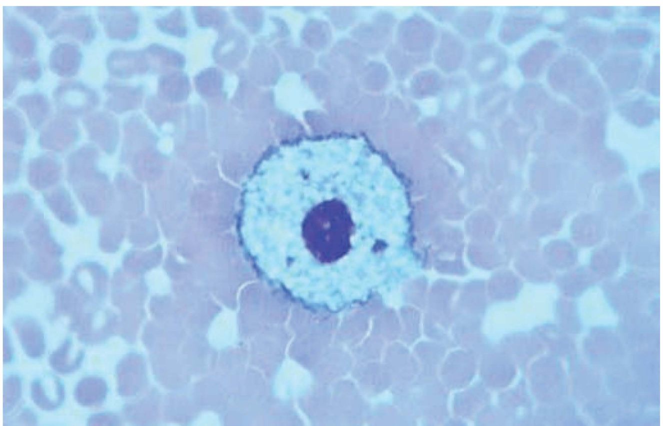

Figure 14-14. Lipid-laden macrophage in synovial fluid (Wright–Giemsa). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2002.)

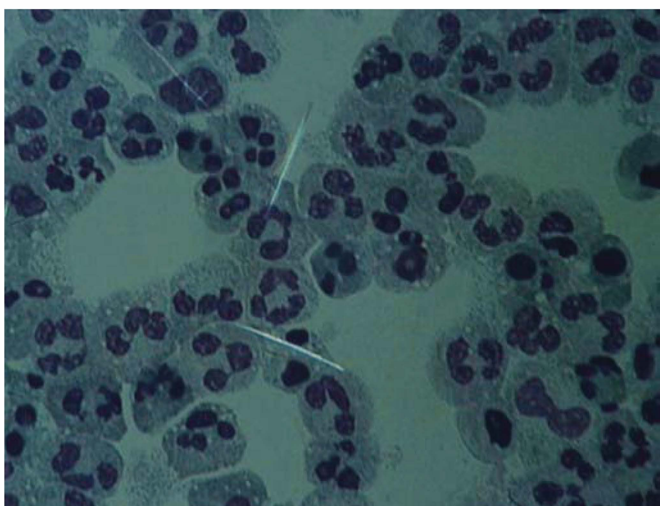

Figure 14-15. Synovial fluid with acute inflammation and monosodium urate crystals (Wright–Giemsa stain and polarized light). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

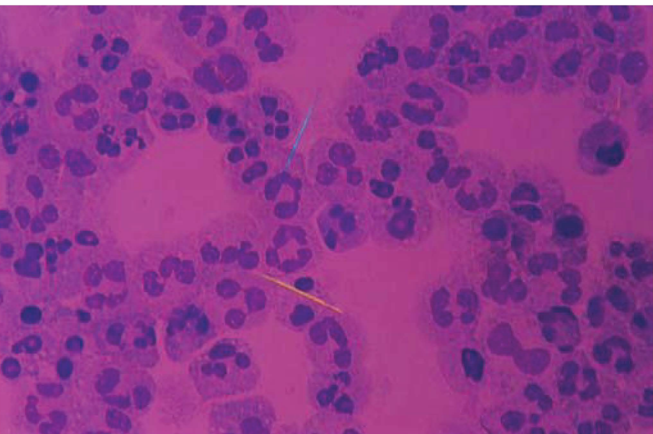

Figure 14-16. Synovial fluid with acute inflammation and monosodium urate crystals. The needle-shaped crystals demonstrate negative birefringence, because they are yellow when aligned with the compensator filter and blue when perpendicular to the filter (Wright–Giemsa stain and polarized/compensated light). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

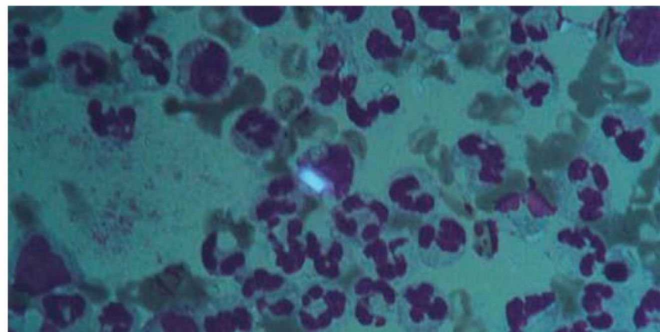

Figure 14-17. Synovial fluid with acute inflammation and calcium pyrophosphate dihydrate crystals (Wright–Giemsa stain and polarized light). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

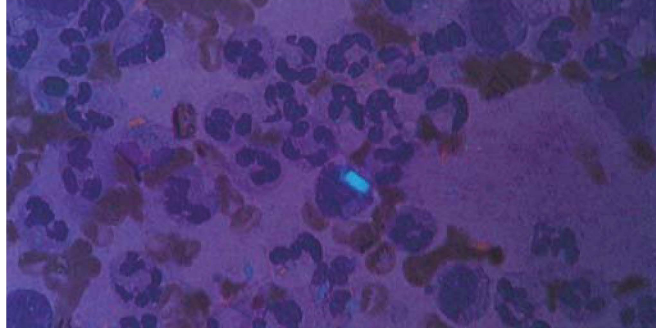

Figure 14-18. Synovial fluid with acute inflammation and calcium pyrophosphate dihydrate crystals. The rhomboidal intracellular crystal (center) demonstrates positive birefringence, because it is blue when aligned with the compensator filter (Wright–Giemsa stain and polarized/compensated light). (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

MICROBIOLOGIC EXAMINATION

Infectious agents that can enter the synovial fluid include bacteria, fungi, mycobacteria, and viruses, with bacteria being the most common. Bacteria and other microorganisms enter the synovial capsule through the bloodstream, deep penetrating wounds, and rupture of osteomyelitis into the joint. In addition, bacteria may be introduced during procedures such as arthroscopy, intra-articular steroid injections, and prosthetic joint surgery.

Gram stain is performed on synovial fluid smears prepared by centrifugation or cytocentrifugation. Diluting synovial fluid with saline helps separate cells that tend to cluster. Even if Gram staining does not suggest the presence of infectious agents, both aerobic and anaerobic cultures should be performed. Synovial fluid Gram stains are positive in only 50% of cases with joint sepsis.

1. The word synovial means resembling:
   a. An oval
   b. Egg albumin
   c. Lipids
   d. Serum
2. Aspiration of joint fluid is indicated for any patient with:
   a. Edematous joints
   b. Inflamed joints
   c. Painful joints
   d. All of these
3. Normal joint fluid is:
   a. Colorless and clear
   b. Red and cloudy
   c. White and hazy
   d. Yellow and hazy

4. A firm mucin clot of synovial fluid indicates the presence of:
   a. Arthritis
   b. Fibrinogen
   c. Hyaluronate
   d. Inflammation

5. No formation of a "string" when dispensing synovial fluid from a syringe indicates that:
   a. Collection was traumatic
   b. Fibrinogen levels are low
   c. Inflammation is present
   d. The fluid is normal

6. A cloudy synovial fluid demonstrating poor viscosity with decreased glucose levels and a WBC count of 180,000 (90% neutrophils) is most likely from a patient with which process?
   a. Crystal-induced
   b. Hemorrhagic
   c. Noninflammatory
   d. Septic or inflammatory

Match the characteristics of synovial fluids with their corresponding Group category.

A. Normal

B. Group I

C. Group II

D. Group III

E. Group IV

F. Group V

7. ______ colorless, clear, 57 WBCs, 10% neutrophils

8. ______ milky, 80,000 WBCs, 40% neutrophils, monosodium urate crystals

9. ______ red, cloudy, 210,000 RBCs, 15,000 WBCs, 45% neutrophils

10. ______ yellow, cloudy, 80,000 WBCs, 85% neutrophils

11. ______ yellow, purulent, 220,000 WBCs, 98% neutrophils

12. ______ xanthochromic, 10,000 WBCs, 30% neutrophils, erythrophagocytosis

Match the cell with its description.

A. LE cell

B. RA cell

C. Reiter cell

D. Tart cell

13. ______ macrophage containing a neutrophil

14. ______ monocyte containing nuclear material

15. ______ neutrophil containing antibody-altered nucleus

16. ______ neutrophil containing immune complexes

Match the crystals with their clinical significance.

A. Apatite

B. Calcium pyrophosphate

C. Corticosteroid

D. Monosodium urate

17. ______ Gout

18. ______ injections

19. ______ osteoarthritis

20. ______ pseudogout

CASE STUDY

Case 14-1 A middle-aged women is exhibiting swelling in both her knees after a fall while skiing. The images below show the results of an arthrocentesis performed in the emergency department a few days later.

1. Provide the physical description for this synovial fluid shown in Figure 14-19.

2. Identify the cells in Figure 14-20.

3. Classify this synovial effusion.

4. What is the most likely diagnosis?

Figure 14-19. First image for Case Study 14-1. Synovial fluid.

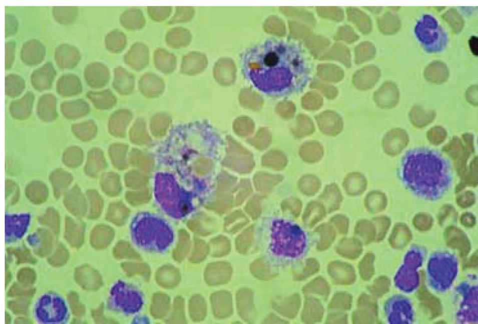

Figure 14-20. Second image for Case Study 14-1. (From McClatchey KD. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.)

Case 14-2 An elderly man is experiencing elbow pain. The images below show the results of an arthrocentesis.

1. Provide the physical description for this synovial fluid shown in Figure 14-21.
2. Identify the crystals in Figure 14-22.
3. Classify this synovial effusion.
4. What is the most likely diagnosis?

Figure 14-21. First image for Case Study 14-2. Synovial fluid.

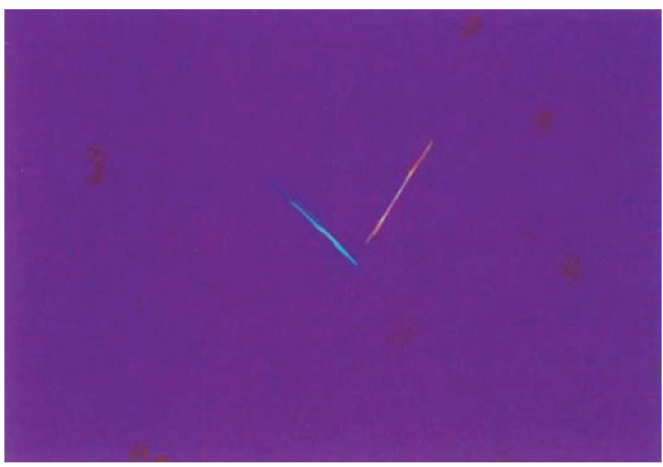

Figure 14-22. Second image for Case Study 14-2. (Courtesy of McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

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Gastric Fluid and Fecal Analysis

15
Chapter

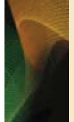
KEY TERMS

Accessory Digestive Organs
Acholic Stool
Alimentary Canal
Chyme
Colorectal Cancer
Creatorrhea
Cystic Fibrosis
Diarrhea
Dysentery
Fecal Leukocytes
Fecal Occult Blood
Gastric Occult Blood
Gastrin
Hematochezia
Hypermotility
Malabsorption
Maldigestion
Melena
Pancreatic Insufficiency
Secretagogues
Steatorrhea
Upper Gastrointestinal Bleeding (UGIB)
Urobilin
Zollinger–Ellison Syndrome

LEARNING OBJECTIVES

1. Describe gastrointestinal tract physiology and fecal formation.
2. Explain mechanisms of diarrhea, including its relationship to reabsorption of intestinal water and hypermotility.
3. Compare disorders of the gastrointestinal tract.
4. Describe collection procedures for gastric fluid and fecal samples.
5. Describe the appearance of normal and abnormal gastric fluid and feces.
6. Describe laboratory testing for gastrointestinal tract disorders: enzymes, fecal carbohydrates, fecal fat, occult blood.
7. Interpret laboratory findings in various gastrointestinal tract disorders.

This chapter describes routine clinical testing of gastric fluid and feces and the information this testing gives clinicians. Examination of gastric contents aids in the differential diagnosis of gastric and duodenal ulcers, and to evaluate the presence of hyper- or hypochlorhydria. Fecal testing provides important information related to gastrointestinal disorders, infections, and several other medical conditions. Macroscopic, microscopic, and chemical testing of feces are routinely performed in the core laboratory for detection of colon cancer, steatorrhea and other malabsorptive digestive disorders, or maldigestion disorders. Tests are also performed in the microbiology department for various stool pathogens; bacterial, viral, and parasitic, as well as for their toxins.



Gastrointestinal Tract Physiology and Fecal Formation

The digestive system is composed of the **alimentary canal**, a continuous tube from the mouth to the anus including the esophagus, the stomach, the intestines, the colon, and the **accessory digestive organs**, including the mouth, much of the pharynx, the teeth, tongue, salivary glands, liver, gallbladder, and pancreas (Fig. 15-1). The gastrointestinal (GI) tract contains and processes food from ingestion, through digestion, and elimination.

The stomach secretes several substances needed for proper digestion. Hydrochloric acid secreted by parietal cells hydrolyzes peptides and disaccharides and converts pepsinogen to pepsin. Intrinsic factor also secreted by parietal cells is needed for the absorption of vitamin B_{12}. Pepsinogen secreted by peptic cells catalyzes the degradation of proteins to proteases and peptones. A few of the other digestive enzymes secreted by the stomach include peptidase, lactase, and lipase. Mucus secreted by goblet cell and mucous glands acts to protect the stomach wall from acids and enzymatic activity.

Food and water ingested becomes **chyme** (a mixture of digestive secretions and partially digested food) in the stomach and small intestines. Enzymes, such as salivary amylase, gastric secretions, and many pancreatic enzymes, are added to the food along the pathway. Gastric secretions are stimulated by neural response sight, smell, or anticipation of food, distention of the stomach by food or fluid, gastric mucosal contact with **secretagogues** (protein breakdown products), and **gastrin** (hormone secreted by gastric mucosa).

After chyme has remained in the large intestine for 3 to 10 hours, it normally becomes solid or semisolid and is then called feces. Along the way, much of the water, nutrients, vitamins, and electrolytes are adsorbed into the circulatory system. Despite about 9 L of water entering the small intestine from ingestion and digestive fluids, only about 0.1 L of water is excreted in feces daily, as most is absorbed via osmosis in the small intestines, with only about one-tenth of the

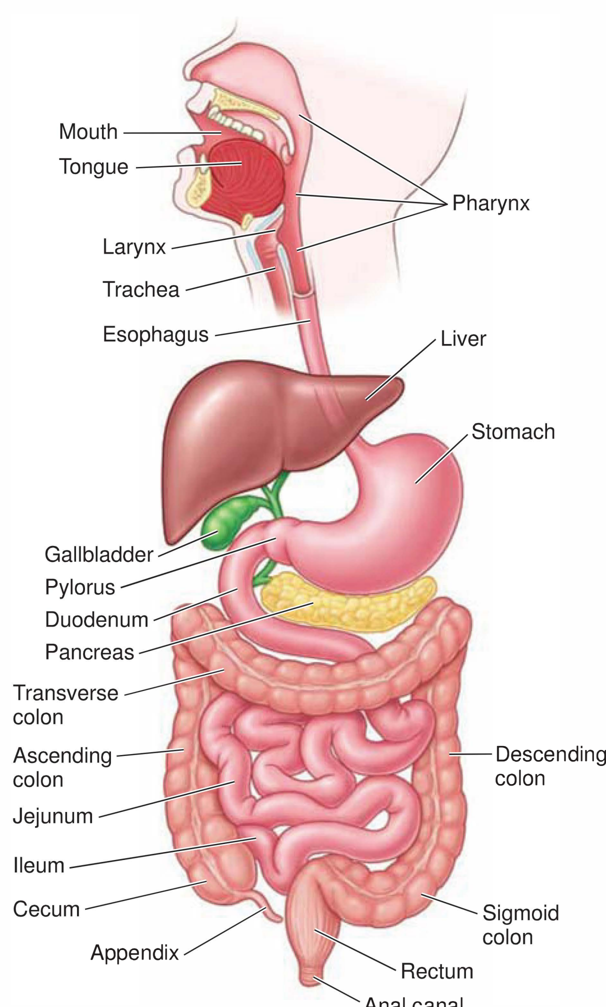


Figure 15-1. Diagram of alimentary tract.

volume, about 0.9 L, also being reabsorbed in the large intestine. Because of the limited ability of the colon to absorb water, if a large volume of water is presented to the colon from the intestines, a large volume of liquid diarrhea is the result.

Disorders of the Gastrointestinal Tract

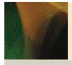
Upper gastrointestinal bleeding (UGIB) (bleeding in the esophagus, stomach, or duodenum) is the most common gastroenterological emergency. UGIB can lead to anemia. Diagnostic testing includes repeated blood hemoglobin levels and occult blood tests. **Fecal occult blood** (nonvisible blood in the feces) tests are not as sensitive as are tests for **gastric occult blood** (nonvisible blood in gastric juices) for the diagnosis of UGIB.

Diarrhea is a common disorder of the GI tract. In diarrhea the frequency and volume of bowel movements are increased

and the bowel movements are more liquid. Diarrhea is associated with infectious agents, toxins, malabsorption, and a variety of GI disturbances. Diarrhea is caused by a large volume of fluid being presented to the large intestine (a) due to increased secretions or (b) due to increased amounts of osmotically active substances remaining in the GI tract or (c) because of **hypermotility** (increased intestinal movement) resulting in decreased intestinal absorption. Diarrhea can result in dehydration and critical electrolyte imbalances.

Malabsorption is a state of abnormal digestion or absorption of a single nutrient or of multiple nutrients through the GI tract that may lead to malnutrition or anemia. If osmotically active substances are not absorbed, it can result in diarrhea. Fats, meat fibers, and carbohydrates all may be improperly digested with malabsorption. **Maldigestion** is impaired digestion which is caused by lack of digestive enzymes.

Colorectal cancer is a relatively common cancer of the GI tract. In colon cancer, increased blood loss in the GI tract is detected by occult blood testing. The term "occult blood" refers to hidden blood or small amounts of fecal blood that are often not visible to the naked eye. These are some of the conditions that can be detected by fecal analysis. In addition, various diseases of the accessory organs can also be detected through fecal testing. An example of this is **cystic fibrosis**, a hereditary disease affecting mucous secretion in the pancreas and lungs. In cystic fibrosis or other **pancreatic insufficiency**, there are decreased pancreatic digestive enzymes such as trypsin, chymotrypsin, and elastase I, resulting in maldigestion. These enzymes can be tested in feces.



Collection of Gastric Fluid

Gastric juices are present in vomitus that can be used to test for gastric occult blood or gastric contents may be collected by nasogastric intubation and gastric aspiration (Fig. 15-2). A nasogastric tube is carefully inserted through the nasal passage, down the esophagus, and into the stomach. Gastric contents are aspirated into a syringe, transferred to a properly labeled sterile container, and sent to the laboratory.

Laboratory Examination

GROSS EXAMINATION

The normal appearance of gastric fluid is a translucent pale gray color. It may contain flecks of blood or a variation of color (brown, green, or yellow) due to reflux of bile during the intubation process. Gastric fluid normally shows a slight viscosity and has a faint acrid or sour odor. Volume normally ranges from 20 to 100 mL.

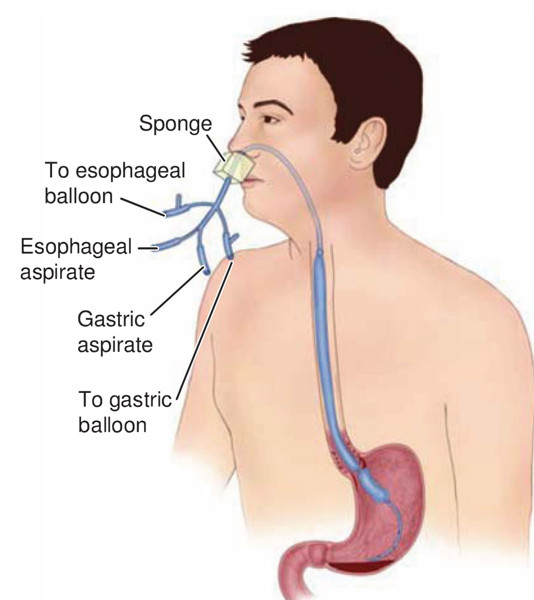


Figure 15-2. Gastric contents collection using nasogastric intubation and aspiration.

CHEMICAL ANALYSIS

Gastric pH and Acid Output

Gastric fluid pH can be measure using a pH meter or pH paper in the appropriate range for gastric fluid, which is acid with a normal range from 1.5 to 3.5. Intragastric pH may also be measured using specialized electrodes inserted through a nasogastric tube and into the stomach, although this procedure is rather invasive and uncomfortable.

The total volume of gastric fluid is measured and a sample is titrated with sodium hydroxide and a color-changing indicator. Most healthy persons secrete 0 to 6 mmol of acid in a volume of 10 to 100 mL.

A marked elevation in acidity is seen in **Zollinger–Ellison Syndrome** (pancreatic tumor that secretes increased amounts of gastrin). Very low acid output is seen in pernicious anemia.

Gastric Occult Blood

Occult blood means "hidden blood" as it is often present but not visible in dark-colored samples and requires laboratory testing for detection. Tests are available for both gastric and fecal occult blood. Tests specific for fecal occult blood are affected by hemoglobin degradation by proteolytic enzymes, loss of hemoglobin antigenicity by the action of enterobacteria, fecal hydration, and the action of various drugs. In addition, fecal occult blood tests (FOBTs) lose sensitivity at low pH and may be unsuitable for use with gastric samples. Therefore, specialized screening tests are used for the detection of occult blood in gastric aspirate or vomitus. Gastroccult Slide Test (Fig. 15-3, procedure Box 15-1) is used for in vitro diagnosis of various gastric conditions, and can be performed on gastric fluid or vomitus. Hemoglobin in the

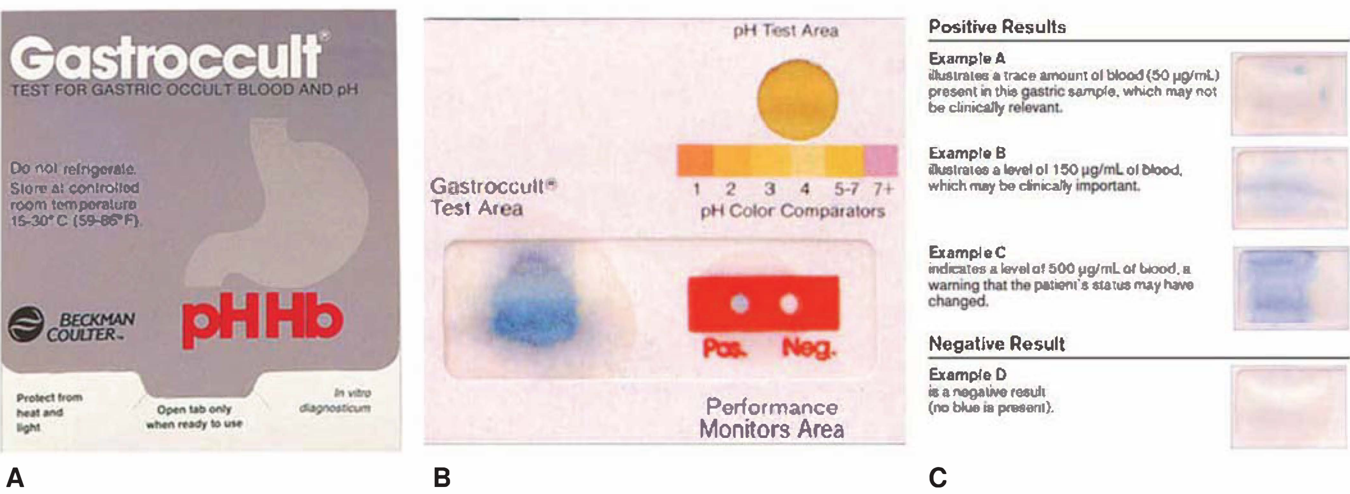


Figure 15-3. Gastroccult test card with interpretation. **A:** Cover of closed Gastroccult card. **B:** Gastroccult test card opened with positive test result. **C:** Interpretation of Gastroccult results. (Images courtesy of Beckman Coulter, Inc.)

specimen acts as a peroxidase facilitating the oxidation of a phenolic compound, alpha guaiaconic acid to form a blue quinone structure as shown in the following reaction:

$$\text{Alpha guaiaconic acid} \xrightarrow[\text{Buffered } H_2O_2]{\text{Hemoglobin}} \text{quinone (blue color)}$$

Gastric Occult Blood Test Limitations/Cautions

- Gastroccult test should NOT be used with fecal samples.
- Hemoccult or any other developer CANNOT be used with Gastroccult test.
- False-positive results can occur from a complicated nasogastric intubation, which may cause nasopharyngeal bleeding.
- False positives may occur after ingestion of undercooked meat, and peroxidase containing foods such as fresh fruits and vegetables.
- False-negative results occur as a result of high doses of vitamin C (ascorbic acid).
- No interference is expected from antacids if aspiration occurs at least 60 minutes after the last dose.

BOX 15-1 Gastroccult Procedure

1. Properly fill out patient identification, collection date, and physician name in spaces provided on front of each slide. This is especially important to do on slides that are not to be developed immediately.
2. Open slide. Apply one drop of gastric sample to pH test circle and one drop to occult blood test area.
3. Determine pH of sample by visual comparison of test area to pH color comparator. This must be done within 30 seconds after applying sample.
4. Apply two (2) drops of Gastroccult Developer directly over the sample and one (1) drop between the positive and negative Performance Monitor areas.
5. Read occult blood results within 60 seconds. The development of any blue color in the occult blood test area is regarded as a positive result. Negative and positive controls results must be in range.
6. Record results according to your laboratory's policies.

Interpretation:

Any blue color is considered positive.
See Figure 15-3C for further interpretation.

Fecal Specimen Collection

The patient needs proper directions on the collection of a stool sample and he or she should be provided with the proper sample collection container. For most fecal specimens, the container simply needs to be clean, dry, sealable, and leak proof. Patients must understand that the fecal sample should not be contaminated with urine or water. Toilet water often contains strong oxidizing cleaners that interfere with laboratory testing, protozoa can be destroyed by urine contamination, and even not adhering to recommended diet recommendations can adversely affect many fecal specimens. If the patient has had enemas or barium sulfate for radiological examinations, these can adversely affect stool samples for microscopy, making them difficult to read.

The type of collection container and the amount of specimen to be collected depend upon the tests to be performed. Special specimen containers are available for fecal occult blood, fecal parasite examinations, and some other fecal examinations.

Special dietary restrictions must be followed for some tests, such as fecal occult blood by guaiac methods. Timing of specimen collection is important in some tests, such as

for parasite examinations or quantitative testing. If quantitative specimens are required, a 3-day fecal collection is recommended as 24 hours is often insufficient for foods to be processed through the alimentary canal. Use a secure lid for the fecal specimen as gas in the fecal specimen can build up, causing the lid to pop open, possibly with some force.

During many GI tract infections, diarrhea causes an increased number of bowel movements. Determining the time to symptom development and the type of symptoms, such as the frequency of eliminations can assist the physician in determining the causative agent or toxin.

Fecal Analysis Methods

The laboratory can screen for a variety of fecal characteristics that can aid healthcare providers in diagnosing and monitoring their patients. From simple gross observations to microscopic examinations and chemical determinations, laboratory testing provides clues to the functioning of the GI tract and diseases that arise in the digestive system.

GROSS EXAMINATION

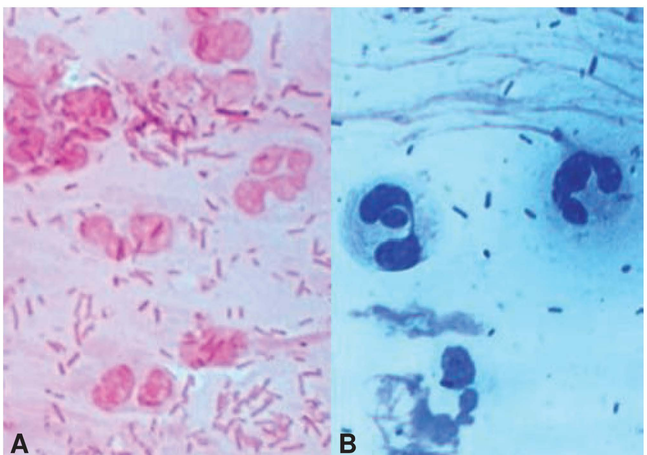
Gross appearance of the feces provides some clues to possible GI disorders. The consistency, whether formed, hardened, or liquid, and color change from the normal dark brown alert the healthcare provider to abnormalities. Black color may indicate older blood from the upper GI tract, whereas bright red blood is more likely to be from the lower GI tract. Bright red blood in feces is known as **hematochezia**. A very pale stool (called an **acholic stool**) often indicates a biliary obstruction. Another common reason for a pale stool is the presence of barium sulfate from a barium enema performed for radiological testing. This barium sulfate will interfere with some fecal examinations, especially microscopic examinations for fat, fibers, or parasites.

A ribbon-like fecal specimen could be associated with GI tract obstruction.

Normal fecal specimen is dark brown; due to the oxidation of urobilinogen in the intestines, the color changes to orange brown **urobilin**. The presence of blood-streaked mucus or mucus with pus or eosinophils often accompanies bacterial or amebic dysentery. **Dysentery** is associated with damage to the intestinal wall due to invasion by these organisms.

MICROSCOPIC EXAMINATION

Fecal leukocytes, especially neutrophils, are commonly associated with dysentery or invasion of the intestinal wall. In amebic infections, eosinophils are also often present. Wet preparations made with methylene blue are used to detect fecal leukocytes or alternatively, dried smears of the stool sample can be stained with Gram stain or Wright stain to examine for leukocytes. The Wright stain improves differentiation of these cells. The presence of even a few leukocytes can be indicative of an invasive condition.



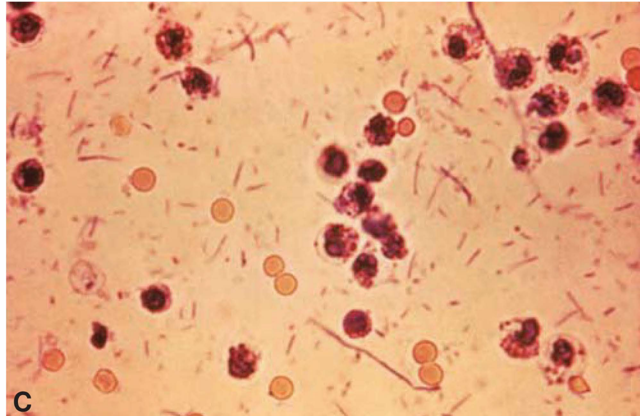


Figure 15-4. Fecal leukocytes. **A:** Gram stain (1,000×), fecal leukocytes. **B:** Wright stain (1,000×), fecal leukocytes. **C:** Wet preparation, Shigella dysentery or bacterial dysentery with fecal white blood cells, red blood cells, and bacteria. (Fig. 15-4C obtained from: http://phil.cdc.gov/phil image 6659.)

Another type of test that can be used to detect fecal leukocytes is a latex agglutination test for lactoferrin, an enzyme found in the granules of granulocytes, also indicative of fecal leukocytes (Fig. 15-4).

FECAL OCCULT BLOOD

Fecal blood is found in infection, in trauma, and in colorectal cancer. Because fecal blood is a frequent and an early symptom of colorectal cancer, the American Cancer Society recommends annual screening on all individuals older than 50 years of age. Early diagnosis is associated with a good prognosis and this testing is beneficial in this early diagnosis.

In addition to colorectal cancer, inflammatory conditions, infectious agents, ulcers, hemorrhoids, and even bleeding gums can cause a positive occult blood test. The excretion of large amounts of blood in the upper GI tract may cause the stool to be dark or black in color. A very large amount of fecal

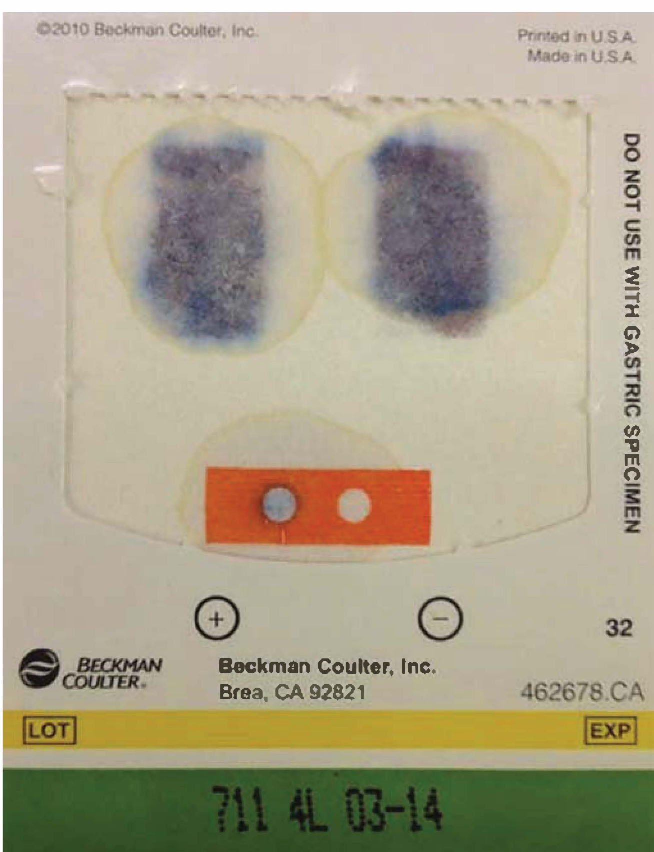


Figure 15-5. Hemoccult positive fecal occult blood test. (Image courtesy of Beckman Coulter, Inc.)

blood is called **melena** and is associated with a black, tarry stool. Lower GI tract bleeds tend to have a brighter red color blood, if visible.

The three types of FOBTs each reacts with a different part of the hemoglobin molecule. These tests include guaiac-type tests, immunochemical tests, and heme-porphyrin tests.

Guaiac Test

The most common method for detection of fecal blood involves a guaiac-impregnated paper in a cardboard holder. Guaiac-type tests such as Hemoccult require the peroxidase-like activity of intact heme. Patients should be instructed to avoid red meat, fish, bananas, cantaloupe, pars, plums, turnips, horseradish, broccoli, cauliflower, and aspirin for the days preceding testing as these substances cause false-positive results with guaiac testing. In addition, patients should not be taking vitamin C, as this may cause a false-negative result.

Patients must be instructed to collect several portions of the same stool sample to maximize blood detection. A thin layer of these stool portions is applied to the front of the guaiac card as directed and sent to the laboratory for testing within 14 days of collection (the stability of the specimen on the Hemoccult slide).

If hemoglobin or another peroxidase or pseudoperoxidase is present in the feces in sufficient amounts, the guaiac paper

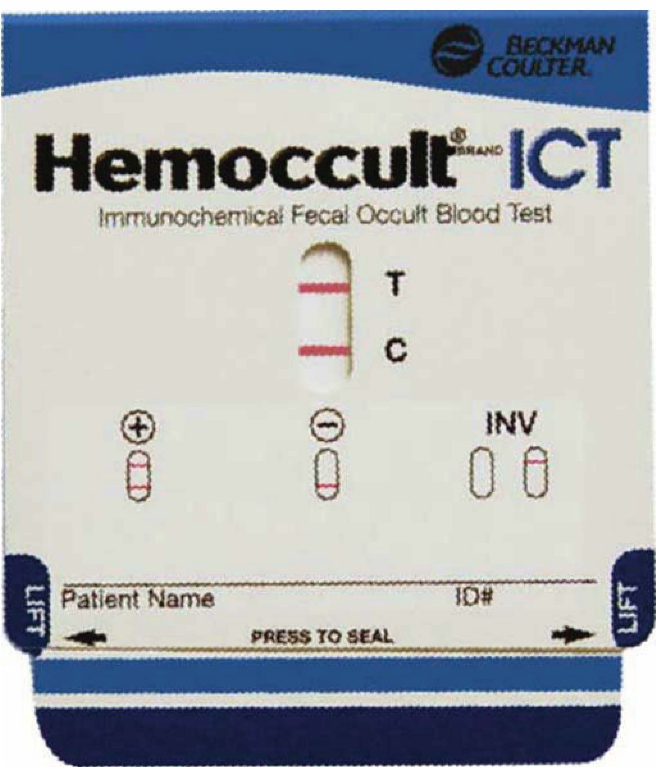


Figure 15-6. Hemoccult-ICT positive fecal occult blood test. (Image courtesy of Beckman Coulter, Inc.)

will develop (usually turns blue as in Fig. 15-5) when hydrogen peroxide developer is applied. The chemical reaction is:

$$H_2O_2 + \text{colorless indicator} \xrightarrow[\textit{or hemoglobin}]{\textit{(pseudo) peroxidase}} \text{blue color (oxidized indicator)} + H_2O$$

Methods other than guaiac are available for detecting fecal blood including benzidine and orthotolidine. A problem with these tests is a higher degree of sensitivity that leads to false positives. The less sensitive guaiac seems to give the best results under most circumstances.

Immunologic-based Test

Guaiac-based FOBTs are being replaced by immunochemical-based tests for occult blood. Immunochemical tests, such as Hemoccult-ICT (Fig. 15-6) react with antigenic sequences on human globin chains.

Immunochemical detection of fecal occult blood is more specific for lower GI bleeding than are guaiac-based tests. Collection of the specimen for Hemoccult-ICT does not require patients to follow any dietary restrictions. As with other card tests, a thin layer of stool is applied to the Hemoccult-ICT card, which must be tested within 14 days of collection. This test should not be used on gastric fluid specimens because globin proteins may be completely destroyed by peptidases of the proximal gut. An automated immunochemical FOBT method is discussed in the Chapter 20.

Heme-Porphyrin–based Test

Heme-porphyrin–based tests are less affected by the anatomic site of bleeding than are the other guaiac-based tests and immuno-chemical FOBT methods. The porphyrin ring remains intact during transit and fecal storage. Heme-porphyrin tests are fluorometric assays based on the conversion of nonfluorescing heme to fluorescent porphyrins. This test also includes porphyrins from intestinally converted hemoglobin.

FETAL HEMOGLOBIN (APT TEST)

Newborns may excrete stools or vomitus containing blood. This blood can originate either from the maternal blood ingested at delivery or from the newborn's own gastrointestinal tract. Differentiating between these two sources of blood is important for the newborn's survival. Black, tarry stool samples are not acceptable for this test as the hemoglobin degradation has taken place. This test determines whether the hemoglobin present is hemoglobin A (maternal) or hemoglobin F (fetal) in origin. The stool or vomitus is mixed with water to yield a pink supernatant. The supernatant is removed and then alkalinized with dilute sodium hydroxide. If the pink color remains after adding the alkali, the blood contains fetal hemoglobin. If the pink color changes to yellow or brown within 2 minutes, the hemoglobin in the sample is maternal hemoglobin.

FECAL FAT TESTING

Conditions that decrease the production of pancreatic enzymes such as cystic fibrosis, pancreatic insufficiency, pancreatitis, and pancreatic carcinoma, as well as the absence of the bile salts that assist lipases in fat breakdown, cause **steatorrhea** (an increase in fecal fat). Steatorrhea is also present in malabsorption. Malabsorption can be caused by bacterial overgrowth, intestinal resection, celiac disease, tropical sprue, lymphoma, Crohn disease, Whipple disease, and giardiasis. In steatorrhea, the stool sample has a pale and greasy appearance and a foul smell.

Before any fecal fat testing, it is important for the patient to have a diet with a normal amount of fat intake. Mineral oils and many creams can cause false-positive results in fecal fat testing and must be avoided. Both qualitative and quantitative fecal fat analyses are available.

Qualitative Fecal Fat Testing

Qualitative tests are performed microscopically for triglycerides (neutral fats), fatty acid salts (soaps), fatty acids, and cholesterol. These fats can be stained with Sudan III, Sudan IV, and oil red O. Two procedures are used to detect these different lipids.

Neutral fats are detected qualitatively by staining with Sudan III in 95% ethanol in a wet preparation and microscopic observation for the number and size of fat globules

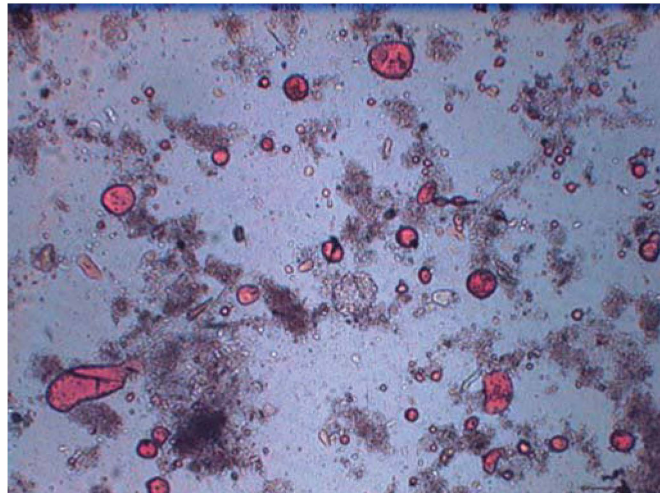


Figure 15-7. Steatorrhea with fecal fat Sudan III stain for neutral fats.

(Fig. 15-7). A normal stool will have less than 60 medium or small-sized orange-red fat globules/high-power field.

Soaps and fatty acids do not stain directly with the Sudan III stain and the same wet preparation must also have acetic acid and be heated before staining and reading microscopically (Fig. 15-8). Normal samples will have less than 100 orange-red fat globules/high-power field and they should not exceed 4 um. A normal amount of neutral fecal fat with an increased amount of soaps and fatty acids is associated with intestinal malabsorption, whereas an increased amount of neutral fats on the first slide is associated with maldigestion or impaired digestion leading to steatorrhea.

Quantitative Fecal Fat Testing

If qualitative fecal fat testing is positive, confirmatory quantitative fecal fat analysis is performed. Generally, this is performed in the chemistry department and it is often a reference test.

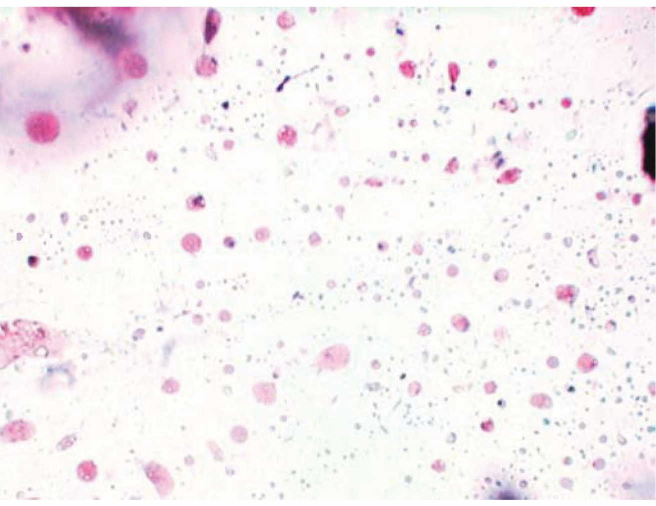


Figure 15-8. Steatorrhea with fecal fat Sudan III stain for fatty acids.

recognized microscopically as they are rectangular with the cross striations that are characteristic of muscle fibers. If no striations are seen, the fibers are not counted as they may be digested. More than 10 fibers/high-power field is considered increased. Meat fiber examination may be done together with microscopic fecal fat analysis. Eosin in 10% ethanol can be used to assist in identifying these meat fibers with striations in a microscopic wet preparation. An increase in the number of meat fibers is **creatorrhea** (Figs. 15-9 and 15-10).

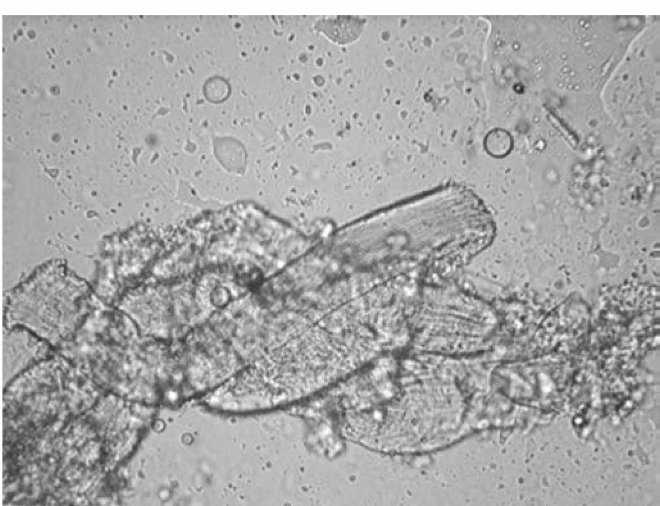


Figure 15-10. Creatorrhea. Undigested meat fibers tangled. The clear striations help identify these meat fibers.

1. All of the following are mechanisms of diarrhea EXCEPT:
   - **a.** Increased osmotically active compounds such as carbohydrates causing increased fecal water and electrolytes
   - **b.** Increased secretions leading to increased fluid presented to the large intestine
   - **c.** Decreased osmosis
   - **d.** Intestinal hypermotility
2. All of these are seen with malabsorption, maldigestion, or hypermotility, EXCEPT:
   - **a.** Steatorrhea
   - **b.** Creatorrhea
   - **c.** Positive APT test
   - **d.** Increased fecal carbohydrates
3. Bright red blood and mucus in feces are most often seen with:
   - **a.** Malabsorption
   - **b.** Dysentery
   - **c.** Creatorrhea
   - **d.** Upper GI tract bleeding
4. Eosinophils are often seen with:
   - **a.** Colorectal cancer
   - **b.** Creatorrhea
   - **c.** Fecal carbohydrates
   - **d.** Parasites such as amebas

5. An advantage of the guaiac tests over the other fecal occult blood tests is that they are:
a. Not overly sensitive
b. The most sensitive
c. The most specific
d. Do not need special diet

6. Even a few fecal leukocytes indicate:
a. Steatorrhea
b. Invasion of the intestinal wall by microbes
c. Malabsorption

7. Steatorrhea is present in:
a. Colorectal cancer
b. Fetal hemoglobin
c. Bacterial invasion of the intestine
d. Giardiasis

8. Which of the following types of fats stains with Sudan III without heat or acid?
a. Soaps
b. Fatty acids
c. Neutral fats (triglycerides)
d. Carbohydrates

9. Clinitest is used to detect this substance in newborn feces:
a. Fatty acids
b. Meat fibers
c. Fetal blood
d. Carbohydrates

10. Who should be screened annually for occult blood?
a. Infants and pediatric patients
b. Adults older than age 50
c. Cystic fibrosis patients
d. All adults

11. Which of these conditions is associated with low gastric acidity?
a. Melena
b. Pernicious anemia
c. Sickle cell anemia
d. Zollinger–Ellison syndrome

12. Which of these statements is TRUE concerning gastric occult blood?
a. A red meat diet can cause a false-negative result
b. Peroxidase containing foods can cause a false-positive result
c. Peroxidase in certain foods inhibits the peroxidase reaction on occult blood test cards
d. A development of a blue color indicates the test is invalid

13. Which of the following tests are acceptable for detecting occult blood in vomitus? (*choose all that apply*)
a. Guaiac-based gastric occult blood test
b. Guaiac-based fecal occult blood test
c. Immunochemical-based fecal occult blood test
d. Orthotolidine-based fecal occult blood test

14. When food and water are ingested, they become a substance in the stomach known as:
a. Amylase
b. Chyme
c. Gastrin
d. Peptidase

15. Accessory digestive organs include all of these EXCEPT:
a. Gall bladder
b. Liver
c. Pancreas
d. Spleen

CASE STUDY

Case 15-1 A premature infant in the neonatal intensive care developed abdominal bloating and bloody diarrhea. The child suffered from apnea and respiratory distress. Radiological examination revealed pneumatosis intestinalis (trapped gas in the intestinal wall). The infant's complete blood cell count showed an elevation of the white blood cell count and elevation of neutrophils with an increase in bands. A stool culture, fecal leukocytes, fecal carbohydrate test, and a fecal pH were ordered. The culture was set up and the fecal leukocyte examination showed a few neutrophils. The Clinitest was performed for the fecal carbohydrates and the result was positive and the fecal pH was 5.0.

1. What condition is the physician most concerned with in this case?

2. What is the significance of a pH 5.0 fecal sample?

Case 15-2 A patient was seen by a gastroenterologist because of visible bleeding into the toilet during a bowel movement. An FOBT was positive, however the physician does not suspect colorectal cancer.

1. What is the most likely cause of positive FOBT in this patient?

2. NOT including dietary substances, what other conditions can cause the FOBT to be positive for blood and yet be a false indicator for colorectal cancer?

3. Explain how one's diet may contribute to false-positive and false-negative FOBT test results.

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Semen Analysis

16

Chapter

KEY TERMS

Agglutination
Antisperm Antibodies
Asthenozoospermic
Azoospermic
Bulbourethral Glands
Epididymis
Flavin
Infertile
Liquefaction
Morphology
Motility
Normozoospermic
Oligozoospermic
Penetration
Prostate Gland
Semen Analysis
Seminal Vesicles
Semenogelin
Sertoli Cells
Spermatogenesis
Spermiogenesis
Spermatozoa
Teratozoospermic
Testis
Varicocele
Vas Deferens
Vasectomy
Viability
Viscosity

LEARNING OBJECTIVES

1. Name the organs involved in the production of semen and explain the involvement of each.
2. Explain the process of spermatogenesis.
3. Explain the proper collection and specimen handling of semen samples.
4. Describe procedures for macroscopic and microscopic analysis of semen.
5. Identify normal and abnormal values for tests comprising a routine semen analysis.
6. Correlate results of semen analysis tests.
7. Identify and describe normal and abnormal spermatozoa morphologies.
8. Identify and describe various sperm motilities.
9. Identify and describe the appearance of viable and nonviable spermatozoa.
10. Classify semen using parameters of count, motility, and morphology.
11. Suggest confirmatory tests for suspected infertility.
12. Explain how confirmatory tests for infertility are performed.
13. Interpret confirmatory infertility tests.
14. Discuss the clinical significance of other cells that may be found in semen besides sperm.
15. Describe types of specimens for which semen detection may be requested.
16. Suggest appropriate laboratory tests to detect the presence of semen.

When a man and a woman have difficulty conceiving a child, one or both of the pair may be **infertile** (unable to conceive). Analysis of semen from the male partner is one form of testing that occurs for the evaluation of an infertile couple. Approximately 40% of infertility cases are due to disorders of the male reproductive system. A semen analysis may disclose one of these male disorders or rule out male infertility. Once a potential problem is revealed, further testing for the cause may contribute to a definitive diagnosis. Establishing the correct cause for infertility is the key to establishing a treatment plan for an infertile couple. Other indications for performing a semen analysis include determining the effectiveness of a vasectomy, rape-case forensic studies, sperm donor evaluation, and paternity cases.

Semen Composition

Semen consists of several fluids produced in various male reproductive organs. The slightly alkaline fluid from **seminal vesicles** comprises over half the volume of semen and contains citric acid, flavins, fructose, and potassium. These substances provide nutritional support for **spermatozoa**. Spermatozoa are formed in the **testis** and are stored in the **epididymis** and **vas deferens**. The process of spermatozoa formation is under control of various hormones, testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). The **prostate gland** contributes a slightly acidic fluid containing acid phosphatase, citric acid, and proteolytic enzymes. These substances account for about 20% of the semen's volume. The remaining reproductive organs, **bulbourethral glands**, epididymis, and urethral glands contribute little additional volume to the semen. Figure 16-1 illustrates the male reproductive system. Upon ejaculation, the fluids from all of these sources form the mixture, semen.

Sperm Formation

Spermatogenesis is the formation of spermatozoa in the **Sertoli cells** of the seminiferous tubules of the testis. Further maturation of sperm occurs in the epididymis. This approximately 74-day process involves several phases: spermatocytogenesis, meiosis, and spermiogenesis. Spermatocytogenesis is a two-step phase in which spermatogonia undergo mitotic division and maturation into spermatocytes. Meiosis is the specific type of cell division that results in haploid gamete cells. **Spermiogenesis** is the phase in which the gamete cell develops a flagellum and transforms from a spermatid to a spermatozoon. Figure 16-2 illustrates the process of spermatogenesis, whereas Figure 16-3 illustrates the stages of human spermatid transformation into spermatozoon.

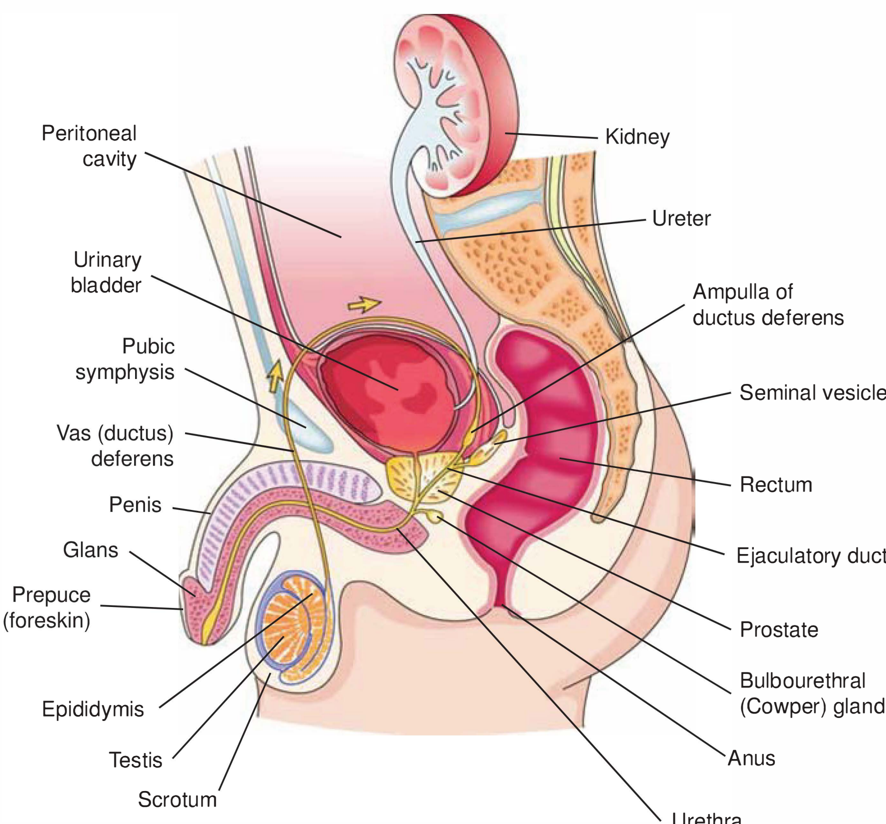

Figure 16-1. Detail of the male reproductive system. (Reprinted with permission from Cohen BJ, Wood DL. *Memmler's the Human Body in Health and Disease*. 9th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2000.)

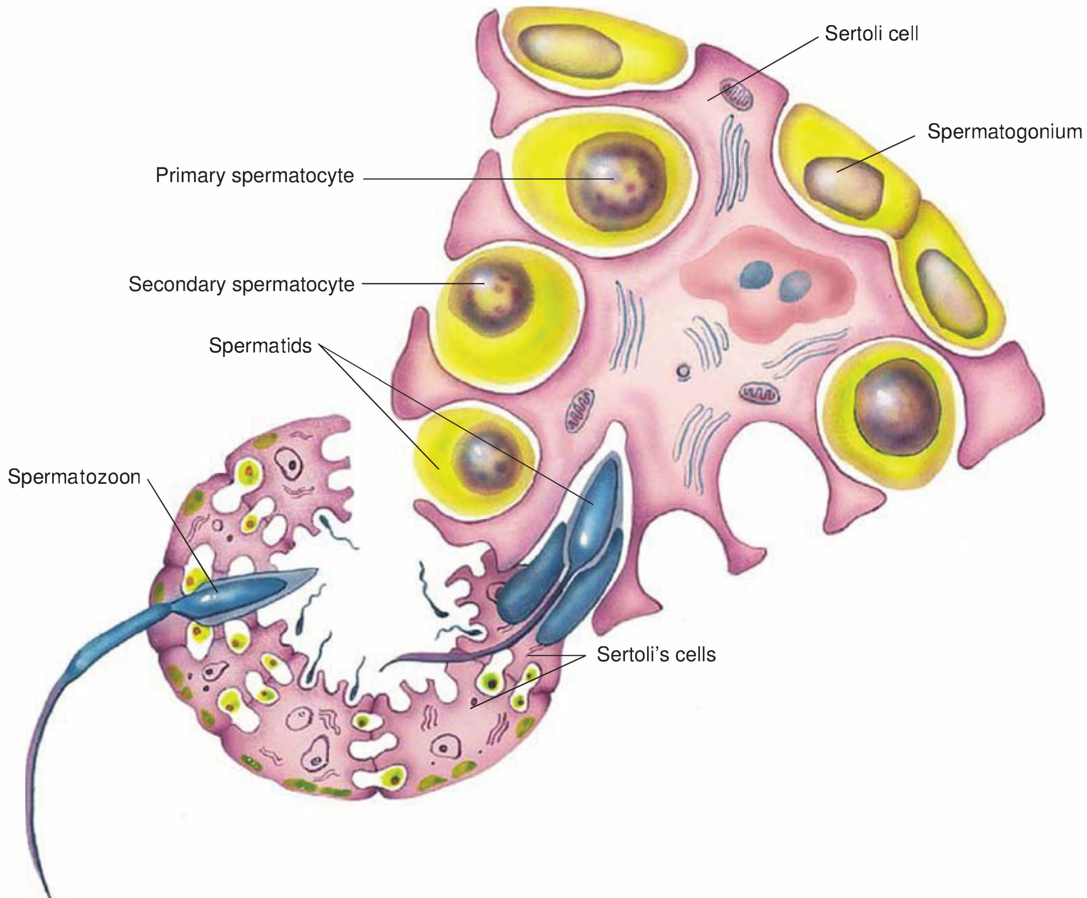

Figure 16-2. The process of spermatogenesis in the seminiferous tubules. (Courtesy of Wolter Kluwer, Skokie, IL.)

Specimen Collection and Handling

The preferred method of semen collection is by masturbation. This procedure ensures the opportunity to collect the entire ejaculate. Collection should be performed after a 48- to 72-hour continence (abstinence from sexual activity) to provide a specimen containing the most accurate sperm count and viability. A private, comfortable room should be provided for specimen collection that allows for quick delivery of the specimen to the laboratory. Written and verbal instructions for the procedure should be provided. Specimen collection containers should be clean glass or

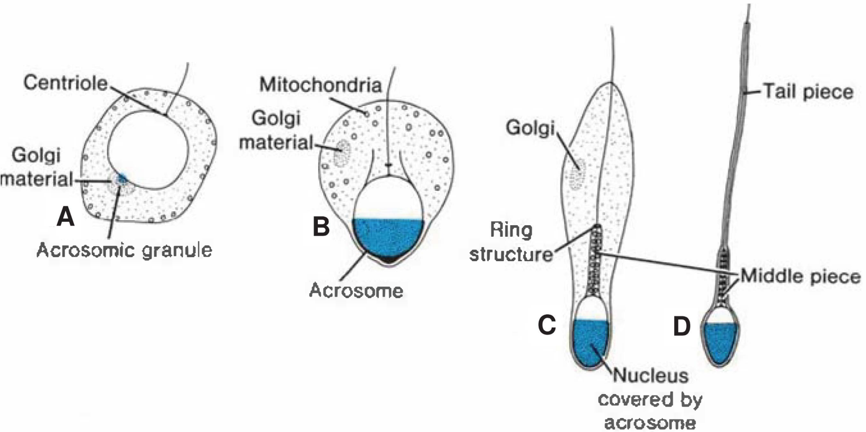

Figure 16-3. Final stages of spermatogenesis. **A:** Immature spermatid with round shaped nucleus, early signs of acrosome and tail development; **B:** Immature spermatid with formation of the acrosome, nuclear condensation developing tail piece; **C:** Spermatid with acrosome covering the nucleus, developing middle piece, and shrinking cytoplasmic membrane; **D:** Mature spermatozoon. (Courtesy of Wolter Kluwer, Skokie, IL.)

Table 16-1 Semen Classifications

CLASSIFICATION	DEFINITION
Azoospermic	A complete absence of sperm
Normozoospermic	Normal sperm count, motility, and morphology
Asthenozoospermic	Normal sperm count with less than normal motility, and morphology
Oligozoospermic	Less than normal sperm count with normal sperm motility, and morphology
Teratozoospermic	Normal sperm count and motility but less than normal morphology

plastic and have a wide opening. Specimens should not be collected in a condom as these often contain spermicidal compounds and lubricants that may interfere with laboratory tests.

If the specimen must be transported from a site distant to the laboratory, it must be kept near body temperature and extremes in temperature must be avoided.

Shortly after ejaculation, the semen coagulates because of the actions of zinc and a clotting enzyme, formed in the prostate, on **semenogelin** (a fibrinogen-like precursor substance) that is produced by the seminal vesicles. **Liquefaction** (coagulated semen becoming more liquid) occurs within 30 to 60 minutes, due to the hydrolysis of semenogelin by prostate-specific antigen (PSA). Ideally, the specimen should arrive in the laboratory as soon after collection as possible so that an accurate liquefaction time may be recorded. The specimen should be labeled with all patient information and time of collection. In addition, the patient should be asked whether any part of the specimen was lost during collection. This information is important to note because the highest concentration of sperm is usually found in the first part of the ejaculate.

Semen analysis can help to establish a cause for male infertility. Classifications of semen quality are shown in Table 16-1.

Laboratory Testing

Semen analysis is often performed immediately after liquefaction of the specimen because delays will alter the motility observation and could lead to erroneous conclusions. The most common parameters evaluated during a **semen analysis** include semen volume, viscosity, pH, and sperm concentration, motility, viability, and morphology. Table 16-2 displays the normal ranges for tests that are routinely performed for a complete semen analysis.

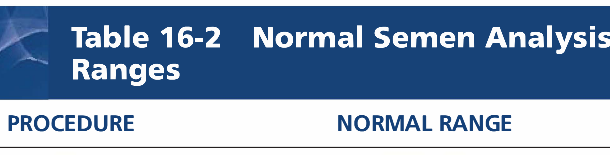

Table 16-2 Normal Semen Analysis Ranges

PROCEDURE	NORMAL RANGE
Liquefaction	Liquefied after 30 minutes
Color	White or grayish white
Viscosity	Viscous
Volume	2.0–5.0 mL
pH	7.2–7.8
Concentration	20–250 million/mL
Agglutination	None
Motility	progressively motile 60%
Viability	75%
Penetration	30 mm
Normal morphology	Laboratory dependent (>70%)
Immature forms	<2%
Leukocytes	None to occasional
Red blood cells	None
Epithelial cells	None to few
Bacteria	None

MACROSCOPIC EVALUATION

Liquefaction

Once the specimen arrives in the laboratory, it is observed for liquefaction time. If coagulation did not occur, it should be reported. A noncoagulating semen in cases of azoospermia may indicate a congenital bilateral absence of the vas deferens and seminal vesicles. If delivery of the specimen to the laboratory took longer than 30 minutes after collection, the specimen may already be liquefied, and there is no opportunity to notice whether it coagulated properly. Normal liquefaction occurs between 30 and 60 minutes. Liquefaction times beyond 60 minutes are considered abnormal. Specimens that do not liquefy must be treated with amylase or bromelin to break up mucus in order to obtain accurate sperm counts. The addition of alpha-amylase solution to semen will not alter motility.

Appearance

Semen is opaque and can exhibit several normal colors. Typical colors include gray, white, and light yellow. The higher the **flavin** concentration of semen, the darker the yellow color may be. A deep yellow color has been associated with certain drugs. Brown or red-colored semen may contain blood. A highly turbid semen specimen usually contains leukocytes and may indicate a reproductive tract infection or inflammation.

Figure 16-4. Normal semen viscosity test.

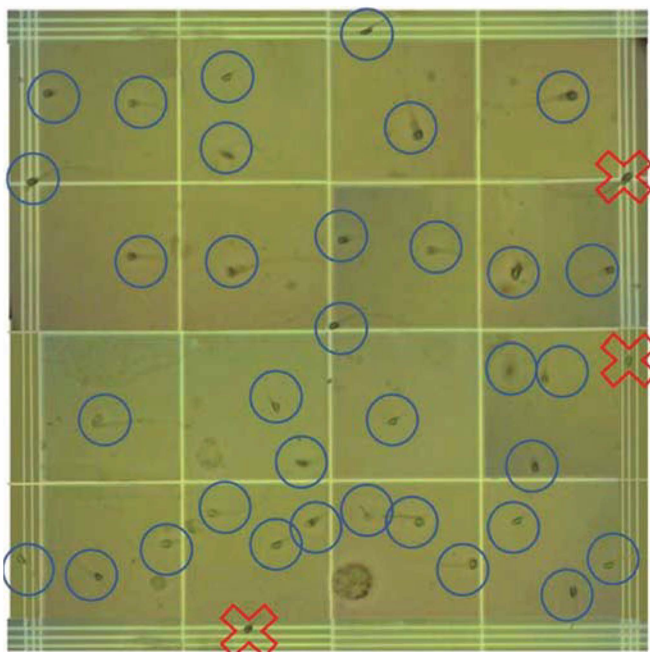

Figure 16-5. Inclusion criteria of counting cells. Count cells (sperm heads, not tails) that touch the upper and left boarders of the counting grid. Do not count cells (sperm heads, not tails) that touch the lower and right boarders of the counting grid. Count only complete sperm.

Volume

Semen volume is measured by using a serological pipette, or small graduated cylinder. Volume is recorded in milliliters to one decimal place (0.1 mL). Normal semen volume ranges from 2 to 5 mL for a complete ejaculate. Volumes both lower and higher than this range have been associated with infertility.

Viscosity

Viscosity (thickness of a fluid) may be assessed while measuring specimen volume or when pipetting the specimen for other tests. Normal semen is slightly viscous and dispenses drop by drop. Increased viscosity is demonstrated by the formation of a string of fluid as the specimen is dispensed from a pipette. Figure 16-4 demonstrates normal semen viscosity. Semen with abnormal viscosity may be watery.

MICROSCOPIC EXAMINATION

Sperm Concentration

Automated methods for counting sperm are available and discussed in the chapter on automation. Most laboratories use manual hemocytometer counting techniques. A manual dilution of 1:20 using distilled water to immobilize sperm may be used.

Professional judgment should be used when determining the area to count on the hemocytometer. The center square millimeter may be sufficient for accurate counts when the sperm concentration is high. Otherwise, it may be necessary to count the four corner square millimeters or even one entire side of the hemocytomer (nine square millimeters) for accurate counts when the sperm concentration is low.

Following the rules for hemocytometer counting needs professional judgment as well, because sperm do not always lie entirely inside or outside the counting area. What usually works best is to determine the placement of the sperm heads on the hemocytometer grid rather than the tails. Figure 16-5 illustrates the inclusion criteria of counting sperm with heads that touch the upper and left borders of the counting grid and exclusion of sperm with heads touching the bottom and right borders.

When using a Neubauer hemocytometer, the simplified formula allows for rapid calculation of sperm concentration: $C = N \times D \times 10/A$ where C is the concentration, N is the number of sperm counted, D is the dilution factor, A is the area in square millimeters (not number of squares). The count is multiplied by ten before dividing by the area to account for the depth between the coverslip and the counting chamber surface.

For example, if the number of sperm counted in nine square millimeters on a 1:100 dilution is 25, the calculation is $(25 \times 100 \times 10)/9 = 2{,}778/\text{mm}^3$. Sperm concentration is often reported in number per cubic centimeter (cc) or milliliters (mL). Therefore, multiplying by 1,000 is necessary to convert the count to the correct unit. In this example, the final count is 2,778,000/cc.

Normal sperm concentrations have been reported to range between 20 and 250 million per milliliter. Oligospermia is a sperm count less than 20 million per milliliter. Azoospermia

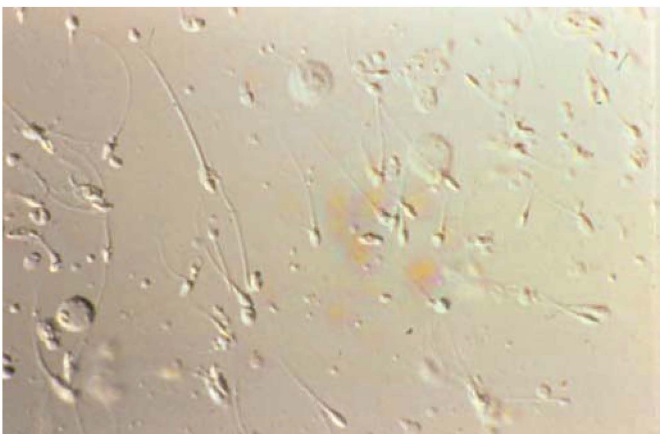

Figure 16-6. Wet mount of semen. Many sperm are present (450×). (Courtesy of Hapner and Spahn, Dayton, OH: Educational Materials for Health Professional Inc., 1981.)

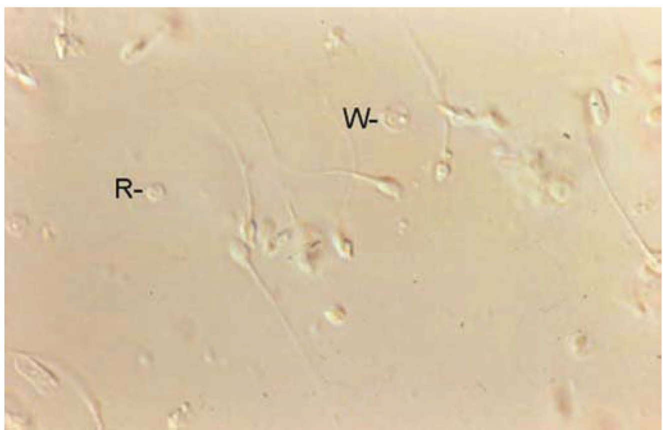

Figure 16-7. Wet mount of semen. Several sperm can be seen along with a red blood cells (R) and a white blood cell (W) (450×). (Courtesy of Hapner and Spahn.)

is the complete absence of sperm. Sperm counts less than normal may be due to chromosomal disorders, ductal obstruction, drugs, gonadotropin deficiency, hyalinization of the seminiferous tubules, maturation arrest, pituitary disorders, radiation, renal failure, and Sertoli-cell-only syndrome. Hormone tests, discussed later in this chapter, may help differentiate among the various causes of azoospermia.

Fertility, however, is possible at counts as low as 1 million sperm per milliliter. Of greater importance in the analysis of semen for fertility evaluation are other microscopic tests. Tests that have a greater bearing on fertility include **morphology** (physical characteristics), **motility** (degree of forward movement), **penetration** (ability to move through mucous), and **viability** (proportion of live sperm).

Motility

Fertilization of an ovum is dependent on the ability of sperm to reach and unite with it. Motility should be evaluated within 1 hour of specimen collection, because motility will decrease over time. One way to evaluate sperm motility is to place a small drop of liquefied semen on a prewarmed slide and coverslip. Observation of sperm movement is best performed using a high dry (45×) objective. Some laboratories prefer to use phase contrast microscopy while evaluating sperm motility, however, bright light microscopy with the condenser turned down is adequate. Figure 16-6 shows the appearance of sperm on a wet mount for the evaluation of motility. Figure 16-7 shows a semen wet mount that includes a red blood cell and white blood cell.

The movement of sperm is evaluated and may be subjectively estimated or counted into three categories. These categories may be called high-motile, low-motile, and nonmotile; or progressive, nonprogressive, and nonmotile. Some laboratories may use as many as five categories: nonmotile, nonprogressive, slow nonlinear progression, moderate linear progression, and strong linear progression. Some laboratories report the percent of sperm in each category, whereas others report only the percent of motile sperm. At least 80% of the sperm demonstrate some forward progress in a normal semen sample. An alternate method used by some fertility clinics is to evaluate sperm motility from a video recording that is played back with a grid overlaying the monitor. This method can also be used to verify questionable motility results. More recent use of technology for sperm evaluation includes the use of high-resolution video photography in combination with computer programs that can calculate velocity, linear progression, and motility efficiency and measure patterns of sperm motion.

Motility can be affected by temperature and other factors, such as the presence of antisperm antibodies. Therefore, a viability test should be performed, especially if a high number of nonmotile sperm are present.

Agglutination

Agglutination (clumping) of sperm may be observed while evaluating a wet mount of semen for sperm motility. A few clumps of sperm or sperm sticking to mucus or other cells can normally be seen in a semen sample. However, true agglutination is present if sperm are distinctly clumped head to head or tail to tail, which may indicate the presence of **antisperm antibodies**. Both IgG and IgA antibodies have been found in the semen of some men with reduced fertility whose sperm demonstrate agglutination. Confirmation with immunologic tests can help determine the specific type of antibody. These tests are discussed later in this chapter.

Viability

Determining whether nonmotile sperm are viable or nonviable is important in establishing a cause for infertility in males. The membranes of dead sperm are damaged and can easily take up eosin stain. The membranes of viable sperm remain intact and do not allow eosin stain to penetrate, leaving the sperm colorless (they will appear white). Eosin stain can be

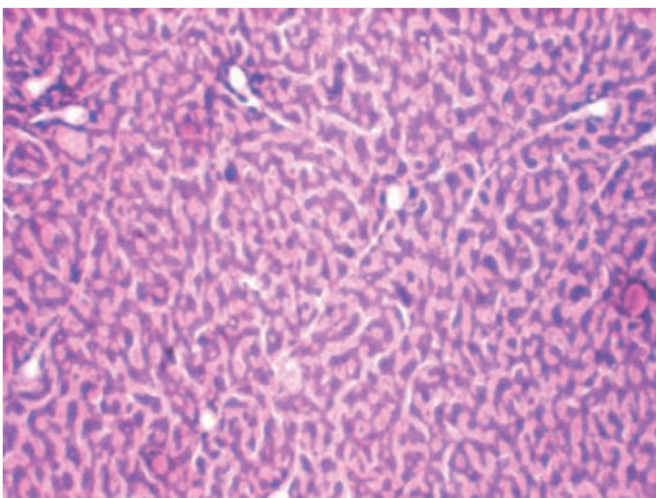

Figure 16-8. Viable sperm do not take up the eosin stain and remain colorless, thus appearing white (eosin/nigrosin stain, 1,000×).

used alone or in conjunction with nigrosin stain. Nigrosin provides a dark background against which the red-colored dead sperm and the white or colorless sperm can be visualized. Figure 16-8 shows the white appearance of the colorless viable sperm, whereas Figure 16-9 shows the red-colored staining of nonviable sperm. At least 100 sperm heads are counted into two categories: red dead and white viable. The percent of viable sperm is reported. Viability and motility do not always correlate. Sperm that are nonmotile may be alive but may have defects of the tailpiece. However, the proportion of motile sperm should not be higher than the proportion of viable sperm. Dead sperm cannot demonstrate motility. Normally, 75% of sperm are viable.

Penetration

Even though sperm may be viable and motile, a couple can still be experiencing male infertility problems if the sperm are incapable of penetrating through cervical mucus. Some physicians consider penetration to be the most important parameter to evaluate in the investigation of infertility. A postcoital cervical mucus specimen is observed by the physician in his office. The presence of many motile sperm contained in this specimen is evidence for normal penetration ability.

A commercially produced product for evaluating sperm penetration, Penetrak, is not currently available and is discussed in Appendix D.

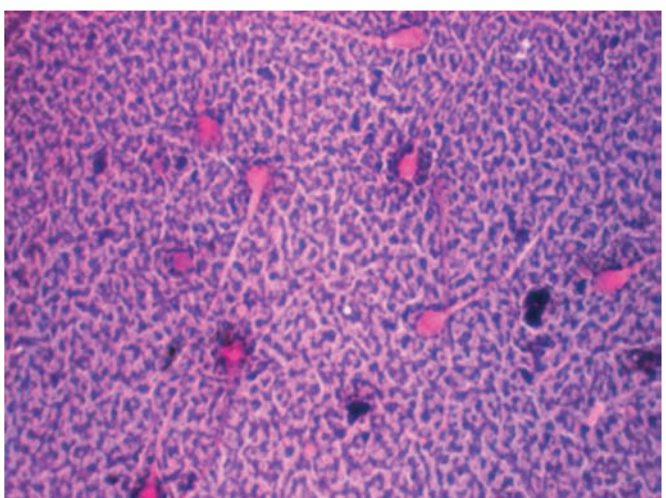

Figure 16-9. Nonviable sperm take up the eosin stain and appear various shades of red (eosin/nigrosin stain, 1,000×).

SPERM MORPHOLOGY

Sperm morphology is evaluated by preparing a stained smear of semen and counting and categorizing all forms of sperm seen. The smear may be made by placing a drop of semen on a slide, placing another slide on top, and pulling them apart in opposite directions. The smear may be fixed with a cytology fixative and then stained with Papanicolaou stain. Giemsa or Wright stain may also be used. Sperm morphologies are classified by counting 100 to 200 sperm using oil immersion. Values for the minimum number of normal sperm vary according to individual laboratories' evaluation criteria. Minimum normal forms for sperm morphology may be 30% to 70%.

Normal Sperm

A normal spermatozoon has a flattened oval head and an elongated tailpiece. The head is about 4 to 5 µm in length and 2 to 3 µm in width and contains a nucleus that comprises 65% of the head. The acrosomal cap may be visible on the stained smear and contains enzymes that assist the sperm's penetration of the ovum. The head appears oval when viewed from the front and appears pyriform when viewed from the side. The side view may be mistaken for an abnormal form by inexperienced observers. The tailpiece is about 50 to 55 µm in length and varies in thickness from 1 µm at near the neck to 0.1 µm at the tip. Four distinct regions comprise the tailpiece: neckpiece, midpiece, main piece, and endpiece. A cellular membrane, plasma lemma, surrounds the entire spermatozoon. Figure 16-10 illustrates the features of a normal spermatozoon. Figures 16-11 to 16-13 show normal sperm stained with Papanicolaou stain. Notice the appearance of sperm observed with a side view.

Abnormal Sperm Morphology

Abnormal sperm morphology occurs as an anomaly of either the head or the tailpiece, or both. Head anomalies include acrosomal abnormalities, constricted heads, double-headed or double-nucleated heads, enlarged or pinheads, nuclear abnormalities, and vacuolation. Tailpiece anomalies include coiled tailpiece, cytoplasmic extrusion mass, lengthened or bent neckpiece, midpiece abnormalities, multiple tails, and variation in tail length. In addition, immature forms of sperm may be

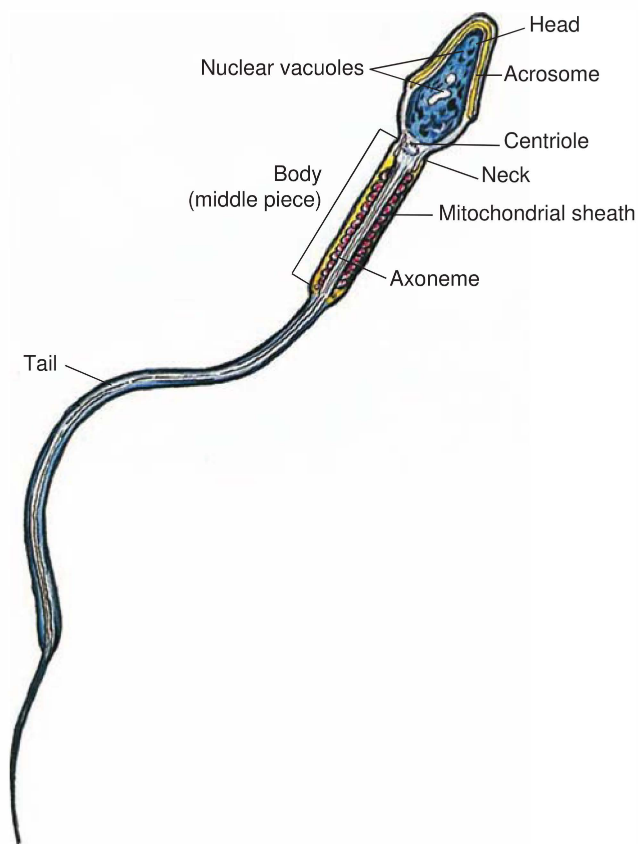

Figure 16-10. Features of a normal spermatozoon. (Courtesy of Wolter Kluwer, Skokie, IL.)

present. Figures 16-14 to 16-25 illustrate some of the more common sperm morphology anomalies. Figure 16-26 shows various immature spermatids that may be seen in semen samples.

Other Cells and Microscopic Findings

Semen may contain cells other than spermatozoa. Immature cells may be present in semen due to premature exfoliation

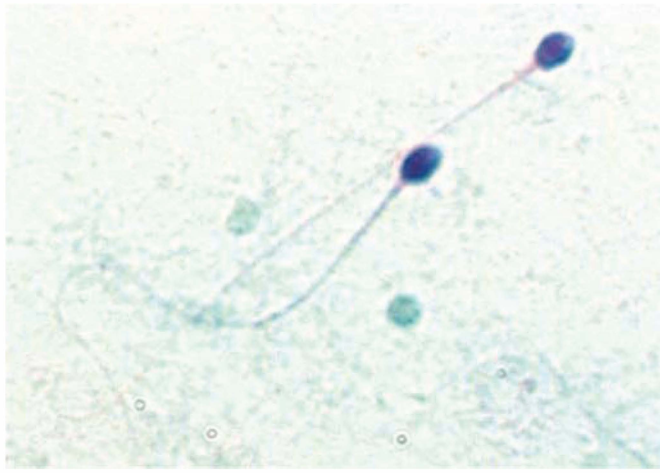

Figure 16-11. Normal sperm (Papanicolaou stain, 1,000×).

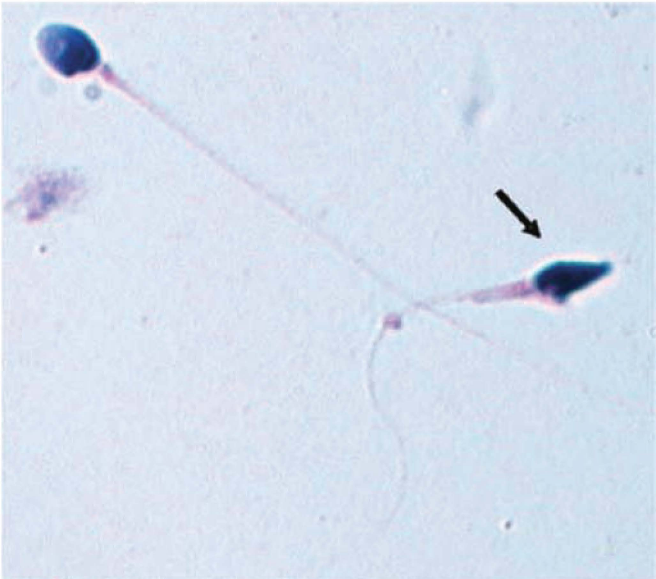

Figure 16-12. Normal sperm. The arrow points to a sperm observed with a side view (Papanicolaou stain, 1,000×).

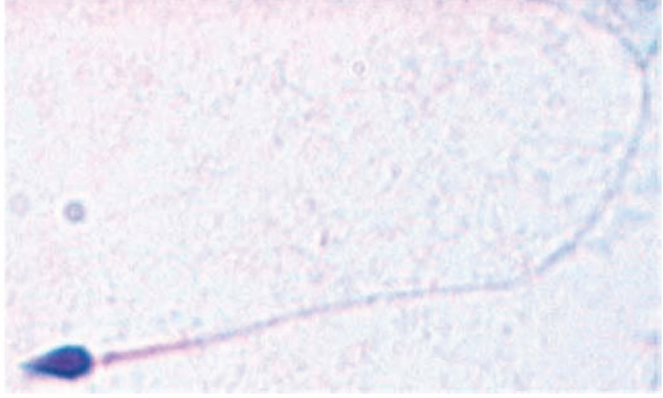

Figure 16-13. Normal sperm, side view (Papanicolaou stain, 1,000×).

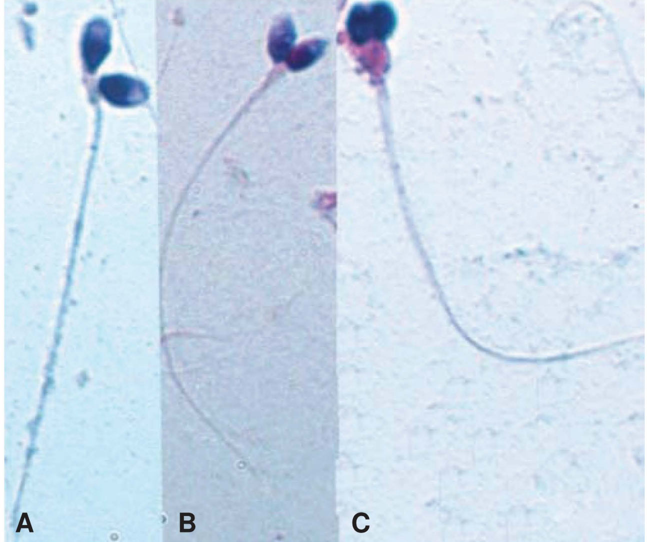

Figure 16-14. Double-headed sperm. Notice the excessive cytoplasmic membrane in image C (Papanicolaou stain, 1,000×).

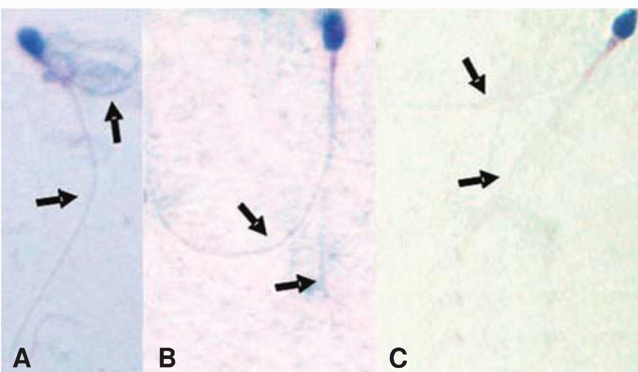

Figure 16-15. Double-tailed sperm. Arrows point to both tails (Papanicolaou stain, 1,000×).

from the seminiferous tubules. In addition, greater than 2% immature spermatozoa may be present during testicular stress, after a viral infection, and as a result of heavy alcohol consumption. Immature spermatozoa may resemble leukocytes and must be properly identified to avoid misdiagnosis of infection. Urethral epithelial cells and white blood cells are usually present in low numbers and can be seen during the hemocytometer count and on morphology smears. An increased number of neutrophils indicates an infection or inflammatory process. Red blood cells are not normally present in semen and should be reported if seen. In addition, bacteria are not a normal finding in seminal fluid and should also be reported.

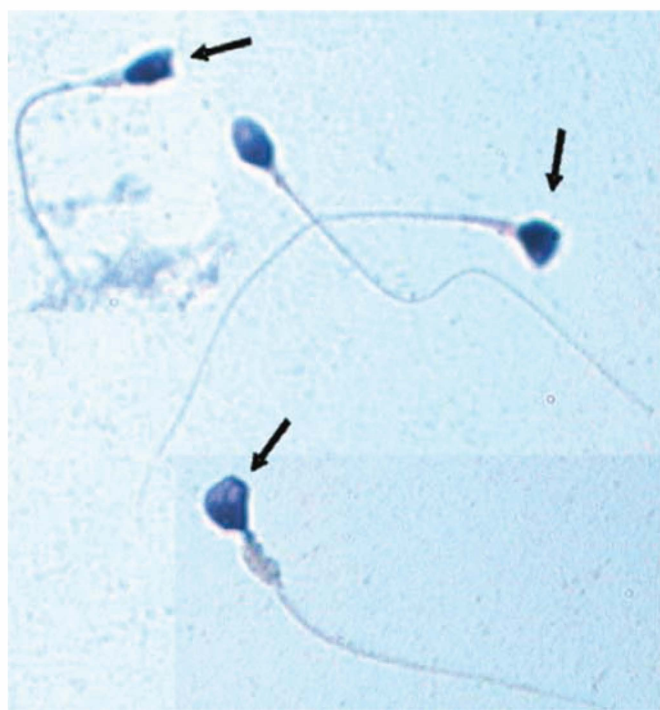

Figure 16-17. Flat-headed sperm. One normal sperm is seen among those with flat heads, indicating the absence of the acrosomal cap (Papanicolaou stain, 1,000×).

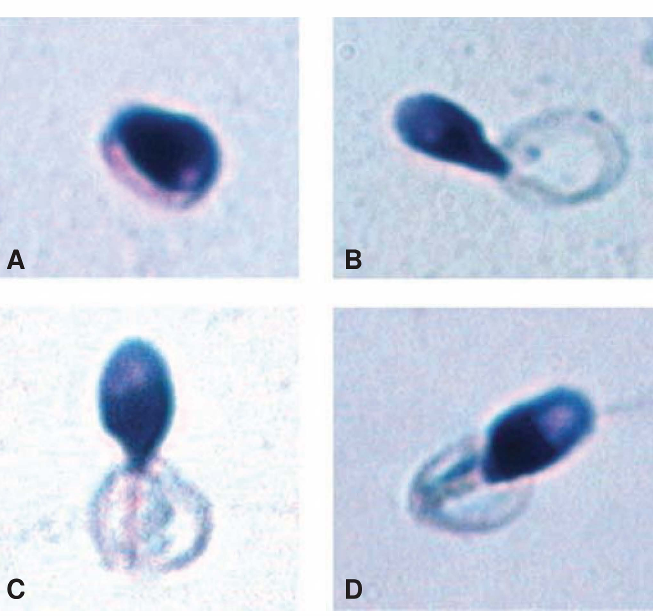

Figure 16-16. Coiled-tailed sperm. Tails may coil completely around the head as seen in image A (Papanicolaou stain, 1,000×).

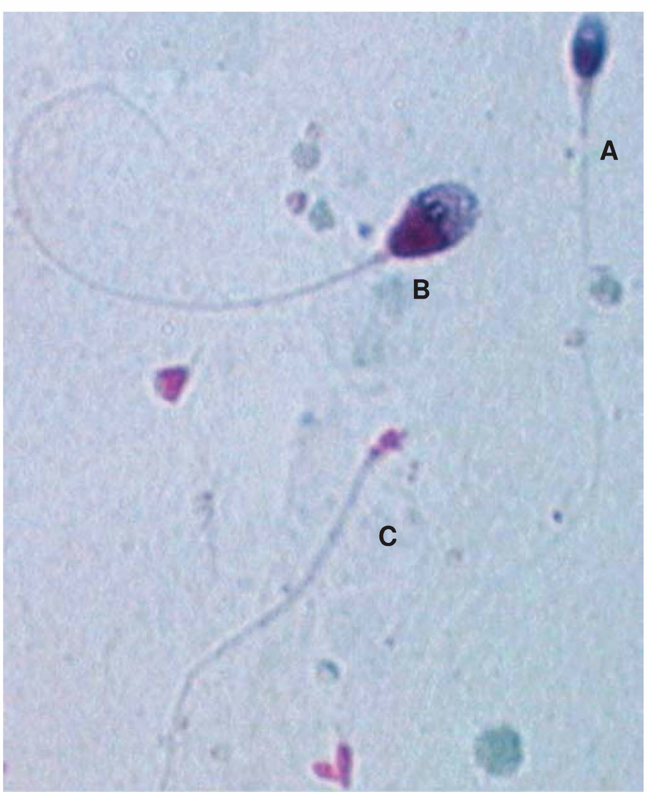

Figure 16-18. Various sperm head sizes. **A:** Normal sperm. **B:** Large head. **C:** Small or pinhead (Papanicolaou stain, 1,000×).

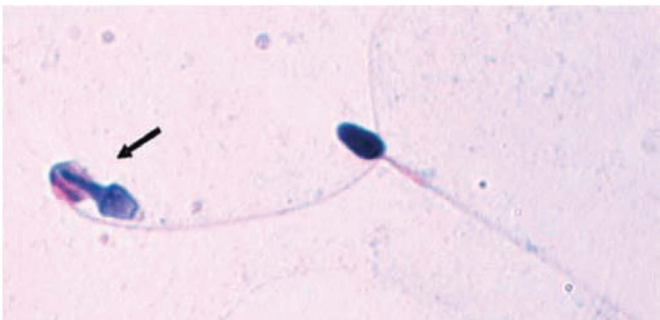

Figure 16-19. Normal sperm shown with sperm at *arrow* that has a constricted (or pinched) head and excessive cytoplasmic membrane (Papanicolaou stain, 1,000×).

CHEMICAL ANALYSIS

pH

The pH of semen should be measured within an hour of collection because semen can become either be more acidic (lactic acid production with high sperm counts) or more alkaline (loss of CO_2 over time) as the specimen ages. Nitrazine paper is the simplest way to measure semen pH. The pH of fresh semen normally ranges from 7.2 to 7.8. Acidic semen pH may be seen in congenital aplasia of vasa deferentia and seminal vesicles, while a male reproductive tract infection produces an alkaline pH.

Acid Phosphatase

Semen acid phosphatase is used to evaluate the secretory function of the prostate. Normal levels of acid phosphatase are equal or greater than 200 units per ejaculate.

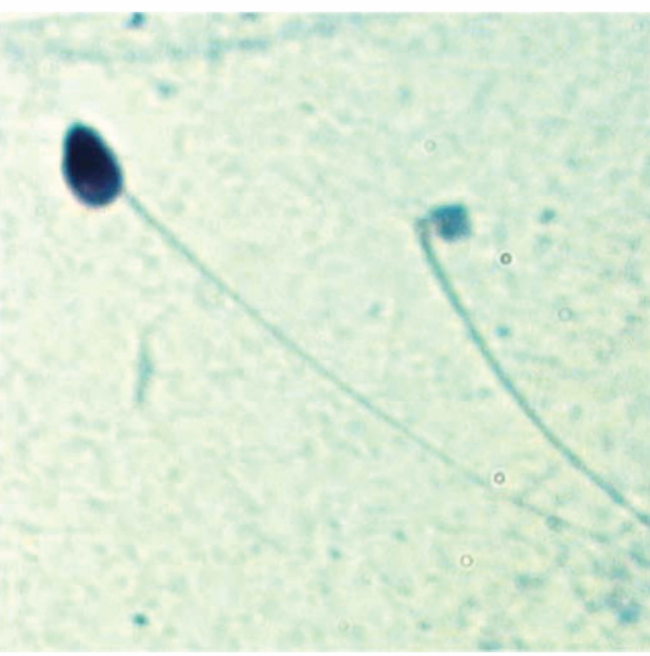

Figure 16-21. These sperm both have bent neck pieces. One has a normal head, whereas the other is a pinhead (Papanicolaou stain, 1,000×).

Fructose

Fructose provides energy for spermatozoa. Semen fructose is produced by the seminal vesicles, with normal levels being equal or greater than 13 μmol per ejaculate and comprises 99% of reducing sugar found in semen. A low semen fructose level indicates the presence of ejaculatory duct obstruction or

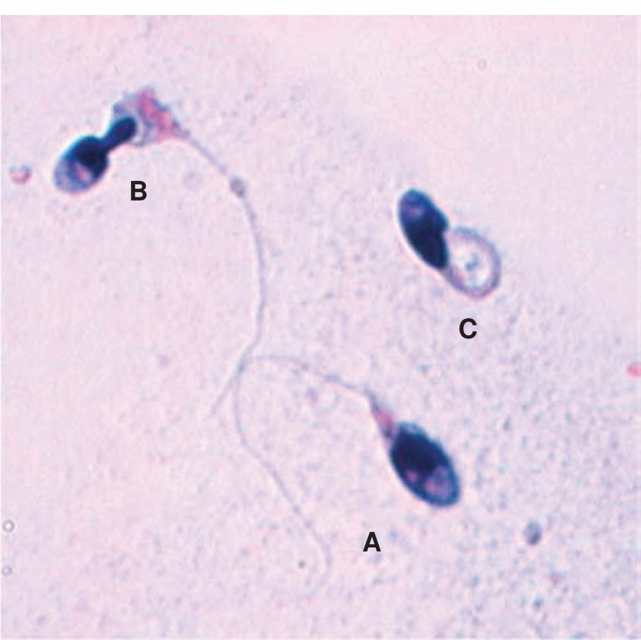

Figure 16-20. Various sperm anomalies. **A:** Normal sperm. **B:** Sperm with constricted (or pinched) head and excessive cytoplasmic.

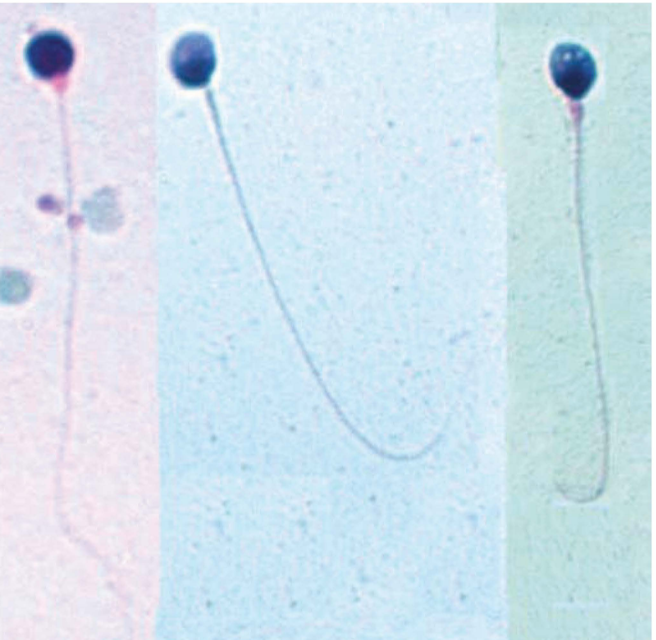

Figure 16-22. These sperm have round heads rather than oval (Papanicolaou stain, 1,000×).

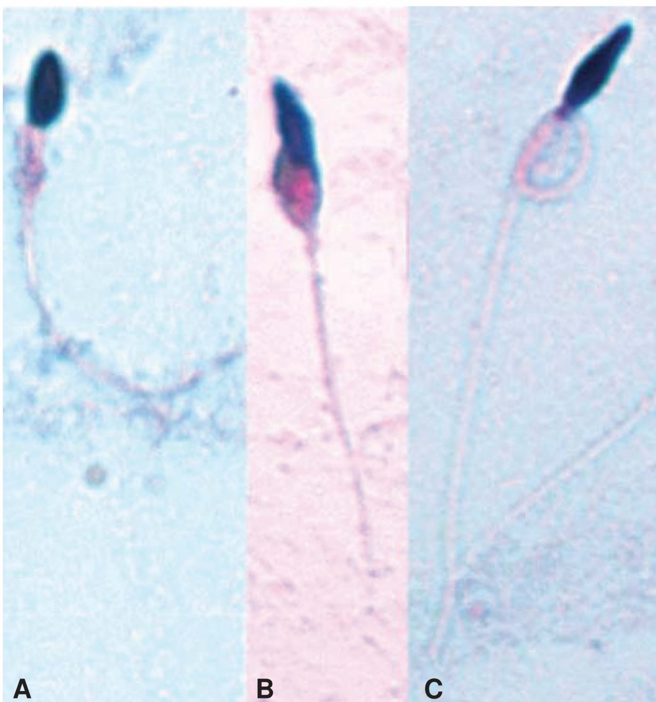

Figure 16-23. These sperm all have tapered heads rather than oval. Image B also shows excessive cytoplasmic membrane and image C has a coiled tail (Papanicolaou stain, 1,000×).

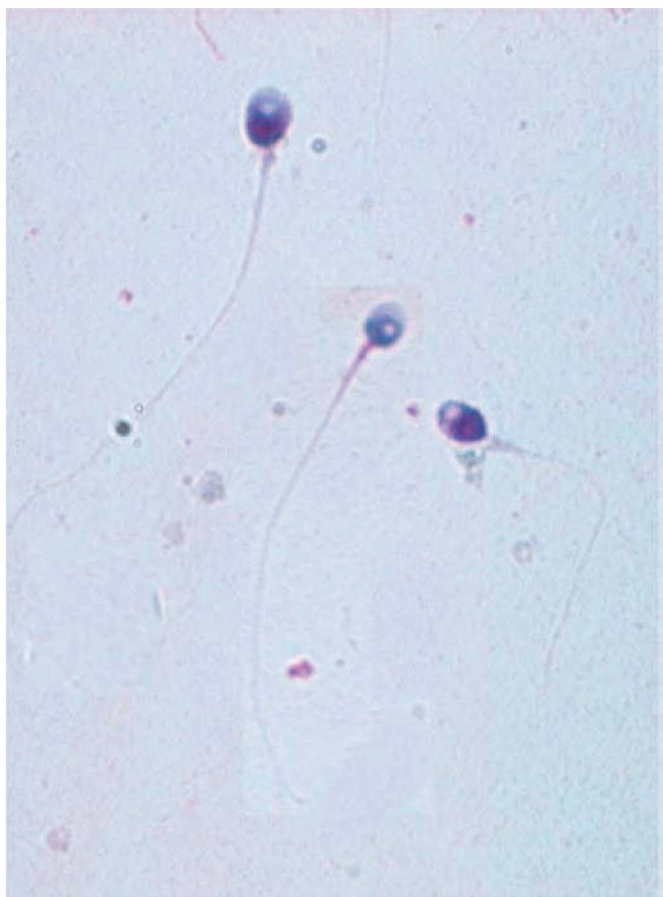

Figure 16-24. The heads of these sperm all contain vacuoles (Papanicolaou stain, 1,000×).

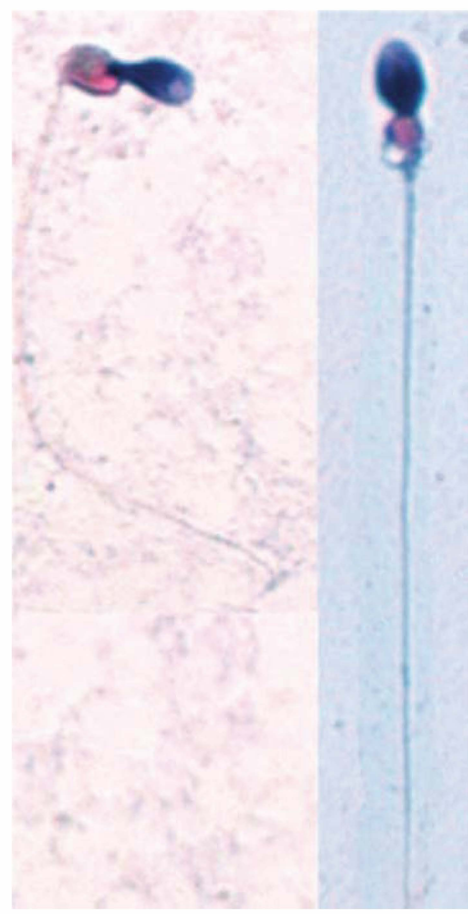

Figure 16-25. The necks of these sperm have excessive cytoplasmic membrane remaining (Papanicolaou stain, 1,000×).

abnormalities in the vas deferens and is accompanied by azoospermia (absence of sperm). Low semen fructose levels have been found to correlate with androgen deficiency, decreased testosterone levels, and genital tract inflammation. In addition, the absence of fructose may indicate ejaculatory duct obstruction, seminal vesicle dysfunction, or hypoplasia.

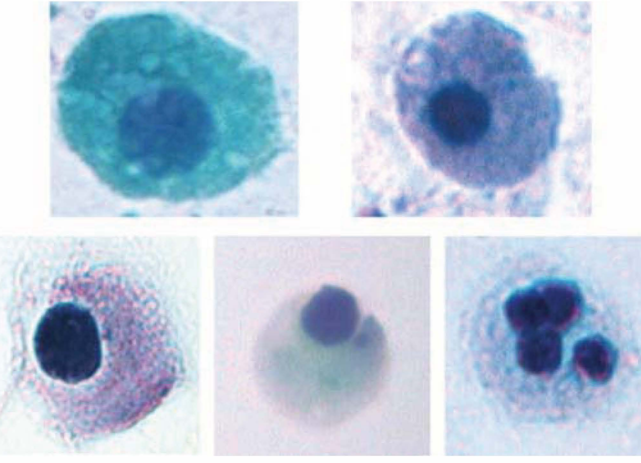

Figure 16-26. Immature spermatids (Papanicolaou stain, 1,000×).

Hormones

Measuring the level of various hormones is helpful in differentiating among causes of azoospermia. These hormones include testosterone, LH, and FSH. Hyalinization of the seminiferous tubules is accompanied by a decreased to normal testosterone level with an increase in both LH and FSH. Gonadotropin deficiency demonstrates decreased levels of all three of these hormones.

In Sertoli-cell-only syndrome, the testosterone and LH levels are normal while FSH is increased. These hormone levels are normal if the cause of azoospermia is ductal obstruction or maturation arrest.

Trace Elements

Trace elements are important for normal spermatogenesis and maintaining antioxidant defense include copper (Cu), selenium (Se), and zinc (Zn). The presence of Se in seminal plasma has positive effect on sperm count, motility, and morphology. Selenium has been found to be deficient in men with **varicocele** (dilatation of pampiniform venous plexus of the spermatic cord). Iron (Fe) may affect sperm morphology as it has been found to be higher in teratozoospermic males.

Other Biochemical Markers of Infertility

Several other chemicals found in semen are the focus of ongoing infertility studies. Malondialdehyde acid and glutathione may affect sperm count and motility. Superoxide dismutase activity may affect semen volume. Neutral alpha-glucosidase, secreted by the epididymis, may affect semen volume and pH. In addition, carnitine and glycerolphosphocholine are markers of epididymal function. Citric acid (citrate) is responsible for the pH of semen and correlates with the physiological function of the prostate gland. Prostate-specific acid phosphatase can be used to evaluate prostate function. In addition to fructose, certain prostaglandins are made in the seminal vesicles and are useful in their evaluation.

If an infection of the spermatic vessels is suspected, polymorphonuclear granulocyte elastase (PMN elastase), released during degranulation of neutrophils, can assist in the diagnosis and monitoring of therapy. Table 16-3 lists these biochemical markers and the organ they assess.

Table 16-3 Semen Biochemical Markers

REPRODUCTIVE ORGAN	SEMEN BIOCHEMICAL MARKER
Epididymis	Neutral alpha-glucosidase Carnitine Glycerolphosphocholine
Prostate	Citrate Zinc PSA
Seminal vesicles	Fructose Prostaglandins
Spermatic vessel infection	PMN elastase

IMMUNOLOGIC ANALYSIS/ANTISPERM ANTIBODIES

Autoimmune antibodies to sperm can form if trauma or infection causes a breakdown of the barrier between sperm and blood. These antibodies are present in both serum and semen. Women can develop isoantibodies to their husbands' sperm. These antibodies may be individual specific or may be reactive to all human spermatozoa. Immunologic testing for antisperm antibodies can be performed as a confirmation when agglutination of sperm is present. Several methods currently exist to test for antisperm antibodies.

Kibrick Method

The Kibrick method involves incubating fresh, liquefied semen with serum from the male or serum from his female partner. Agglutination is observed macroscopically, if antisperm antibodies are present.

Isojima Method

The Isojima method tests for sperm immobilizing antibody. Comparison is made between sperm motility of fresh, liquefied semen and that of semen incubated with either rabbit or guinea pig complement. A sperm immobilization value is calculated by dividing the percent of motile sperm in the fresh specimen by the percent of motile sperm in the incubated sample. A value of 2 indicates the presence of antibodies.

Immunobead Assay

Immunobead assays are used to detect the presence of sperm antibodies on the surface of sperm. These assays can determine whether antisperm antibodies are directed against head, midpiece, or tail and whether the antibodies are IgA, IgG, or IgM. In addition, the immunobead assay method allows for calculating the proportion of sperm in an ejaculate that is antibody bound.

ELISA Methods

Enzyme-linked immunosorbent assay (ELISA) techniques can be used to detect antibodies to prostasomes (prostate-secreted organelles that adhere to spermatozoa).

MICROBIOLOGY

Urogenital infections, caused by various microorganisms, are responsible for about 15% cases of male infertility. Microorganisms

1. What is the optimal place and method for collection of a semen specimen?

2. What type of container should be provided for the collection of a semen specimen?

3. If semen collection occurs at the physician's office or patient's home, what care should be taken during transport?

4. Over half the volume of semen is produced in the:
   - a. Epididymis
   - b. Prostate gland
   - c. Seminal vesicles
   - d. Vasa deferentia

5. Fructose is contained in the portion of semen produced by the:
   - a. Bulbourethral glands
   - b. Epididymis
   - c. Prostate gland
   - d. Seminal vesicles

6. The process of spermatozoa formation is under control of all of these hormones EXCEPT:
   - a. Human chorionic gonadotropin
   - b. Luteinizing hormone
   - c. Follicle-stimulating hormone
   - d. Testosterone

7. Semen analysis plays an important role in:
   - a. Determining the effectiveness of a vasectomy
   - b. Establishing a cause for infertility
   - c. Forensic studies of suspected rape
   - d. All of these

8. Normal semen color(s) includes (*select all that apply*):
   - a. Clear
   - b. Gray
   - c. White
   - d. Yellow

9. Which semen volume is within normal limits?
   - a. 0.5 mL
   - b. 1.5 mL
   - c. 4.0 mL
   - d. 6.0 mL

10. Which semen pH is within normal limits?
    - a. 6.8
    - b. 7.0
    - c. 7.6
    - d. 8.0

11. Which of the following describes a normal spermatozoa head?
    - a. Constricted near the acrosome
    - b. Elongated and tapered
    - c. Flattened oval
    - d. Round and small

12. Viable sperm appear using the eosin–nigrosin stain.
    - a. Black
    - b. Orange
    - c. Red
    - d. White

13. Which statement is NOT true concerning semen fructose?
    - a. Decreased fructose levels may indicate androgen deficiency.
    - b. Fructose levels are independent of testosterone levels.
    - c. Fructose comprises 99% of semen reducing sugars.
    - d. Fructose levels are assessed in cases of azoospermia.

14. Which test is useful in evaluating the presence of infection in the spermatic vessels?
- **a.** Carnitine
- **b.** Glycerolphosphocholine
- **c.** Malondialdehyde acid
- **d.** Polymorphonuclear granulocyte elastase

15. Which test is useful in evaluating the function of the epididymis?
- **a.** Malondialdehyde acid
- **b.** Neutral alpha-glucosidase
- **c.** Prostaglandins
- **d.** Superoxide dismutase activity

16. An asthenozoospermic semen is one that displays a:
- **a.** Complete absence of sperm
- **b.** Normal sperm count with less than normal motility, and morphology
- **c.** Less than normal sperm count with normal sperm motility, and morphology
- **d.** Normal sperm count and motility but less than normal morphology

17. What substance(s) can help establish the presence of semen? (*select all that apply*)
- **a.** Acid phosphatase
- **b.** Prostate-specific antigen
- **c.** Semenogelin
- **d.** Testosterone

18. Identify the morphologies of the labeled spermatozoa in Figure 16-27.

A. ______________________

B. ______________________

C. ______________________

D. ______________________

E. ______________________

F. ______________________

G. ______________________

H. ______________________

I. ______________________

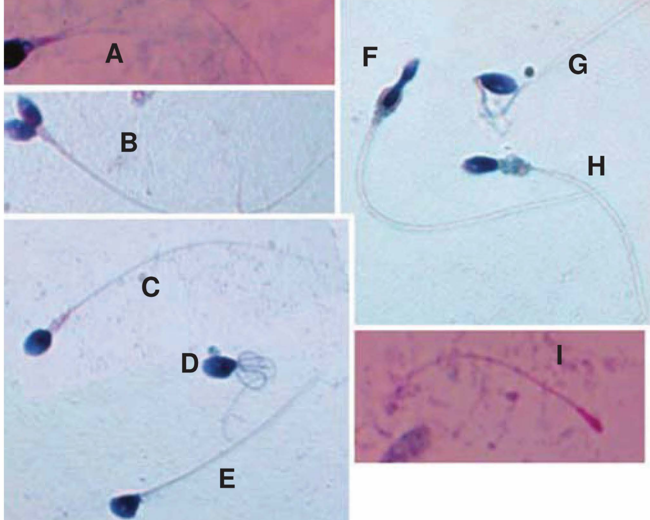

Figure 16-27. Figure for review questions 16 to 18.

CASE STUDY

Case 16-1 Evaluate the following semen analysis results and answer the questions below.

Liquefaction: coagulation did not occur
Color: white
Viscosity: watery
Volume: 5.0 mL
pH: 7.2
Concentration: no sperm seen

1. Which tests on this semen yielded abnormal results?
2. These results correlate with what diagnosis?
3. What follow-up test should be performed and what is the expected result?

Case 16-2 The following semen analysis results were obtained on a specimen that was collected at home in a condom and delivered to the laboratory 1 hour after collection.

Liquefaction: liquefied upon receipt
Color: white
Viscosity: viscous
Volume: 3.0 mL
pH: 7.4
Concentration: 150 million/mL
Motility: 10% progressive
10% nonprogressive
80% nonmotile
Viability: 60% viable

1. Explain how this specimen could be liquefied upon receipt in the laboratory.
2. Explain the correlation between the low number of motile sperm and the number of viable sperm.
3. Discuss the reliability of these results in determining a state of infertility.

Case 16-3 The following semen analysis results were obtained on a specimen that was collected at a fertility clinic.

Liquefaction: 15 minutes
Color: white
Viscosity: viscous
Volume: 1.0 mL
pH: 7.4
Concentration: 10 million/mL
Motility: 80% progressive
10% nonprogressive
10% nonmotile
Viability: 100% viable
Morphology: 80% normal

1. Which tests on this semen yielded abnormal results?
2. What may be an explanation for these abnormal results with the remaining tests being normal?
3. If these are truly accurate results, how is this semen classified?

Case 16-4 A semen specimen is submitted to the laboratory for postvasectomy evaluation. The specimen is suspected to not be semen.

1. Suggest testing that can confirm whether or not this specimen is semen.

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Amniotic Fluid

Chapter 17

KEY TERMS

Acetylcholinesterase
Alpha Fetoprotein
Amniocentesis
Cytogenetic Studies
Fetal Lung Surfactants
Fetal Neural Tube Defects
Foam Stability
Hemolytic Disease of the Newborn
Erythroblastosis Fetalis
Hydramnios
Hydronephrosis
Lamellar Body
Liley Graph
Meconium
Oligohydramnios
Potter Complex
Respiratory Distress Syndrome
Vernix Caseosa

LEARNING OBJECTIVES

1. Describe the production and components of amniotic fluid.
2. Explain the procedure and reasons for performing amniocentesis.
3. Define terminology associated with variation in amniotic fluid volume.
4. Discuss how to differentiate amniotic fluid from maternal urine.
5. Describe the testing available for genetic and congenital abnormalities.
6. Explain the disease process of hemolytic disease of the newborn.
7. Summarize testing available to detect hemolytic disease of the newborn.
8. Compare and contrast the amniotic fluid testing available for fetal lung maturity.
9. Discuss the risks for the fetus in preterm delivery and explain assessment of fetal risk using the Liley graph.

Amniotic fluid is found around the developing fetus, inside a membranous sac, called the amnion. This fluid serves to cushion and protect the developing fetus and also serves a key role in the exchange of water and molecules between the fetus and the maternal circulation. The laboratory performs several crucial tests on amniotic fluid to assess the status of the fetus. These tests can be divided into these groups: (a) tests to diagnose genetic and congenital disorders before birth, (b) tests to detect fetal distress from **hemolytic disease of the newborn (HDN)** or from infection, (c) tests to assess fetal lung maturity, and (d) assessment of the ability of the fetus to survive early delivery. This chapter focusses on amniotic fluid analysis. Tests for the presence of amniotic fluid in vaginal secretions are discussed in the Chapter 18.

Anatomy and Physiology of Amniotic Fluid Formation

The amniotic fluid is formed from the placenta. Amniotic fluid has a composition similar to that of the maternal plasma with a small number of cells from the skin, urinary tract, and digestive tract of the newborn and biochemical substances produced by the fetus. The volume of amniotic fluid increases steadily throughout the pregnancy up to a maximum of 1,100 to 1,500 mL at 36 weeks of gestation.

When fetal urine production begins, the chemical composition of the amniotic fluid changes. This change corresponds to the increased production of creatinine at about 36 weeks of gestation. Prior to 36 weeks of gestation, the amniotic fluid creatinine level is 1.5 to 2.0 mg/dL and after 36 weeks, it rises greater than 2.0 mg/dL. Exchange between amniotic fluid and the maternal plasma circulation equals the amount of amniotic fluid every 2 to 3 hours (Fig. 17-1).

At the commencement of fetal urine production, fetal swallowing of amniotic fluid begins and this regulates the formation of fetal urine. Decreased fetal swallowing results in an increase in amniotic fluid volume, known as **hydramnios**. Abnormally decreased amounts of amniotic fluid, **oligohydramnios**, can occur with premature rupture of the membranes causing a loss of amniotic fluid and result in a number of congenital malformations such as **Potter complex**. Potter complex includes pulmonary hypoplasia, contractures of the limbs, and nodular appearance of the amnion. Fetal urinary tract obstruction can cause **hydronephrosis** (dilation of the pelvis and calyces of one or both kidneys). See Figure 17-2. The fetus also secretes lung liquid and fetal pulmonary substances into the amniotic fluid through fetal breathing movements that circulate amniotic fluid.

Amniocentesis, Specimen Collection and Handling

Amniotic fluid is obtained by **amniocentesis**, a procedure using needle insertion into the amniotic sac, usually transabdominally with simultaneous use of ultrasound (Fig. 17-3). The addition of ultrasound has helped make this procedure safer especially if performed after 14 weeks of gestation.

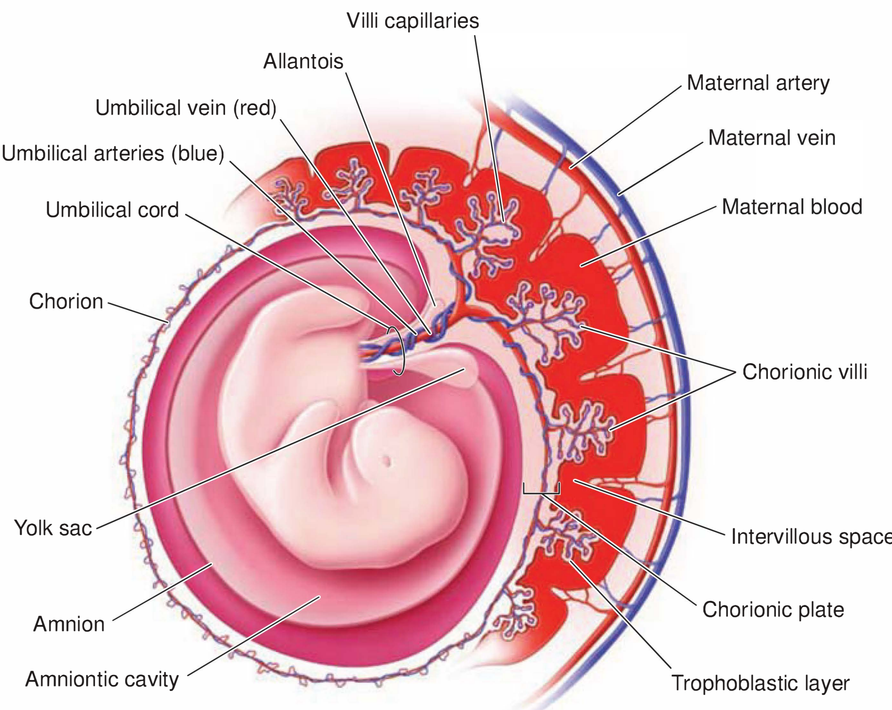

Figure 17-1. Schematic drawing of the amniotic cavity. (Nath JL. *Using Medical Terminology: A Practical Approach.* Philadelphia, PA: Lippincott Williams & Wilkins; 2006.)

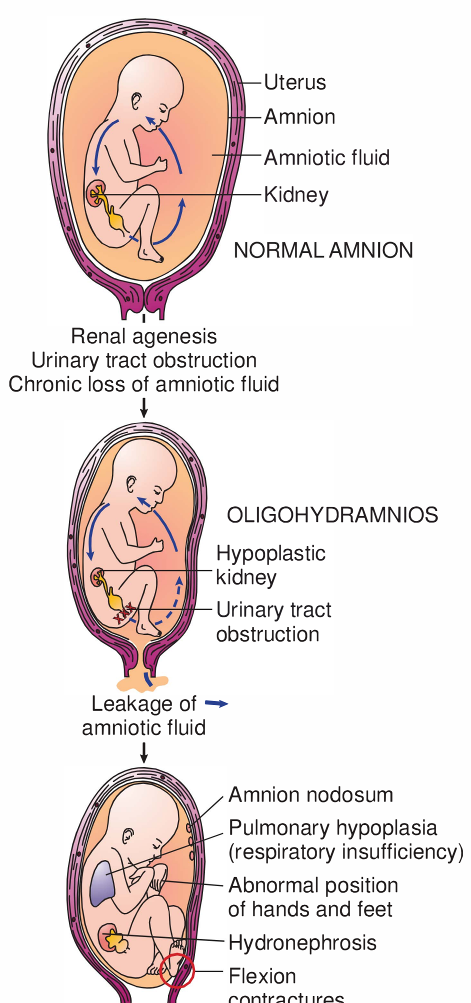

Figure 17-2. Potter complex results from leakage of amniotic fluid, oligohydramnios. Congenital hydronephrosis results from urinary tract obstruction.

Amniocentesis is generally performed between 15 and 18 weeks of gestation for genetic studies although it may be used later in the pregnancy in cases of fetal distress. The amount collected is usually 10 to 20 mL (maximum 30 mL), with collection into several different syringes to prevent the contamination of all specimens with the blood from initial puncture.

Immediately after collection, the fluid is dispensed into sterile plastic specimen containers. Glass containers are less desirable as cells have more of a tendency to adhere to the glass surface. Proper handling and transport of specimens depend on the tests that are ordered.

- Specimens for cell culture and chromosomal studies must be stored at body or room temperature to keep fetal cells alive.
- Specimens for bilirubin analysis must be protected from light. An amber plastic container may be used or the tube may be wrapped in foil.
- Specimens for phospholipid analysis should be transported on ice and centrifuged at 500 g and the supernatant saved for testing.

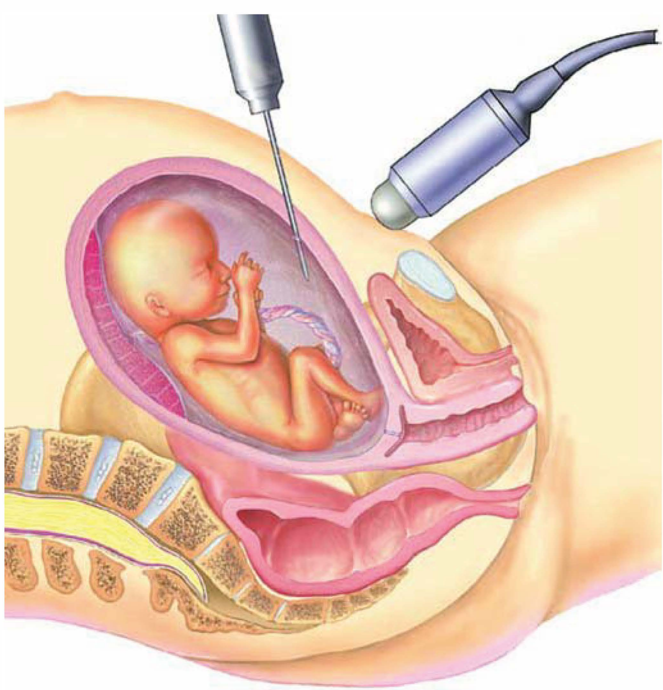

Figure 17-3. Amniocentesis is performed under ultrasound guidance. A needle is inserted through the mother's abdomen into a pocket of amniotic fluid, which is then aspirated with a syringe.

- If blood is present, the specimen should be centrifuged to prevent hemolysis from altering the test results.
- All amniotic fluid samples for chemical analysis that must be stored for any length of time must be centrifuged. If samples for chemical analysis need to be stored more than 24 hours, they must be stored frozen.

Amniotic Fluid Examination

DIFFERENTIATION OF AMNIOTIC FLUID FROM MATERNAL URINE

In case of possible premature membrane rupture or maternal bladder puncture or rupture, it may be necessary to differentiate amniotic fluid from urine. To differentiate these two fluids, chemical levels of creatinine, urea, glucose, and protein can be of assistance. Levels of creatinine and urea are much higher in urine than in amniotic fluid. Glucose and protein levels tend to be higher in amniotic fluid than in urine. Detection of amniotic fluid in vaginal secretions is included in the Chapter 18.

MACROSCOPIC AND MICROSCOPIC EXAMINATION

Physical Characteristics

Normal amniotic fluid is colorless to pale yellow and slightly cloudy. A dark yellow or amber color is associated with

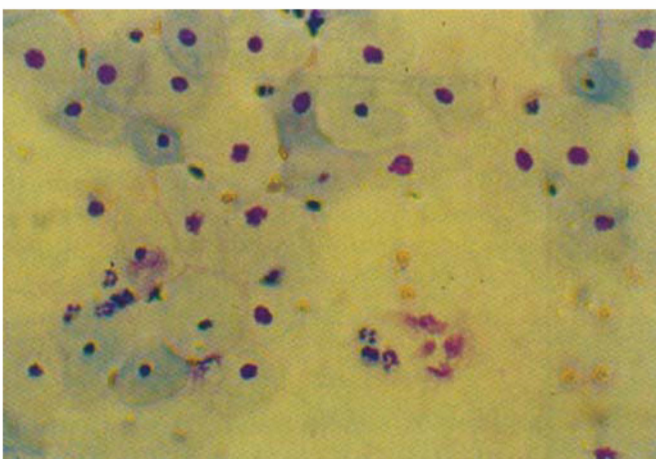

Figure 17-4. Numerous squamous epithelial cells, and other cells observed in cytocentrifuge preparation of amniotic fluid (Wright stain 200×). (Courtesy of McBride, *Textbook of Urinalysis and Body Fluids*. LWW; 1998.)

bilirubin, whereas a green color indicates **meconium**, the newborn's first fecal bowel movements. Blood can appear as pink or red and the source of the blood, whether fetal or maternal, can be distinguished by the Kleihauer–Betke test for fetal hemoglobin. A very dark red-brown amniotic fluid is associated with fetal death.

Microscopic Examination

Microscopic cytological examination of amniotic fluid may yield information on the diagnosis of ruptured membranes or chorioamnionitis. During the early stages of pregnancy, amniotic fluid contains little or no particulate matter. By the 16th week of gestation a number of cell types are present as they begin to shed from the surfaces of the amnion, skin, and tracheobronchial tree. As pregnancy continues other fetal cells appear such as hair, **vernix caseosa** (covering of fetal epidermis).

Smears of amniotic fluid may be made by cytocentrifugation and stained with Papanicolaou, hematoxylin and eosin, or Wright stains. Figure 17-4 shows a few of the cells from amniotic fluid that can be visualized this way. Microscopic evaluation of amniotic fluid may be of limited value, but may provide additional information on the diagnosis of fetal maturity and disorders.

TESTS FOR GENETIC AND CONGENITAL DISORDERS

Cytogenetics

Cytogenetic studies (analysis of chromosomes and DNA) are a common reason for performing amniocentesis. Amniotic fluid contains fetal cells that can provide material for genetic testing and provide valuable information related to the sex of the fetus and any potential genetic abnormalities. Congenital neural tube disorders can be detected as well as Down syndrome and anencephaly prior to birth. Cytogenetic methods used for testing of amniotic fluid include chromosome karyotyping, fluorescence in situ hybridization (FISH), and polymerized chain reaction (PCR).

Alpha Fetoprotein

Fetal neural tube defects such as anencephaly and spina bifida cause elevated **alpha fetoprotein** (AFP) in amniotic fluid and in the maternal circulation. AFP is present in the fetal serum and is secreted in the fetal urine and thus appears in the amniotic fluid. In normal fetal development, AFP peaks at about 16 weeks of gestation and then declines gradually to term. With neural tube disorders, the neural tube is open and AFP is released from the cerebrospinal fluid directly into the amniotic fluid, resulting in amniotic AFP levels that are much higher than normal. AFP is also typically elevated in the maternal serum with fetal neural tube disorders.

Acetylcholinesterase

Acetylcholinesterase (AChE) is also tested, usually in conjunction with AFP, in neural tube disorders. AChE testing is more specific than AFP testing for neural tube disorders. Because blood interferes with AChE testing, amniotic fluid must be free of blood or hemolysis for this test to be accurate, however.

FETAL DISTRESS TESTING

Hemolytic Disease of the Newborn

HDN, also known as **erythroblastosis fetalis**, is caused when mother develops antibodies to an antigen on the fetal erythrocytes and these maternal antibodies cross the placenta to destroy many fetal red blood cells (RBCs). Most frequently, HDN is caused by the sensitization of an Rh-negative mother to fetal Rho[D] antigen, although rarely, other antigens are involved. The destruction of these fetal RBCs results in the appearance of elevated unconjugated bilirubin in the amniotic fluid. With this hemolytic disease process, the high unconjugated bilirubin triggers early production of fetal hepatic glucuronyl transferase activity and this unconjugated bilirubin is converted to conjugated bilirubin. The conjugated bilirubin is not cleared by the placenta, and variable amounts of the conjugated bilirubin are found in the amniotic fluid. Modern preventive measures such as prenatal screening and the administration of RhoGam (Rho[D] immune globulin) to mother during pregnancy have dramatically lowered the incidence of this disease but have not totally eliminated HDN.

Measurement of amniotic fluid bilirubin is performed through spectrophotometric analysis. The absorbance spectrum of amniotic fluid is measured between 365 and 550 nm. The amount that the curve deviates from a straight line at 450 nm (the ΔA_{450}) is directly proportional to the amount of bilirubin in the amniotic fluid. The ΔA_{410} corresponds to oxyhemoglobin, which is the major contaminant of concern. This

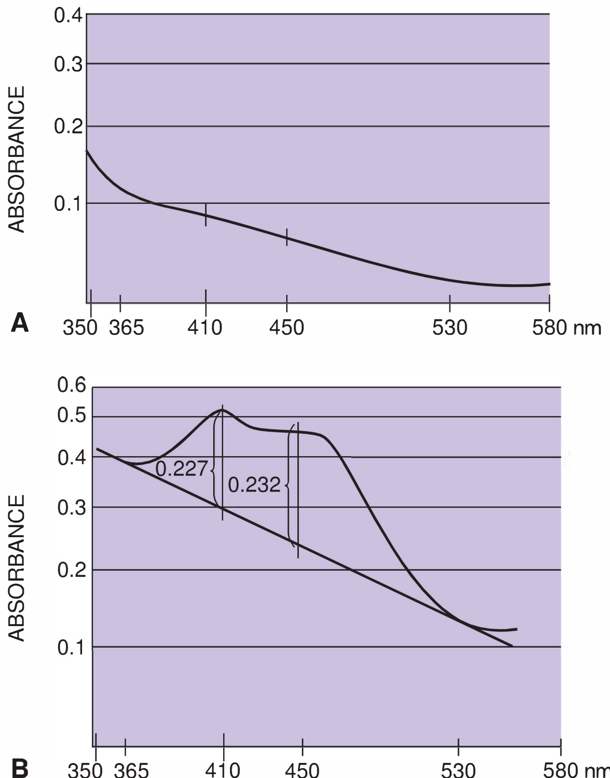

Figure 17-5. Spectrophotometric scan of amniotic fluid indicating bilirubin and oxyhemoglobin peaks. Note the near linearity of the normal curve in **(A)**. In **(B)**, note the elevated bilirubin peak (at 450 nm) and the oxyhemoglobin peak (at 410 nm). The base is drawn from 550 nm to 365 nm. (From Burtis CA, Ashwood ER. *Tietz Textbook of Clinical Chemistry*. 2nd ed. Philadelphia, PA: WB Saunders Company; 1994.)

constituent can be minimized by centrifugation to remove blood upon receipt. The bilirubin concentration correlates to the severity of HDN (Fig. 17-5).

Infection

Evidence is mounting of the importance of microorganisms in the amniotic fluid contributing to the incidence of preterm delivery and spontaneous abortion. Even maternal bacterial vaginosis and trichomoniasis have been linked to preterm birth. Gram stain, wet mount, culture, and molecular tests may be used on amniotic fluid to look for potential infectious agents.

Respiratory Distress Syndrome

Respiratory distress syndrome is a potential cause of death in the premature newborn. Enormous strides have been made in understanding the pathophysiology and management of respiratory distress syndrome, leading to improvements in morbidity and mortality in infants with the condition. It is still a serious concern in premature births, however, and results in many potential complications and comorbidities.

When fetal lungs are immature, they lack sufficient lung surfactant to allow the alveoli of the lungs to function throughout the normal cycle of inhalation and exhalation. Surfactant prevents the alveoli from collapsing by decreasing the surface tension enough to allow them to inflate with air. The surfactant is packed by the cell in structures called **lamellar bodies** which extend into the alveolar airspaces. The lamellar bodies then unfold into a complex lining of the alveolar space. This layer reduces the surface tension of the fluid that lines the airspace. There is a correlation between the levels of **fetal lung surfactants** (a soap-like substance that helps to lower alveolar surface pressure and fetal lung maturity and lung stability). Several fetal lung tests are available to assess fetal lung maturity before birth in order to manage the patient and prevent respiratory distress syndrome.

FETAL LUNG MATURITY

Lecithin: Sphingomyelin Ratio

Fetal lung surfactants include these three phospholipids: lecithin (also known as phosphatidylcholine), sphingomyelin, and phosphatidylglycerol. Lecithin is the major lung surfactant. The ratio of lecithin to sphingomyelin is used to assess fetal lung maturity. Up until the 33rd week of gestation, the levels of these two phospholipids are relatively equal. After 34 weeks of gestation, the level of sphingomyelin decreases, whereas the level of lecithin increases significantly. A lecithin:sphingomyelin (L/S) ratio of 2.0 or greater is associated with fetal pulmonary system maturity.

Phosphatidylglycerol

Phosphatidylglycerol is another lung surfactant that is measured to assess fetal lung maturity. Phosphatidylglycerol is not normally detectable in the amniotic fluid until 35 weeks of gestation. Phosphatidylglycerol production is delayed in cases of maternal diabetes. An advantage to testing for phosphatidylglycerol is that the presence of blood and meconium in the amniotic fluid does not invalidate this test result.

The Amniostat-FLM (Irving Scientific of Santa Ana, California) is a commercial product that uses antibodies to phosphatidylglycerol to detect this fetal lung surfactant. An advantage to this immunological test is that it is not affected by blood or meconium that might be present in the amniotic fluid.

Foam Stability

Foam stability is a screening test for fetal lung surfactant in amniotic fluid. In this test, a fixed amount of amniotic fluid is mixed with an increasing volume of 95% ethanol in a series of tubes with alcohol concentrations ranging from 0.43 to 0.55. The mixtures are shaken vigorously for 30 seconds, and the contents are allowed to settle for 15 seconds and the samples are examined for an uninterrupted ring of foam in the tube. The highest concentration of 95% ethanol that is able to support a ring of foam is known as the foam stability index. The principle of the test is that more surfactant is needed to

maintain the foam in greater concentrations of ethanol and more fetal lung surfactant is needed to support fetal lung function at birth. An index of 0.47 or higher is considered to indicate enough fetal lung surfactant for fetal lung maturity.

Lamellar Bodies

Fetal lung surfactants are produced by fetal type II pneumocytes of the fetal lung and are stored as lamellar bodies after about 20 weeks of gestation. Lamellar bodies are about the size of small platelets. Lamellar bodies are storage forms of lung phospholipids and they enter the fetal lungs and the amniotic fluid at about 20 to 24 weeks of gestation. They reach levels of about 50,000 to 200,000 lamellar bodies/microliter of amniotic fluid by the third trimester of pregnancy. Amniotic fluid samples must be free of blood, hemoglobin, and meconium for accurate lamellar body testing.

Lamellar bodies affect the optical density of amniotic fluid and a measurement of the optical density of 0.150 at 650 nm has been shown to correlate with an L/S ratio of 2.0 and to correlate with the presence of phosphatidylglycerol.

Lamellar body counts provide a reliable estimate of fetal lung maturity. Lamellar body counts can be performed easily with many hematology analyzers using the platelet count channel. As the methods employed by each hematology system vary considerably, sample preparation and lamellar body count cut-off values vary for assessment of fetal lung maturity. Lamellar body counts of approximately 50,000 per microliter correspond to adequate fetal lung surfactant levels and 15,000 per milliliter correspond to inadequate surfactant levels, however these lung maturity thresholds need to be established in your laboratory with your instrument. The CLSI final document, *Assessment of Fetal Lung Maturity by the Lamellar Body Count (C58-A),* published in November 2011, provides guidelines for the use of automated lamellar body counting and establishing your testing verification and validation.

Assessment of Fetal Risk and Survivability with Premature Delivery

Of paramount importance to the ability of the preterm infant to survive after delivery is the fetal lung maturity. If the fetus is in danger in utero and needs intervention, the risks they face must be evaluated as well as the risk of early delivery. Tests for fetal lung surfactants and amniotic fluid creatinine level are most helpful to establish fetal maturity and fetal survival risks.

In 1961, Liley proposed testing of amniotic fluid to assess fetal risk in cases of HDN. He developed a graph that is still used today to assess fetal risk in these cases (Fig. 17-6). In the **Liley graph**, a semilogarithmic plot of the amniotic fluid ΔA_{450} against fetal gestational age, three zones are designated to assign disease severity: zone I—normal values, zone II—moderate hemolysis, and zone III—severe hemolysis with risk of death. Using this graph can guide physicians in assessing fetal maturity and can also assist in treatment decisions.

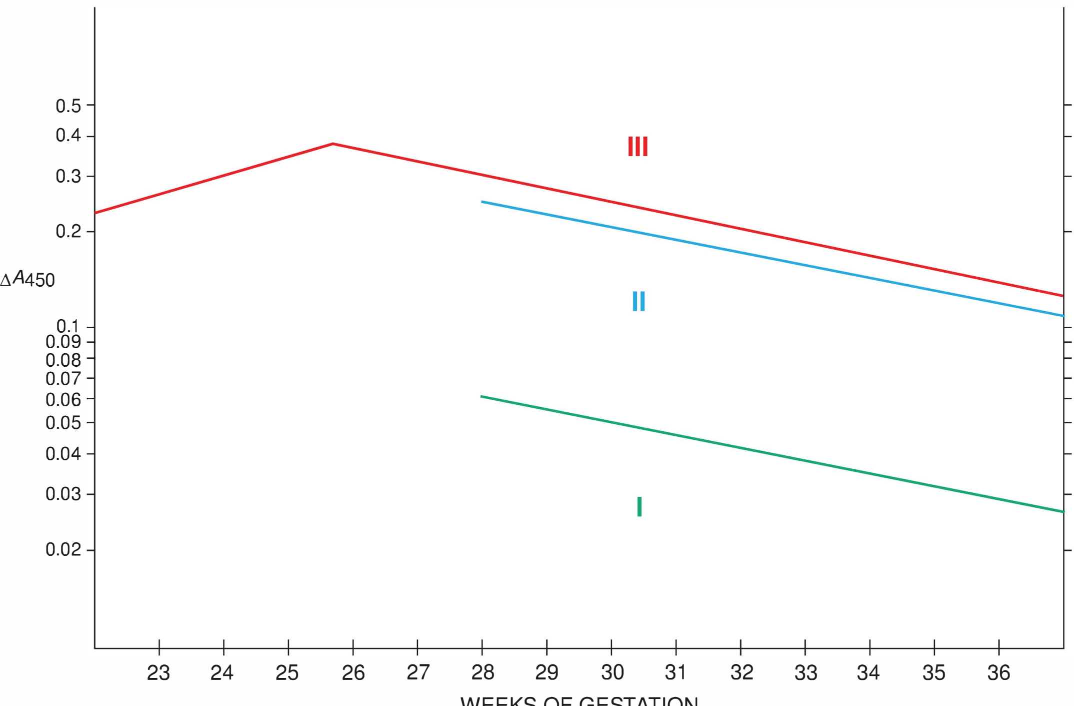

Figure 17-6. Liley graph for assessment of fetal risk. The Liley graph is a three-zone chart with modification for the interpretation of amniotic fluid change in absorbance at 450 nm versus weeks of gestation. The graph divides the patient's readings into three zones of gestational risk, with zone III posing the greatest risk for the developing infant. (From Burtis CA, Ashwood ER. *Tietz Textbook of Clinical Chemistry.* 2nd ed. Philadelphia, PA: WB Saunders Company; 1994.)

1. Reasons for analyzing amniotic fluid include the following EXCEPT:
   a. To diagnose genetic and congenital neural tube disorders
   b. To assess fetal liver maturity
   c. To assess fetal lung maturity
   d. To detect fetal distress from hemolytic disease of the newborn
2. The following is true about amniotic fluid specimen collection and handling:
   a. Fifty milliliters of amniotic fluid is typically collected
   b. Glass containers are used for cytogenetic studies
   c. Typical amniotic fluid is colorless to pale yellow and slightly cloudy
   d. Amniotic fluid is always refrigerated
3. Bilirubin is detected spectrophotometrically in amniotic fluid at:
   a. 365 nm
   b. 550 nm
   c. 410 nm
   d. 450 nm
4. A potential complication caused by early delivery of the premature newborn:
   a. Hemolytic disease of the newborn
   b. Neural tube defects
   c. Respiratory distress syndrome
   d. Excess lamellar bodies
5. All of these phospholipids have a role in fetal lung maturity EXCEPT:
   a. Lecithin
   b. Sphingomyelin
   c. Phosphatidylglycerol
   d. Lamellar bodies
6. A ΔA_{450} value that falls into zone I indicates:
   a. A normal finding without significant hemolysis
   b. Moderate hemolysis
   c. Severe hemolysis
   d. High fetal risk
7. Decreased fetal swallowing can result in:
   a. Hydraminos
   b. Hydronephosis
   c. Oligohydramnios
   d. Oligohyponephrosis
8. Amniocentesis is performed:
   a. Through the umbilicus
   b. Transabdominally
   c. Transvaginally
9. Which test is most specific for neural tube disorders?
   a. Acetylcholinesterase
   b. Alpha fetoprotein
   c. Phosphatidyl glycerol
   d. Lecithin to sphingomyelin ratio
10. Which test on amniotic fluid can aid in the diagnosis of HDN?
    a. Alpha fetoprotein
    b. Bilirubin
    c. Phosphatidylglycerol
    d. Surfactant
11. The Kleihauer–Betke test is for:
    a. Alpha fetoprotein
    b. Fetal hemoglobin
    c. Phosphatidylglycerol
    d. Maternal hemoglobin
12. Which lecithin:sphingomyelin ratio indicates fetal lung maturity?
    a. Equal to or less than 0.5
    b. Less than 1.0
    c. Less than 2.0
    d. Equal to or greater than 2.0

CASE STUDY

Case 17-1 A 23-year-old female has had some bleeding during pregnancy and visited her obstetrician. The physician was concerned that the baby that she was carrying may have hemolytic disease of the newborn. The mother was Rh-negative and had a positive indirect Coombs test. She had not yet sought prenatal care and thought that she may have previously suffered a miscarriage. The physician estimated her pregnancy to be about 34 weeks along from questioning her. An amniocentesis was performed and the amniotic fluid was sent for a bilirubin scan and an L/S ratio. The bilirubin scan showed a ΔO.D. elevation of 0.25 at 450 nm. The L/S ratio was 2.3. Using a Liley graph, the physician estimated her baby's risk to help him decide whether he should try intrauterine transfusion or deliver the infant early for treatment.

1. Does the bilirubin scan confirm the physician's suspicion of hemolytic disease of the newborn?
2. What substances peak at 410 nm and 450 nm and what do these substances indicate?
3. Using the Liley graph, would you classify the fetal risk as nonaffected or mildly affected (zone I), moderately affected (zone II), or severely affected with intervention required (zone III).
4. Does the lecithin:sphingomyelin (L/S) ratio indicate fetal lung maturity if the physician decides to deliver this baby early?

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18 Chapter

Vaginal Secretions

KEY TERMS

Amine or Whiff Test
Atrophic Vaginitis
Bacterial Vaginosis
Candidiasis
Chorion
Clue Cells
Decidua
Fern Test
Fetal Fibronectin
KOH Preparation
Placental Alpha Microglobulin-1
Rupture of Fetal Membranes (ROM)
Trichomoniasis
Vaginitis
Wet Mount

LEARNING OBJECTIVES

1. Explain the procedure collection and handling of vaginal secretion specimens.
2. Discuss the origin of substances present in amniotic fluid used in the diagnosing of rupture of fetal membrane (ROM).
3. Describe tests for detecting amniotic fluid in vaginal secretions.
4. Differentiate among various forms of vaginitis.
5. Describe laboratory tests for detection of atrophic vaginitis, desquamative inflammatory vaginitis, bacterial vaginosis, trichomoniasis, and candidiasis.
6. Summarize laboratory findings in normal vaginal secretions, atrophic vaginitis, desquamative inflammatory vaginitis, bacterial vaginosis, trichomoniasis, and candidiasis.
7. Recognize sources of error when performing laboratory testing of vaginal secretions.

Glands in the cervix normally produce a clear mucus that may turn slightly white or pale yellow upon exposure to air. The amount of vaginal secretions may vary throughout the menstrual cycle. Noticeable changes in the color, consistency, or amount of vaginal secretions may be linked to various conditions and infections. Vaginal secretions are also examined for the presence of amniotic fluid as evidence of fetal membrane rupture.

Specimen Collection and Handling

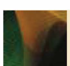
Vaginal secretions are collected by a healthcare provider during a pelvic examination. The method of collection and container used is specific for the testing that is to be performed. A warmed speculum is used to visualize the vaginal fornices and the specimen is collected by swabbing the area required for testing—vaginal pool, vaginal wall, or cervical os (or cervical opening to the uterus) (Fig. 18-1).

The swab used is dependent upon the test to be performed. If the specimen is for bacterial culture, polyester-tipped swabs on a plastic shafts should be used as other substances are toxic to certain pathogens (Table 18-1).

Swabs must be placed immediately into a properly labeled tube or container, containing transport media appropriate for the test to be performed, and be stored and transported at the correct temperature.

Table 18-1 Toxicity of Swabs to Microorganisms

MATERIAL	ORGANISM AFFECTED
Cotton swab	*Neisseria gonorrhoeae*
Wooden shaft	*Chlamydia trachomatis*
Calcium alginate swab	Herpes simplex virus



Rupture of Fetal Membranes

The **rupture of fetal membranes (ROM)**, tearing of the amniotic sac with release of amniotic fluid, normally occurs at the onset of labor once the fetus has arrived to full-term at 37 weeks of gestation. Preterm premature rupture of fetal membranes (PPROM) is the ROM before 37 weeks of gestation. Patients with PPROM must be diagnosed correctly and managed quickly to avoid fetal and maternal complications. Preterm delivery is the leading cause of neonatal morbidity and mortality in the United States.

Several methods exist to test for evidence that the membrane surrounding the fetus has ruptured. Traditional procedures for the diagnosis of ROM include pooling of amniotic fluid observed during speculum examination, nitrazine test, and a ferning test.

LABORATORY EXAMINATION

Fern Test

The **fern test** is used to differentiate amniotic fluid from maternal urine. With this test, vaginal fluid is spread out on a glass slide and allowed to dry at room temperature. This slide is observed for fern-like crystals that are a positive screen test for amniotic fluid (Fig. 18-2).

Placental Alpha Macroglobulin-1 (PAMG-1)

Placental alpha microglobulin-1 (PAMG-1) is a protein expressed by the cells of the decidual part of placenta. During pregnancy, PAMG-1 is secreted into the amniotic fluid but

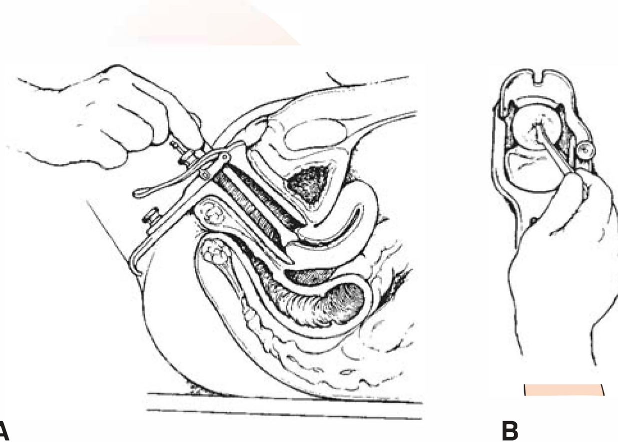


Figure 18-1. **A:** A warmed speculum allows for visualization and insertion of swab. **B:** The swab is used to sample the vaginal pool and nontraumatically "scrape" the cervical os and walls of the vagina (depending on test requirements).

Figure 18-2. "Ferning" seen in vaginal secretions is positive for the presence of amniotic fluid due to ROM.

is present only at very low levels in cervicovaginal secretions when fetal membranes are intact. PAMG-1 is present in amniotic fluid throughout all three trimesters of pregnancy, and appears in vaginal secretions after the ROM.

Traditional procedures for the diagnosis of ROM include pooling of amniotic fluid observed during speculum examination, nitrazine test, and a ferning test. A more current test used by healthcare professionals to aid in the detection of ROM is AmniSure. This test can be performed when pregnant women report signs, symptoms, or complaints suggestive of such a rupture. A positive AmniSure test is indicative of amniotic fluid in vaginal secretions, meaning there is a rupture of the amniotic membranes.

AmniSure is a one-step immunochromatographic assay using monoclonal antibodies to detect PAMG-1. During the test procedure, PAMG-1 from the sample sequentially binds to a monoclonal antibody conjugated with labeled particles and is carried to the test area where the complex is bound by a second monoclonal antibody, resulting in a positive result line. If no PAMG-1 is present, the test area will not display a line. A line in the control area must be present for either a positive or negative result to be valid.

PROCEDURE. A vaginal swab is used non-invasively to take a sample of vaginal secretions. The swab is then immediately placed into a vial with solvent to extract the PAMG-1 from the swab. To perform the test, the test strip (dipstick) is inserted into the vial and the result is read after 10 minutes. See Box 18-1. (Note: Please see instructions for use for full details on the test procedure and limitations.)

PERFORMANCE RESULTS. According to the FDA-cleared package insert, AmniSure has a sensitivity of 98.9% and a specificity of 98.1%.

Insulin-like Growth Factor Binding Protein-1

Insulin-like growth factor binding protein-1 (IGFBP-1) is secreted by the decidual cells of placenta and is present in amniotic fluid in high concentration. When fetal membranes rupture, IGFBP-1 is present in vaginal secretions. The Actim PROM test detects IGFBP-1 using the principle of lateral flow immunochromatography that is visually interpreted.

Fetal Fibronectin

Fetal Fibronectin (fFN) is an adhesive glycoprotein produced by fetal cells and is found in the space between the **chorion** (fetal sac) and the **decidua** (uterine lining). fFN binds the fetal sac to the uterine lining and begins to break down toward the end of pregnancy. The presence of fFN that has leaked into the vagina may indicate that a preterm delivery is likely to occur, although a positive result is often inconclusive.

fFN is tested on patients between 22 and 34 weeks of gestation. A vaginal swab is collected from the pregnant patient, at the posterior fornix of the vagina or the ectocervical region of the external cervical os. Specimens from other locations are not acceptable. The fFN test must be performed within 30 minutes of collection.

A negative fFN result indicates that there is little possibility of preterm delivery within the next 7 to 10 days. The test is repeated weekly for high-risk pregnancies. Positive fFN results are interpreted with caution and may mean that preterm labor and delivery may occur soon.

FALSE RESULTS. False-positive fFN results may be obtained on specimens from patients in whom the cervix has been disrupted during sexual intercourse, digital cervical examination, vaginal probe ultrasound, or other disruptive events. Specimens should not be collected less than 24 hours after intercourse. Creams, disinfectants, lubricants, and soaps can interfere with the absorption of the specimen by the swab, as well as the antigen–antibody reaction of immunologic tests.

AUTOMATION OF fFN. Qualitative measurement of fFN has been automated using a cassette into which the specimen is placed. The specimen flows from an absorbent pad, across a nitrocellulose membrane (by capillary action), through a reaction zone containing murine monoclonal anti-fFN antibody, FDC-6. This antibody is conjugated to blue microspheres. The sample then flows through a zone containing goat polyclonal anti-human fibronectin antibody, which captures any fibronectin-conjugate complexes, resulting in a positive test line. Remaining sample continues to flow through a zone containing goat polyclonal anti-mouse IgG, which captures unbound conjugate resulting in a control line. The lines on the cassette are not to be read or interpreted visually. Measurements must be performed on the analyzer (Fig. 18-3).

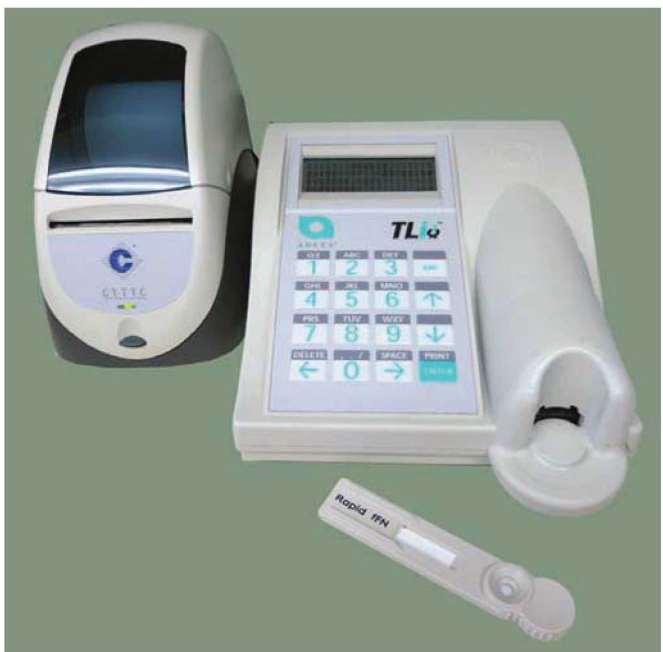
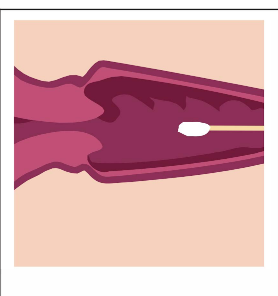
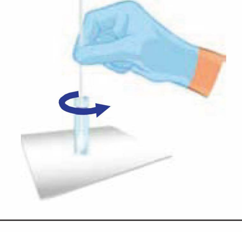
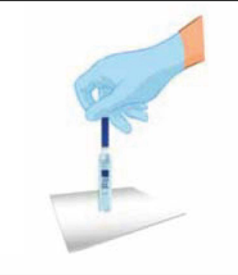


Figure 18-3. The TLi Analyzer, by Adeza Biomedical, is used to perform qualitative measurements of fetal fibronectin.

BOX 18-1 AmniSure Procedure

NOTE: You must follow all directions to get an accurate reading of the results.
The test should not be used earlier than 6 hours after the removal of any disinfectant solutions or medicines from the vagina.
Placenta previa and performing digital exams prior to sample collection can lead to inaccurate test results.

 	1. Take the solvent vial by its cap and shake well to make sure all liquid in the vial has dropped to the bottom. Open the solvent vial and put it in a vertical position.
	2. To collect a sample from the surface of the vagina use the sterile Polyester swab provided. Remove the sterile swab from its package following the instructions on the package. The Polyester top should not touch anything, prior to its insertion into the vagina. Hold the swab in the middle of the stick and, while the patient is lying flat on her back carefully insert the Polyester top of the swap into the vagina until the fingers contact skin (no more than 5–7 cm deep). Withdraw the swab from the vagina after 1 minute.
	3. Place the Polyester tip into the vial and rinse the swab in the solvent by rotating for one minute.
	4. Remove and dispose of the swab.
	5. Tear open the foil pouch at the notches and remove the Amnisure ROM Test strip.
	6. Dip the white end of the test strip (marked with arrows) into the vial with solvent. Strong leakage of amniotic fluid may make the results visible early (within 5 minutes), while a very small leak will take the full 10 minutes.
	7. Remove the Test Strip if two stripes are clearly visible in the vial or after 10 minutes sharp. Read the results by placing the test on a clean, dry, flat surface. Do not read or interpret the results after 15 minutes have passed since dipping the Test Strip into the vial.

Two lines: **There is a rupture**

One line: **No membranes ruptured**

No lines: **Test is invalid; take another test**

The darkness of the stripes may vary. The Test is valid even if the stripes are faint or uneven. Do not try to interpret the test result based on the darkness of the stripes. © QIAGEN, all rights reserved.

Vaginitis

Vaginitis includes a variety of conditions that cause symptoms such as vulvar itching, irritation, burning sensation, malodor (often describe as "fishy," and vaginal discharge). Conditions categorized as vaginitis include atrophic vaginitis, desquamative inflammatory vaginitis, bacterial vaginosis, candidiasis, and trichomonas.

Vaginal epithelium consists of parabasal cells and basal cells overlayed with layers of mature squamous cells. The most superficial layers consist of mature squamous epithelial cells that are large, flat, polygonal-shaped cells with a centrally located nucleus and agranular cytoplasm. Parabasal cells are immature epithelial cells that are smaller than squamous cells and are usually round in appearance. They contain less cytoplasm, which can display basophilic structures. Basal cells make up the deepest layer of vaginal epithelium, are

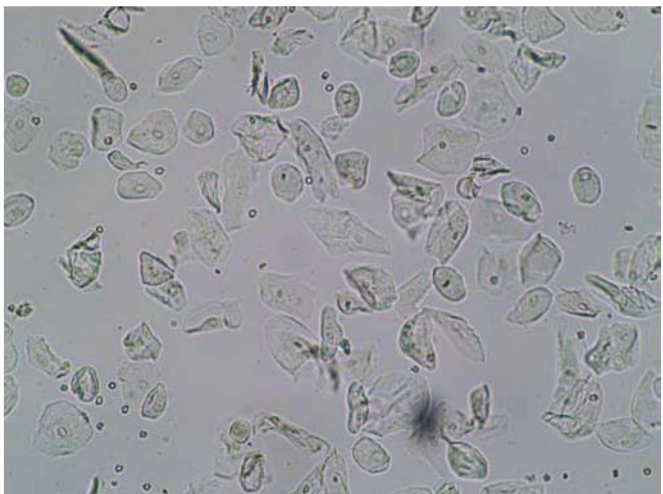
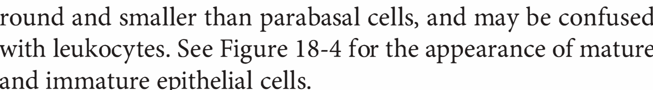


Figure 18-4. Immature epithelial cells (parabasal cells) as well as mature squamous epithelial cells. The immature cells are smaller and round. (Wet mount)



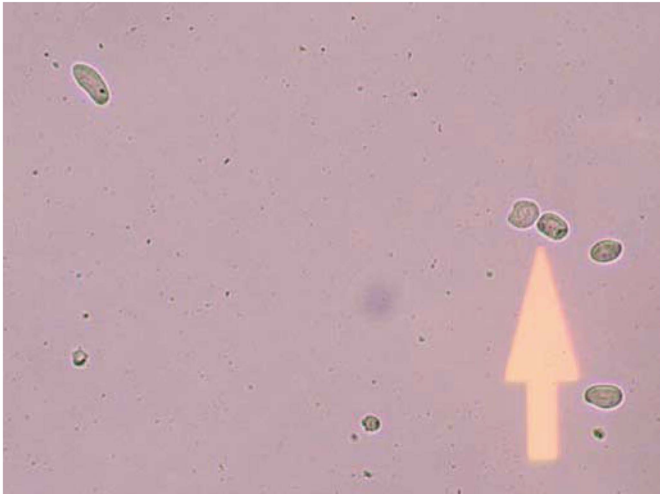


Figure 18-5. A predominance of parabasal cells in vaginal secretions is consistent with estrogen deficiency. In severely estrogen-deficient women, the vaginal fluid is almost acellular. (Wet mount)

round and smaller than parabasal cells, and may be confused with leukocytes. See Figure 18-4 for the appearance of mature and immature epithelial cells.

Squamous epithelial cells slough off and can be seen in vaginal secretions. During childbearing years, vaginal secretions contain a predominance of squamous epithelial cells. The normal process of epithelial glycogen conversion to lactic acid helps maintain a pathogen-free environment.

ATROPHIC VAGINITIS

Atrophic vaginitis is a condition caused by physiologic and structural changes that occur to the vulvovaginal mucosa primarily as a result of a decrease in estrogen levels at menopause. Symptoms of atrophic vaginitis include vulvovaginal dryness, vulvar itching or pain, recurring urinary tract infections, and abnormal vaginal discharge. As estrogen decreases after menopause, so do the number of squamous cells. The resultant rise in pH provides an environment in which pathogens can overgrow.

After menopause vaginal secretions will contain a predominance of parabasal cells and can even be acellular in severe cases of estrogen deficiency (Fig. 18-5). Quantitation of parabasal cell numbers can be used as an assessment of genital atrophy and evaluation of hormonal therapy.

DESQUAMATION INFLAMMATORY DISEASE

Desquamative inflammatory vaginitis is a sterile vaginitis. It can be found in women of any age and is displayed as virginal discomfort, irritation, increased vaginal discharge, and painful sexual intercourse. Vaginal secretions demonstrate an abnormal pH of 7.4 and show a high number of white blood cells (WBCs) and an increased number of parabasal cells (Fig. 18-6). Gram stains show an absence of Lactobacilli; and bacterial, viral, and fungal cultures are negative.

Desquamative inflammatory vaginitis must be differentiated from mucosal blistering disorders and all infectious forms of vaginitis.

BACTERIAL VAGINOSIS

Bacterial vaginosis is the most common vaginal infection in women. In bacterial vaginosis, the vaginal flora is altered. Normally, Lactobacillus predominates in the healthy vaginal flora (Fig. 18-7). In vaginosis, other bacteria such as *Gardnerella*

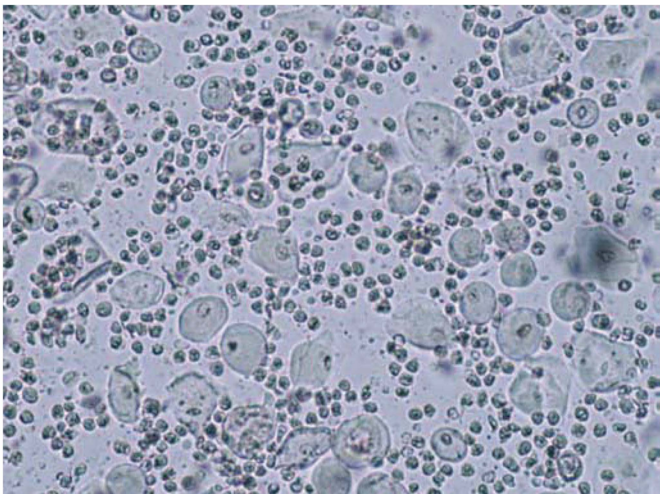


Figure 18-6. Purulent vaginal secretion containing increased numbers of white blood cells, parabasal cells and absent lactobacilli are common findings in desquamative inflammatory vaginitis as well as other forms of vaginitis. (Wet mount)

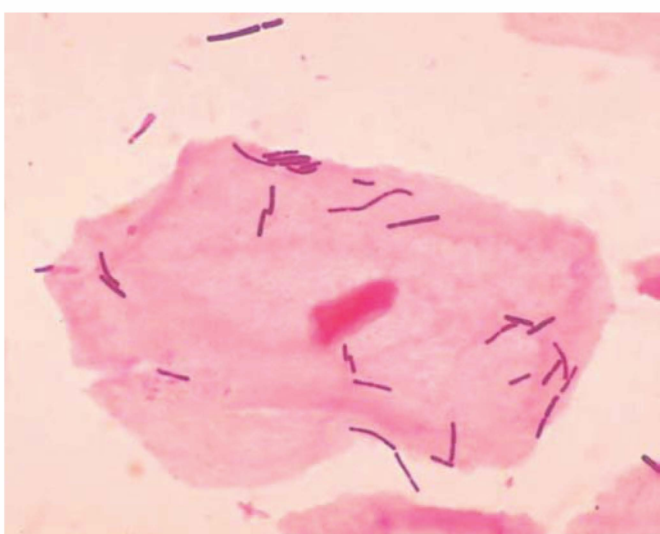


Figure 18-7. Gram stain (1,000×). Lactobacillus predominating in a healthy vagina with squamous epithelial cells. (CDC, PHIL image, http://phil.cdc.gov/phil image 10g0029_lores.jpg.).

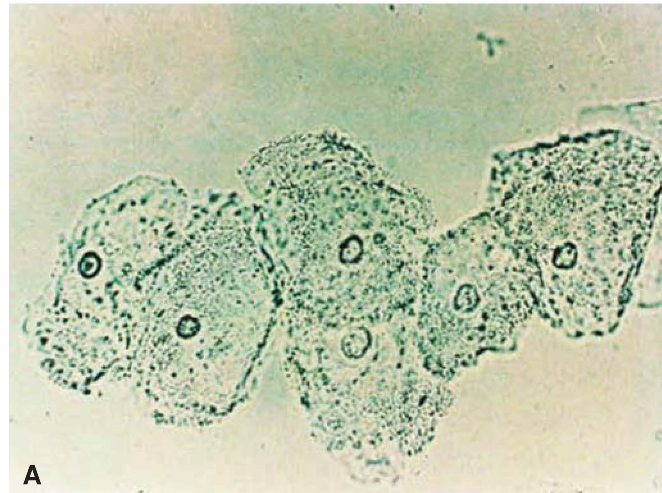
vaginalis, or Mobiluncus species, or the anaerobic Prevotella species predominate.

The overgrowth of other anaerobic bacteria is also associated with bacterial vaginosis. Studies of women with vaginosis have shown a correlation of bacterial vaginosis with an increased risk for premature birth and low–birth-weight infants.

In bacterial vaginosis, the vaginal discharge is gray or off-white and thin, with characteristics of a transudate. There is a characteristic lack of WBCs as there is no invasion of the subepithelial tissue, but there is an increase in exfoliation of epithelial cells. To diagnose bacterial vaginosis, three of the following characteristics should be seen: (a) "**clue cells**," sloughed off squamous epithelial cells covered with numerous small thin, curved gram-variable bacilli, (b) a vaginal pH greater than 4.5, (c) a positive **amine or "whiff" tests** (detection of a characteristic odor upon exposure to KOH), and (d) a malodorous, homogeneous vaginal discharge. Of these tests, the most reliable indicator of bacterial vaginosis is the characteristic microscopic appearance of "clue cells," together with an altered microbial flora, with a reduction in the typical long, thin Lactobacillus, and an overgrowth of the small, thin, curved gram-variable bacilli of species such as Gardnerella, Mobiluncus, and Prevotella (Fig. 18-8).

TRICHOMONIASIS

Trichomoniasis is a common parasitic infection of the vaginal mucosa in females and of the urogenital tract of males that is cause by *Trichomonas vaginalis*. Women usually complain of yellow-green vaginal discharge, although women can be asymptomatic and men are usually asymptomatic. In pregnant women, *Trichomoniasis* is a risk factor for preterm rupture of membranes and preterm labor and delivery. The



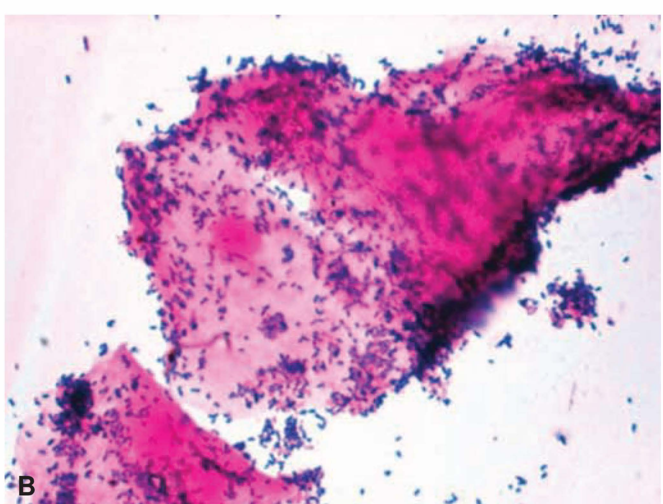


Figure 18-8. **A:** Wet mount and **(B)** Gram stain of "clue cells." These are squamous epithelial cells that are literally covered with numerous small curved bacilli. These cells slough off because of bacterial alteration in bacterial vaginosis. (From Sweet RL, Gibbs RS. *Atlas of Infectious Diseases of the Female Genital Tract*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005, Asset 55832 c10f10.)

wet mount (microscopic examination of a drop of freshly collected fluid) is helpful to detect the majority of cases of *Trichomoniasis*. Culture and/or DNA probe for *Trichomonas* are useful when the wet mount is negative and trichomoniasis is strongly suspected. In *Trichomonas* infection, the bacterial flora is also altered and the pH is abnormally elevated to 5.0 or 6.0. The amine or "whiff" tests may also be positive with *Trichomonas* due to the altered bacterial flora and vaginal pH. WBCs are also frequently seen in the wet preparation of trichomoniasis. See Figure 18-9 for the characteristic appearance of these organisms.

CANDIDIASIS

Candida albicans causes the majority of cases of vulvovaginal **candidiasis**, a common vaginal fungal infection in women. Again, this infection occurs when there is an alteration in the

Figure 18-9. Trophozoites of *Trichomonas vaginalis* obtained from in vitro culture, stained with Giemsa. (CDC, DpDx Laboratory Diagnosis of Parasites of Public Health Concern Parasite Image Library, http://www.dpd.cdc.gov/dpdx/HTML/Image_Library.htm.).

normal bacterial flora and the normal vaginal environment. While *C. albicans* can be found normally in the vagina, it is generally in small numbers but greatly overgrows in candidiasis. This is frequently caused by antibiotic treatment and can occur in celibate as well as sexually active women. It is also more common in immunosuppressed patients. Women with candidiasis frequently complain of a whitish, curd-like vaginal discharge. Microscopic examination reveals an increased number of yeast cells and pseudohyphae with a concomitant increase in WBCs (Fig. 18-10).

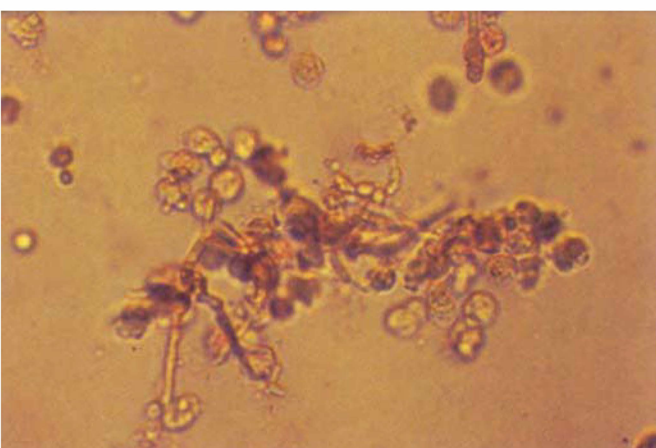


Figure 18-10. Wet preparation of *Candida albicans* yeast and pseudohyphae with WBCs. Yeast (including pseudohyphae), RBCs, and WBCs (200×). (From McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998.)

OTHER DISORDERS

Other tests that are performed for the diagnosis of female genital disorders, such as microbial cultures and molecular testing for *Chlamydia trachomatis, Neisseria gonorrhoeae,* and herpes, and cytology for cervical cancer, are beyond the scope of this chapter. These tests usually require cervical swabs (rather than vaginal secretions) be collected that are appropriate for the methods being performed in the laboratory.

LABORATORY EXAMINATION OF VAGINAL SECRETIONS

Physical Characteristics

APPEARANCE. Normal vaginal fluid appears white and may have a flocculent discharge containing a few WBCs. If the patient is menstruating, the discharge will be reddish due to the presence of red blood cells. Abnormal appearance of vaginal secretions includes a thin, white/gray or gray discharge that is associated with bacterial vaginosis. A "cottage cheese" appearance to the vaginal discharge is seen in *Candida* infections. Infection with *Trichomonas* may produce a yellow-green frothy discharge; and *Chlamydia* infections present with yellow opaque discharge.

pH. Normally, vaginal secretions have a pH of 3.8 to 4.5, due to the growth of Lactobacillus species and its acidic byproducts. With the alterations of bacterial flora that occur in bacterial vaginosis and infection with Trichomonas, the pH rises and Lactobacillus numbers decrease. The vaginal pH also rises in postmenopausal women due to decreased Lactobacillus species that can occur with atrophic vaginosis. In candidiasis, the pH is largely unchanged, between 3.8 and 4.5.

Microscopic Examination

WET MOUNT. The saline wet mount is used to examine vaginal secretions for the presence of "clue cells," microorganisms, leukocytes, and the proportions of epithelial cells and immature epithelial cells (parabasal cells). A preparation is made of vaginal secretions with a drop of isotonic saline and a coverslip and the slide is examined under low power and high dry power.

A saline wet mount of vaginal secretions can also be used to find *T. vaginalis* trophozoite parasites. Trichomonad trophozoite forms are associated with vaginal infections and are motile flagellate protozoans in the saline wet preparation. These organisms are also sometimes seen in urinalysis wet preparations. Trichomonads are readily identifiable by their characteristic jerky movement due to both their five flagella and their undulating membrane. When these cells die, they ball up and become difficult to distinguish from WBCs, so it is important to process and read all specimens for wet mount examination immediately, within half an hour to avoid missing these organisms. The yeast and pseudohyphae of candidiasis

1. Which of the following materials is known to be toxic to specific microorganisms? (*choose all that apply*)
   a. Calcium alginate
   b. Cotton
   c. Dacron
   d. Wood
2. The decidual cells of placenta produce this substance found primarily in amniotic fluid. (*choose all that apply*)
   a. Placental alpha microglobulin-1
   b. Insulin-like growth factor binding protein-1
   c. Fetal fibronectin
   d. Murine monoclonal anti-fFN antibody
3. Which substance functions as an adhesive between the chorion and decidua? (*choose all that apply*)
   a. Placental alpha microglobulin-1
   b. Insulin-like growth factor binding protein-1
   c. Fetal fibronectin
   d. Murine monoclonal anti-fFN antibody
4. The fern test is performed by:
   a. Fluorescence
   b. Immunochromatography
   c. Latex agglutination
   d. Microscopic examination
5. This cell is an abnormal finding indicating bacterial vaginosis:
   a. "Clue cells"
   b. Parabasal cells
   c. Red blood cells
   d. White blood cells
6. In bacterial vaginosis, in trichomoniasis, and in post-menopausal women, the vaginal pH is:
   a. Above 4.5
   b. Between 3.8 and 4.5
   c. Below 3.8
   d. It is above 4.5 in some of these and below 3.8 in others
7. Organisms associated with premature birth include: (*select all that apply*)
   a. Gardnerella
   b. Lactobacillus
   c. Mobiluncus species
   d. Prevotella
8. A predominance of these cells in vaginal secretion is indicative of a postmenopausal vaginitis:
   a. "Clue cells"
   b. Parabasal cells
   c. Red blood cells
   d. Squamous epithelial cells
9. "Clue cells" are indicative of all of these EXCEPT:
   a. *Gardnerella vaginalis*
   b. Lactobacillus species
   c. Mobiluncus species
   d. Prevotella species
10. All of the following tests are used to detect bacterial vaginosis EXCEPT:
    a. Fern test
    b. Vaginal secretions pH
    c. Wet prep for "Clue cells"
    d. "Whiff test"

CASE STUDY

Case 18-1 A 25-year-old pregnant female arrived at her hospital's Labor and Delivery because she feels she will deliver soon. She claims to be 30 weeks into her pregnancy and is having "odd" sensations triggered by recent intercourse. The L&D staff performed a digital examination and then collected a vaginal swab and send it to the laboratory for a fetal fibronectin test. The test demonstrates a positive result; however, there was no event of preterm delivery within the expected 7- to 10-day period. Delivery occurred normally at 34 weeks.

1. What may have caused the false-positive results obtained?
2. What other tests can be used to screen for ruptured of fetal membranes?

Case 18-2 A 20-year-old pregnant female presented to the clinic complaining of yellow green vaginal discharge. She stated that her partner was asymptomatic. Vaginal secretions were collected for wet preparation and cervical secretions were collected for molecular probes for gonorrhea and Chlamydia. The wet preparation showed moderate white blood cells and motile flagellates with jerky movement. The patient was treated with metronidazole.

1. What infection does this patient have?

2. What risks does this infection pose to the developing fetus, if any?

3. What techniques are used to detect this organism?

4. What are the expected vaginal pH and amine test results in this case and why is the pH altered?

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Miscellaneous Urine and Body Fluid Tests

19
Chapter

KEY TERMS

Acute Interstitial Nephritis (AIN)
Bronchoalveolar Lavage (BAL)
Bronchial Washings
Beta-Human Chorionic Gonadotropin Hormone (ß-hCG)
Calcofluor White
Middle Ear Effusion
Otitis Media with Effusion
Pregnancy Testing
Sterile Pyuria
Urine Eosinophils
Vitreous Fluid

LEARNING OBJECTIVES

1. Explain what is detected in a pregnancy test and what may affect test results.
2. Explain the importance of testing for urine eosinophils.
3. Describe the collection of bronchial washings and the bronchoalveolar lavage.
4. Describe normal and abnormal findings for tests performed on bronchial specimens.
5. Describe methods for detection and identification of various microorganisms found in bronchial specimens from patients with various conditions.
6. Describe testing of middle ear effusions.
7. Describe the procedure for collection of vitreous fluid.
8. Suggest testing that may be performed on vitreous fluid.
9. Suggest reasons for which other body fluids may be tested.

The clinical laboratory has a role in providing clinicians with critical test results from a variety of body fluids. This chapter covers miscellaneous urine testing and body fluids that have not been covered previously, or that are currently tested less frequently, but that yield crucial evidence of the patient's status. Laboratory medicine is constantly expanding its capabilities and thus, continually adding new tests and new types of specimens.

Additional Urine Tests

URINE PREGNANCY

Pregnancy testing may be performed on urine or on blood. The substance tested in pregnancy is **beta-human chorionic gonadotropin hormone (ß-hCG)**, a hormone that is secreted in urine within 2 to 3 days after implantation of the embryo (or approximately 8 to 10 days after fertilization). Levels of this hormone rise rapidly after conception and remain elevated in pregnancy, peaking in the first trimester of pregnancy. Some tests performed on serum can detect pregnancy much earlier, within days of conception. One reason that serum is able to detect pregnancy earlier is that the levels of the hormone ß-hCG vary a great deal due to the concentration of the urine, yet the levels are relatively stable in serum. Still, collecting a urine specimen is easier and urine pregnancy test kits are available over the counter. The best specimen for urine pregnancy testing is the first morning urine, which is the most concentrated specimen. For optimal results, urine-specific gravity should be 1.015 or higher. False results may occur with large amounts of blood, protein, or bacterial contamination. Enzyme immunoassays are the most popular type of test kit (Fig. 19-1), but whatever the method, follow the manufacturer's guideline. Results are reported as ß-hCG negative or ß-hCG positive. Test kits may show a positive result in a urine sample in as little as 10 days after conception.

URINE EOSINOPHILS

Eosinophils (Fig. 19-2) are seen in the urine of patients with **acute interstitial nephritis (AIN)**, which is usually drug-induced acute renal failure with **sterile pyuria** (increased urine leukocytes but no bacteria present). Because effective treatment is achieved by discontinuing the drug, the finding of **urine eosinophils** is important. Refer to Chapter 2 for additional information about acute interstitial nephritis.

Urine eosinophils may also be seen in other acute genitourinary tract disorders such as cystitis, glomerulonephritis, pyelonephritis, kidney transplant rejection, *Schistosoma haematobium* infection, and prostatitis. A sample of fresh centrifuged urine is concentrated further using a cytocentrifuge. The entire sediment, along with a drop of albumin, is used to prepare two cytocentrifuge slides. These are air dried then stained with Wright stain. The entire area must be examined for the presence of eosinophils.

Bronchoalveolar Lavage and Bronchial Washings

Bronchoalveolar lavage (BAL) and **bronchial washings** are body fluids that are generally collected to assess the cellular composition and to detect infectious agents present in the lower respiratory tract. These procedures and the analysis of specimens collected may vary by laboratory.

Figure 19-1. ICON pregnancy test cartridge. (Image courtesy of Beckman Coulter, Inc.)

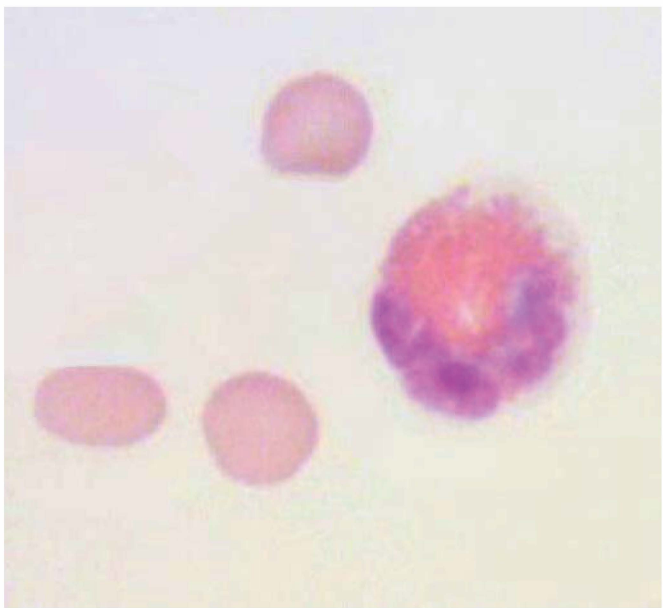


Figure 19-2. Eosinophils in urine. Wright stain of cytocentrifuge preparation (400×).

DISEASE CORRELATIONS

BAL is particularly helpful in the diagnosis of fungi such as *Pneumocystis jiroveci* or *Aspergillus* species or other fungi in the alveolar cellular layer of immunocompromised patients. Immunocompromised hosts are susceptible to many organisms that normally do not cause infection as well as being susceptible to the generally recognized pathogens of the lower respiratory tract.

BAL has been found to be more sensitive for detecting infectious organisms such as *Pneumocystis carinii* or *Aspergillus* species than traditional biopsy procedures. In addition, bronchial washings are less likely to contain material from the alveoli, where *Pneumocystis* exudate is found, and are therefore less effective in recovering the organism, compared with BAL.

SPECIMEN COLLECTION

These specimens are obtained in surgery. A lighted optical instrument, the **bronchoscope**, is used to examine the tracheobronchial tree and can help detect obstructions, pneumonia, carcinoma, hemoptysis, foreign bodies, or abscesses. These instruments can be equipped with suction catheters, brushes, or biopsy attachments for specimen collection. The bronchoscope consists of fiber optics (flexible tube using specialized glass fibers) that form a bidirectional light system through which light enters the interior of the bronchi and returns a magnified image.

The bronchoscope is advanced into a bronchial segment until it occludes the lumen. For washings, 20 to 60 mL of saline are infused and then recollected by aspiration. Bronchial washings obtain material from the more proximal areas of the bronchoalveolar tree. The BAL is used at more distal sites to retrieve cellular alveolar material, which is more representative of the alveoli (Fig. 19-3).

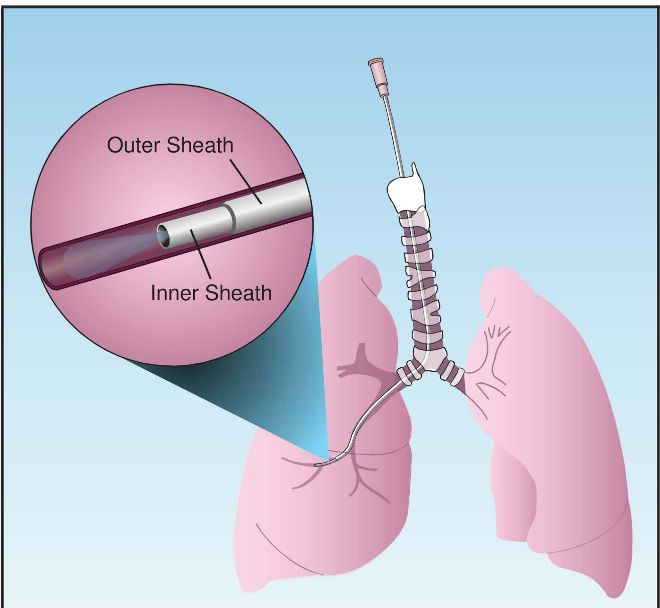


Figure 19-3. This bronchoalveolar lavage is being performed with a protected catheter, but without the aid of a bronchoscope. (From Marino PL. Marino's the ICU Book. Philadelphia, PA: Wolters Kluwer, 2013.)

LABORATORY EXAMINATION

BAL specimens are obtained for routine bacterial, fungal, and mycobacterial examination and culture, and for cytological studies. In the laboratory, the fluid volume of each specimen is measured and analyses conducted.

Microscopic Evaluation

CELL COUNTS AND SPECIAL STAINS. Cell counts are performed using a hemocytometer; and cytocentrifugation gives best cellular preparations for staining for cellular differentiation. Although Wright stain could be used on these specimens, cytological stains (Gomori methenamine silver [GMS] and Papanicolaou [PAP]) and microbiological stains (calcofluor white) are more commonly used on these specimens.

Cells seen in bronchial washings and BAL include ciliated columnar epithelial cells (Fig. 19-4) and squamous epithelial cells. Cytological screening is performed to determine the presence of squamous cell carcinoma (Fig. 19-5) and adenocarcinoma (Fig. 19-6). Alveolar macrophages are present and may contain carbon particles in patients who smoke or lipid globules if the patient has aspirated milk. Leukocytes may also be present in bronchial washings. Lymphocytes are rarely present in normal bronchial washings, but are increased in interstitial lung disease, drug reactions, pulmonary lymphoma, and nonbacterial infections. Neutrophils, usually present in low numbers are increased in people who smoke, have inhaled toxins, or have diffuse alveolar damage. Eosinophils are rarely present.

A variety of microorganisms, bacteria, fungi, and mycobacteria, can be found in these samples in lower respiratory tract infections, as well as cytomegalovirus (see Figs. 19-7 and 19-8) and cysts of *P. jiroveci*, (see Figs. 19-9 and 19-10).

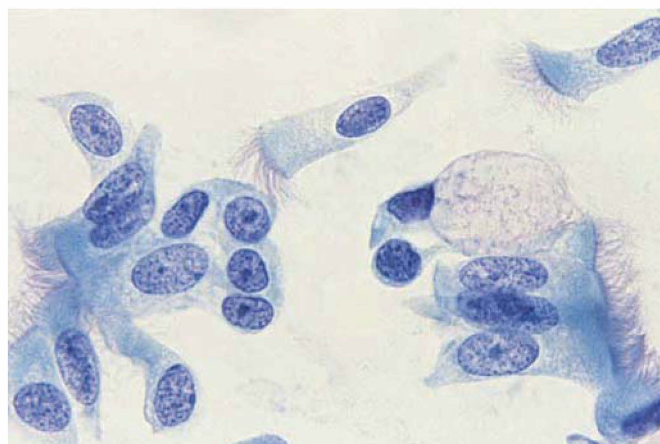


Figure 19-4. Normal bronchial epithelial cells in a bronchial brush specimen are shown. Note the ciliated columnar cells with uniform and basally located nuclei. The chromatin is finely granular and evenly dispersed, the nuclear membrane is smooth and regular, and the nucleoli are inconspicuous. (Papanicolaou stain) (From Rubin E MD, Farber JL MD. Pathology, 3rd Edition. Philadelphia: Lippincott Williams & Wilkins, 1999.)

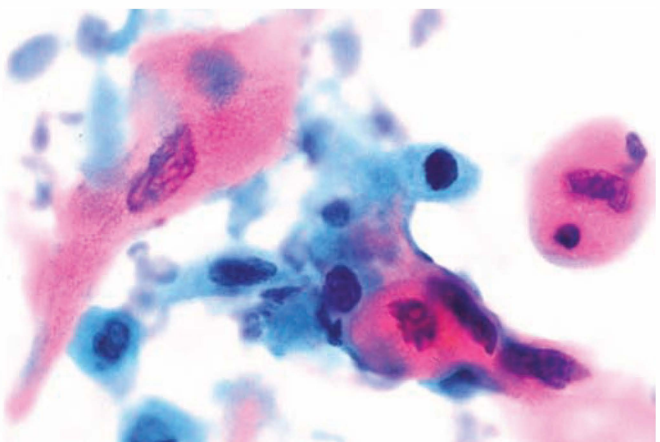


Figure 19-5. Squamous cell carcinoma in a bronchial brush specimen. Highly atypical squamous cells show marked variation in size and shape. The nuclei are hyperchromatic and irregular. (Papanicolaou stain) (From Rubin R, Strayer DS. *Rubin's Pathology: Clinicopathologic Foundations of Medicine.* 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2008.)

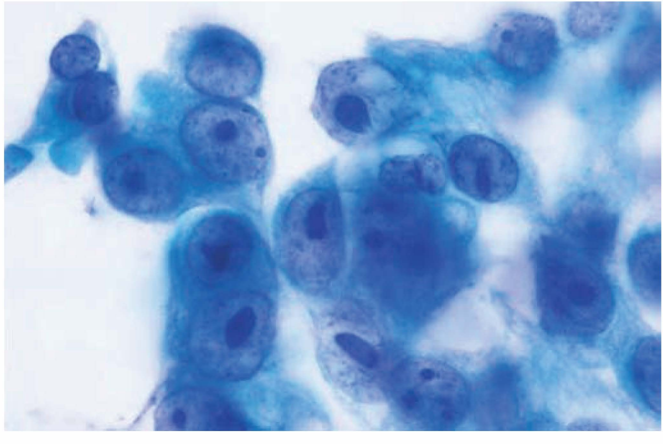


Figure 19-6. Adenocarcinoma cells in bronchial brush specimen. A cluster of epithelial cells with highly atypical nuclei, prominent nucleoli, and cytoplasmic vacuoles is seen. (Papanicolaou stain) (From Rubin R, Strayer DS. *Rubin's Pathology: Clinicopathologic Foundations of Medicine.* 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2008.)

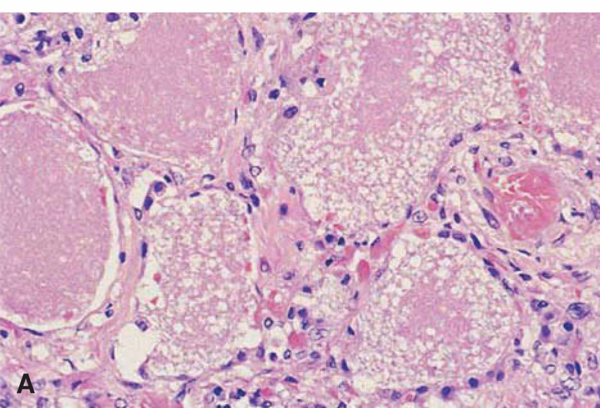
WET MOUNTS AND CALCOFLUOR WHITE STAIN. Wet mounts are useful to detect fungal elements and cells that may be present in these samples. Stains can be used along with wet mounts or stains can be used on smears. A technique that is particularly helpful to detect *P. jiroveci, Candida albicans,* and other fungi is the **calcofluor white** wet preparation. The calcofluor white stain is a fluorescent stain that has increased sensitivity in the detection of these organisms and detection of fungi. It can be combined with KOH to dissolve cells in order to see fungal structures more easily (Fig. 19-11).

Ear Fluid

The middle ear lies between the tympanic membrane and the internal ear (Fig. 19-12). **Middle ear effusion (MME)** (excess fluid in the tympanic cavity) is often seen during chronic **otitis media with effusion (OME).** The effusions that are present in the ear display a complex composition that includes secreted mucus glycoproteins, protein, lipid, and many inflammatory substances. OME can be caused by anatomical factors, obstruction of the eustachian tubes, ear infections, allergic reactions, and impaired immunologic status. Some researchers suggest that gastroesophageal reflux may also contribute to OME. Middle ear effusion specimens are collected using a suction cannula and can be tested for the presence of high levels of pepsin and pepsinogen to establish this correlation.

Vitreous Fluid

Vitreous fluid (fluid of the vitreous body) is collected by vitrectomy during which a balanced salt solution is infused at the same time fluid is being aspirated (Fig. 19-13). Vitreous specimens may be required from patients with diabetic retinopathy to assess production of specific proteins as well as the presence of T-lymphocytes by flow cytometry. Cytological studies and flow cytometry performed on vitreous fluid can help establish the presence of malignant infiltrates. Patients with retinal detachment may have higher levels of certain



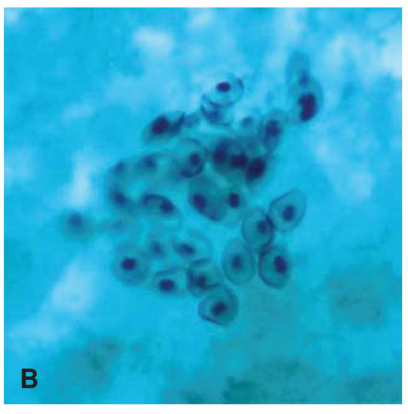


Figure 19-7. *Pneumocystis jiroveci* pneumonia. **A:** The alveoli are filled with a foamy exudate, and the interstitium is thickened and contains a chronic inflammatory infiltrate (Hematoxylin & Eosin stain). **B:** A centrifuged bronchoalveolar lavage specimen impregnated with silver shows a cluster of Pneumocystis cysts. (From Rubin E MD, Reisner H. *Essentials of Rubin's Pathology.* 6th ed. Philadelphia: Wolters Kluwer, 2013.)

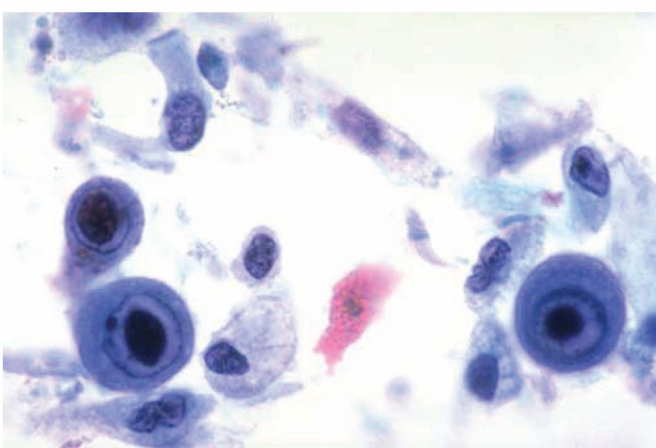


Figure 19-8. Cytomegalovirus (CMV) infection in bronchial washings. Note the large basophilic nuclear inclusions surrounded by a halo and marginated chromatin, forming the typical target-shaped appearance. (Papanicolaou stain) (From Rubin E MD, Farber JL. *Pathology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 1999.)

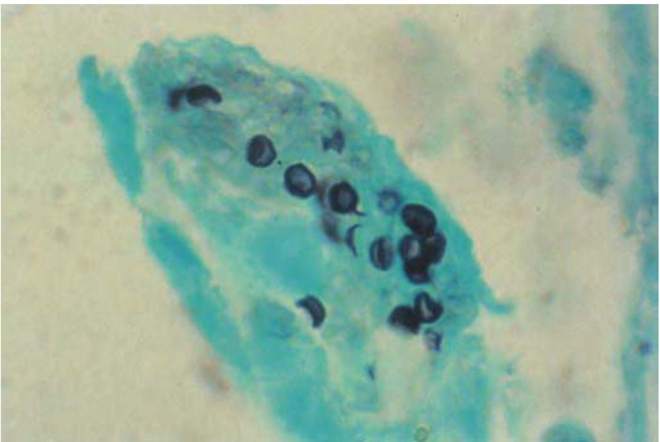


Figure 19-9. Cell block preparation of BAL showing cysts of *P. jiroveci,* GMS-P stain (1,000×). (From McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott; 1998:264.)

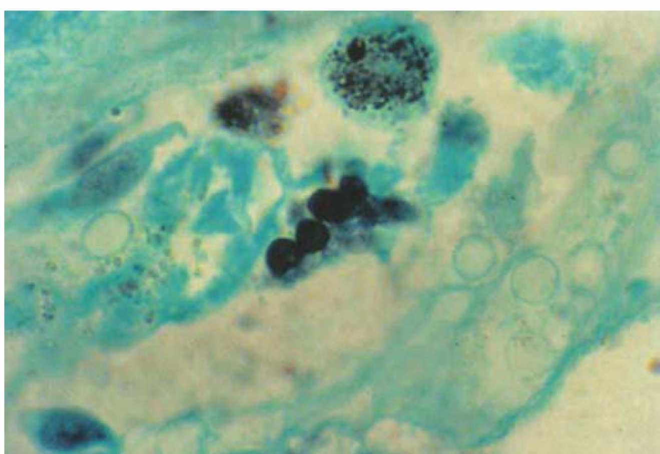


Figure 19-10. Cell block preparation of BAL showing cysts of *P. jiroveci,* GMS-P stain (1,000×). (From McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach.* Philadelphia, PA: Lippincott; 1998:265.)

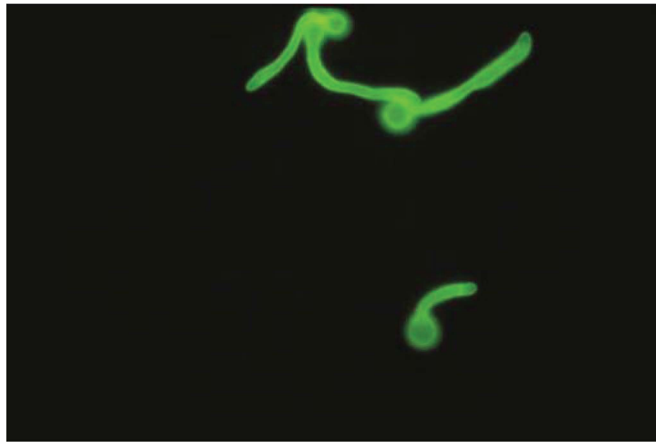


Figure 19-11. *Candida albicans* with germ tube development in a calcofluor white preparation. (CDC, PHIL image, http://phil.cdc.gov/phil image 295.)

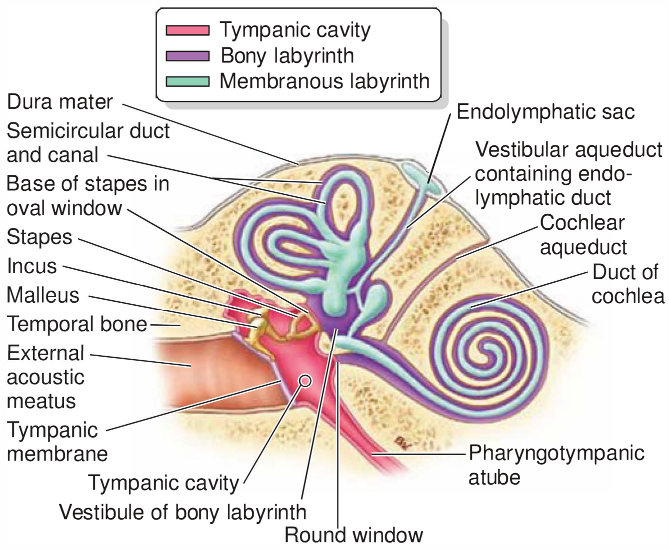


Figure 19-12. Schematic of the middle ear. (From Moore KL. *Clinically Oriented Anatomy*. Philadelphia, PA: Wolters Kluwer, 2013.)

amino acids in their vitreous fluid than persons with normal retinal attachment.

Other Fluids

Other body fluids are collected and analyzed for the presence of abnormalities. Saliva is often used to screen for drugs of abuse but can also be used to test for hepatitis. Fluid collected from cysts can be used to determine the cause of cyst formation such as infection or malignancy. As with more routinely collected fluids, cytology, cell counts, chemistry tests, flow cytometry, and microbial cultures can be performed on most body fluids.

1. Which statements regarding pregnancy testing are true? (*select all that apply*)
   a. Dilute urine is the preferred specimen.
   b. Serum tests are more reliable than urine tests.
   c. Beta-human chorionic gonadotropin hormone is increased during early stages of pregnancy.
   d. For urine pregnancy tests, the specific gravity should be less than 1.015.

2. Urine eosinophils may be present in urine during which of these disorders? (*select all that apply*)
   a. Cystitis
   b. Glomerulonephritis
   c. Pyelonephritis
   d. Prostatitis

3. Which of these specimens is best for the detection of *Pneumocystis jiroveci*?
   a. Bronchoalveolar lavage
   b. Vaginal secretions
   c. Bronchial washings
   d. Amniotic fluid

4. Normal bronchial epithelial cells:
   a. are ciliated columnar cells with uniform and basally located nuclei.
   b. have basophilic nuclear inclusions surrounded by a halo and marginated chromatin.
   c. show marked variation in size and shape and nuclei are hyperchromatic and irregular.

5. The effusions that are present in the ear comprise secreted substances that include all of the following EXCEPT:
   a. Enzymes
   b. Mucus
   c. Lipid
   d. Protein

6. Otitis media with effusion can be caused by any of these conditions. (*select all that apply*)
   a. Abnormalities in ear anatomy
   b. Gastroesophageal reflux
   c. Infections of the ear
   d. Obstruction in the eustachian tubes

7. A vitrectomy is used to collect fluid from the:
   a. Alveoli
   b. Ear
   c. Eye
   d. Nasopharynx

8. Test that can be performed on any body fluid include: (*select all that apply*)
   a. Cell counts
   b. Chemistries
   c. Cultures
   d. Cytology

CASE STUDY

Case 19-1 A 32-year-old man with acquired immunodeficiency syndrome developed fever, chills, shock, blood clots, and punctuate skin lesions during hospitalization. A bronchoscopy with bronchoalveolar lavage (BAL) and a skin biopsy were performed and were sent to the laboratory for bacterial and fungal smears and culture and for histological stains. The BAL and the skin biopsy showed the same organisms, shown in Figs. 19-11 and 19-12.

1. What organisms might be expected in a BAL sample from an immunocompromised patient?
2. What structures are seen in Figs. 19-14 and 19-15?
3. What group of organisms does this belong to?

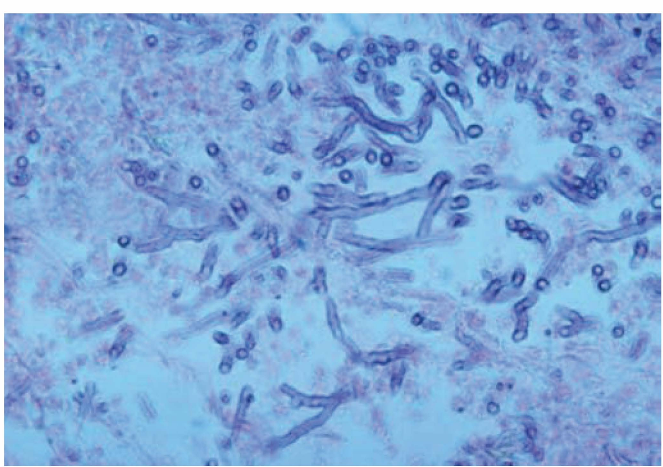


Figure 19-14. This image depicts histopathologic changes indicating aspergillosis of the lung caused by *Aspergillus fumigatus*. Methenamine silver stain reveals hyphae of *A. fumigatus*. Inhalation of airborne conidia of *A. fumigatus* can cause aspergillosis in immunosuppressed hosts. (CDC, PHIL image, http://phil.cdc.gov/phil image 3952.)

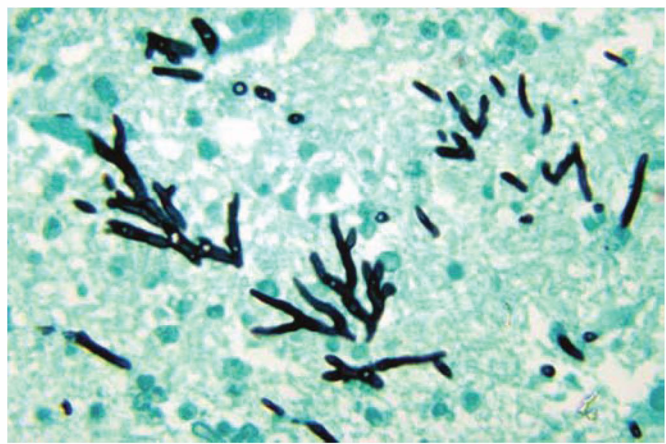


Figure 19-15. Methenamine silver stain. Disseminated infection in an immune-compromised host with the opportunistic fungus, *A. fumigatus*. Note the characteristic dichotomous branching of the hyphae. (CDC, PHIL image, http://phil.cdc.gov/phil image 4228.)

Case 19-2 A 51-year-old man was complaining of a 30-lb weight loss in the past month, cough, fever, shortness of breath, abdominal pain, chest pain, decreased appetite, and feeling weak and faint when ambulatory. X-rays showed pulmonary lesions. He had been traveling in Africa, so his physician ordered tests for malaria, which were negative. A slightly elevated white blood cell count was noted. The patient underwent a bronchoscopy with bronchoalveolar lavage and bronchial washings. A calcofluor white with KOH was performed and bacterial, fungal, and mycobacterial cultures were ordered as well as histologic examination using special stains. The calcofluor white preparation showed small encapsulated intracellular yeast. Figures 19-16 to 19-18 are from this patient.

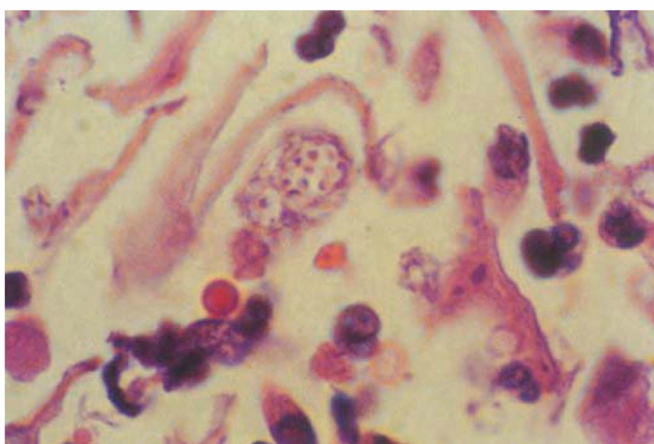


Figure 19-16. Bronchial washing cell block. H&E stain (1,000×). (From McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998:263.)

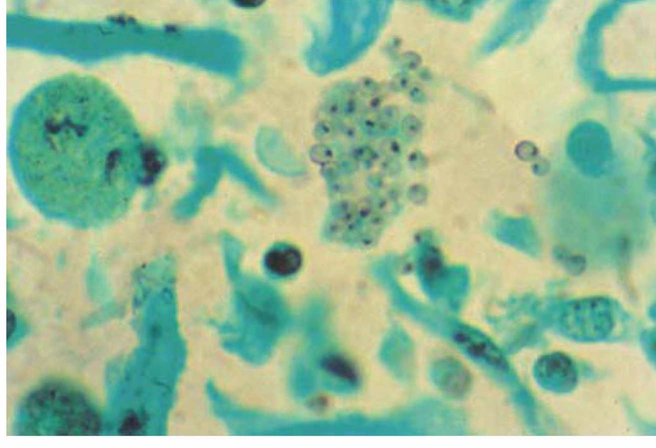


Figure 19-17. Bronchial washing cell block. GMS-C stain (1,000×). (From McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998:263.)

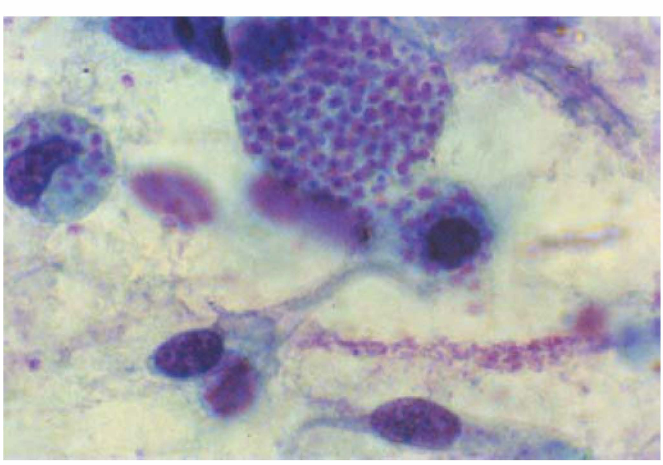


Figure 19-18. Bronchial washing cell block. PAP stain (1,000×). (From McBride LJ. *Textbook of Urinalysis and Body Fluids: A Clinical Approach*. Philadelphia, PA: Lippincott; 1998:263.)

1. What types of conditions can be detected using bronchoscopy with bronchial washings and BAL collections?

2. What group of organisms is causing this patient's condition—bacteria, fungi, or mycobacteria?

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Automation of Urinalysis and Body Fluids Examination

Chapter 20

KEY TERMS

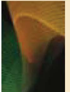
Automated Urine Sediment Analyzers
Barcode-Labeled Specimen
Completely Automated Urine Analyzers
Semiautomatic Strip Readers

LEARNING OBJECTIVES

1. State the rationales for using automated systems for urinalysis and body fluids examination.
2. List and describe the available automated urinalysis systems.
3. List and describe the available automated body fluid analysis systems.

Time-saving equipment and equipment that can accommodate large numbers of samples have been developed for every area of the clinical laboratory. Automating laboratory procedures allow for better standardization of test performance and reduce not only the turnaround time but also transcription errors. In addition, many automated instruments interface with laboratory information systems, providing for quick entry of data into patients' electronic medical records.

The use of automation in the urine and body fluids analysis helps reduce technologist's interpretation variability. Automated equipment for performing urine and body fluid analysis takes the form of semiautomated or automated. Nearly each manufacturer of reagent strips has developed its own instrument. Some manufacturers have also developed automated systems for performing microscopic analysis on urine and/or body fluids. This chapter contains a brief explanation of the basic principles of some of these instruments.



Rationale for Automating Urinalysis and Body Fluids

Significant sediment findings may be missed if laboratory protocols direct laboratory personnel to skip microscopic evaluation when negative reagent strips findings are obtained. Crystals, renal tubular epithelial cells, parasites, and yeast do not have chemical indicators present on reagent strips currently in use. These findings also do not always have other abnormalities present that would lead to the performance of a microscopic evaluation. In addition, interfering substances still do play a role in occasionally masking the presence of red and white blood cells.

Automation of the microscopic portion of the urinalysis not only helps detect unexpected sediment but also helps standardize the identification and enumeration of urinary sediment. Eliminating inaccuracies in manual timing of reactions and visual subjectivity of reagent pad color interpretation helps make urinalysis more reliable and less dependent on the technologist. With automation, not much time is needed to perform a complete urinalysis than a dipstick screening only. Some laboratories do not perform a microscopic examination when dipstick findings are normal. This policy may be helpful in managing workflow in understaffed laboratories, but some significant microscopic findings may be missed.

Automated Urinalysis Systems

Several brands of urinalysis automation are currently available. The current choices available include strip readers, **semiautomatic strip readers**, **automated urine sediment analyzers**, and **completely automated urine analyzers** with both chemical and sediment analysis capabilities. Table 20-1 lists some of these urinalysis instruments currently available.

Table 20-1 Automated Urinalysis Instruments

Waived Urine Chemistry Instruments
Roche Diagnostics Criterion II
Siemens Medical Solutions Diagnostics CLINITEK 50
Siemens Medical Solutions Diagnostics CLINITEK 101
Siemens Medical Solutions Diagnostics CLINITEK Status

Semiautomated Urine Chemistry Instruments
Dirui Urine Analyzer H-50, 100, 200
Iris iChem 100
Roche Diagnostics URISYS 1800
Roche Diagnostics Chemstrip Criterion II
Siemens Medical Solutions Diagnostics CLINITEK 200

Fully Automated Urine Chemistry Instruments
Iris iChem Velocity
Roche Diagnostics URISYS 2400
Siemens Medical Solutions Diagnostics CLINITEK Atlas

Automated Sediment Analysis
Arkry AX-4030
Iris iQ200ELITE
Iris iQ200SELECT
Iris iQ200SPRINT
Sysmex UF-1000i Urine Cell Analyzer

Totally Automated Urinalysis Systems
Arkray AU-4050
Iris iQ200 Automated Urinalysis System
iRICELL3000 (iChem Velocity plus iQ200SPRINT)
Siemens Medical Solutions Diagnostics ADVIA Urinalysis WorkCell System (CLINITEK Atlas plus the Sysmex UF-100)

Semiautomated instruments require manual dipping of the reagent strip into the urine followed by placement on the instrument. Identification of the specimen is keyed in before sampling of the specimen. Instruments that fully automate reagent strip reading use a **barcode-labeled specimen**. Although sampling is automated, tubes must still be decapped prior to placement on these instruments. Automated urine sediment analyzers use similar bar code identification and specimen handling requirements. Reagent strip readers and sediment analyzers can be used in tandem for a fully automated urinalysis.

IRIS INTERNATIONAL, INC.

IRIS International, Inc. manufactures the iQ, iChem, Velocity, and iRICELL series of instruments that provide semiautomation of urine chemistry and fully automated reagent strip reading and urine sediment analysis. The Iris instruments can be used independently or in pairs for a complete urinalysis. In September 2012, IRIS International became a wholly owned subsidiary of Beckman Coulter, Inc.

Figure 20-1. Iris Automated Urinalysis iRICELL workcell. Product Images © 2009 IRIS International, Inc. All rights reserved. IRIS, the Iris logo, iChem, iQ, iRICELL, and Velocity are trademarks of IRIS International, Inc. and are registered in the USPTO.

Figure 20-3. Iris Automated Urinalysis iRICELL workcell with iQ200SELECT. Product Images © 2009 IRIS International, Inc. All rights reserved. IRIS, the Iris logo, iChem, iQ, iRICELL, and Velocity are trademarks of IRIS International, Inc. and are registered in the USPTO.

The Velocity reads the specimen's barcode, aspirates the sample, and dispenses urine onto each pad of the reagent strip. Color assessment of each reaction uses the same principle of reflectance as described previously. Timing remains consistent from sample to sample. The Velocity is capable of assessing the color of a specimen by using wavelengths of light to obtain the tone and hue of a urine specimen. Light scatter is used to determine the turbidity of the specimen. Specific gravity is measured by assessing refractive index of LED-emitted light as it passes through the specimen. The Velocity uses dual wavelength reflectance to measure the pH and chemical constituents of urine.

The iQ200 series reads the specimen's barcode, aspirates the sample, and performs urine sediment identification. The identification is done by enveloping a lamina of the sample with a suspension fluid that moves past the objective lens of the microscope. A digital camera, illuminated by a strobe lamp, captures 500 frames per sample. The Auto-Particle Recognition software uses size, shape, contrast, and texture to classify images. Digital images are reviewed by a technologist and correlated to chemical and physical findings before reporting. Electronic archiving of results allows results to be reviewed by multiple users for confirmation of results, quality control, or used in training sessions.

The combination of Velocity and an iQ200 provides a fully automated urinalysis workcell, the iRICELL (Fig. 20-1). The iRICELL is available in three speeds: at 101 samples per hour (Fig. 20-2), at 70 samples per hour (Fig. 20-3), and at 40 samples per hour (Fig. 20-4). Figure 20-5 shows a close-up of the tube carrier and barcode reader on the iQ.

SIEMENS MEDICAL SOLUTIONS DIAGNOSTICS

Siemens Medical Solutions Diagnostics manufactures the CLINITEK series of urine chemistry instruments. Semiautomated instruments perform reagent strip analysis on a test-by-test basis, while the fully automated instrument is a load and walk away system. The CLINITEK Status (Fig. 20-6) is compact and simple to use yet provides automated Multistix test strip analysis. In addition to automatic reading, interpretation, and documentation of Multistix test strip results, the CLINITEK Advantus Analyzer (Fig. 20-7) provides ease of operation along with special features such as autochecking for strip identification and humidity exposure, and test lockout when QC is needed.

Figure 20-2. Iris Automated Urinalysis iRICELL workcell with iQ200ELITE. Product Images © 2009 IRIS International, Inc. All rights reserved. IRIS, the Iris logo, iChem, iQ, iRICELL, and Velocity are trademarks of IRIS International, Inc. and are registered in the USPTO.

Figure 20-4. Iris Automated Urinalysis iRICELL workcell with iQ200SPRINT. Product Images © 2009 IRIS International, Inc. All rights reserved. IRIS, the Iris logo, iChem, iQ, iRICELL, and Velocity are trademarks of IRIS International, Inc. and are registered in the USPTO.

Figure 20-5. Close-up of the iQ barcode reader and tube carrier.

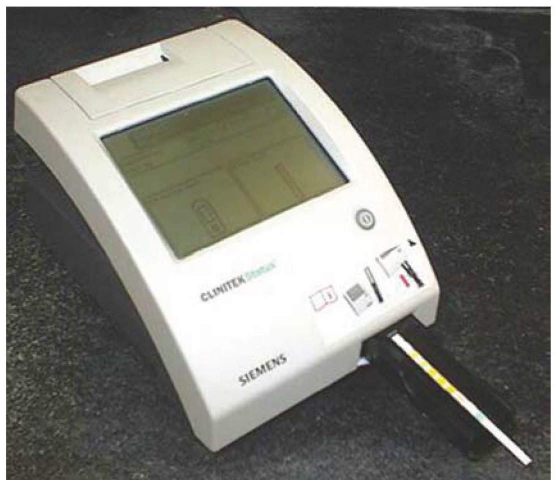


Figure 20-6. CLINITEK® Status. (Used with permission from Siemens Medical Solutions Diagnostics.)

Figure 20-8. CLINITEK Atlas® Automated Urine Chemistry Analyzer with rack. (Courtesy of Siemens Medical Solutions Diagnostics.)

Siemens Medical Solutions Diagnostics' fully automated instrument, the CLINITEK Atlas Automated Urine Chemistry Analyzer is available with rack (Fig. 20-8) or carousel (Fig. 20-9), which provide walkaway convenience. The CLINITEK Advantus is designed for high-volume urine testing, using Siemens' proven technology in dry-pad urine chemistry analysis and continuous reagent roll for simplified loading and reduced interruptions. The CLINITEK Advantus features a built-in liquid level sensing for simplified sample preparation and has a modular design for compatibility with laboratory automation systems. The CLINITEK Advantus may be combined with the Sysmex UF-1000i Urine Cell Analyzer to provide complete automation of both chemical and microscopic aspects of the urinalysis procedure.

Figure 20-7. CLINITEK Advantus® Analyzer. (Courtesy of Siemens Medical Solutions Diagnostics.)

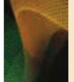
Figure 20-9. CLINITEK Atlas® Automated Urine Chemistry Analyzer with carousel. (Courtesy of Siemens Medical Solutions Diagnostics.)

Figure 20-10. Fully Automated Urine Cell Analyzer UF-1000i. (Image courtesy of Sysmex America, Inc.)

SYSMEX

The Sysmex Fully Automated Urine Cell Analyzer UF-1000i (Fig. 20-10) uses flow cytometry technology to classify and quantitate the cellular elements found in the urine sediment.

After the cells are mixed with fluorescent stains, heated and placed in suspension, they are covered in sheath fluid and ejected through a nozzle into the flowcell. Here each urine cell is illuminated by a focused semiconductor laser beam, causing the individual cells to fluoresce and scatter light. The forward and lateral-scattered light reflects the size and surface characteristics of the cell. The fluorescent light emitted reflects the more detail of the cell surface and highlights the internal structures, and the specific fluorescent antibodies in the different stains reflect the DNA and/or RNA properties of the cell nucleus. The forward scattered light, lateral scattered light, and fluorescent light released by the cells are captured by the detector block, then converted into electrical signals, which are then analyzed for height, amplitude, and intensity. The cells are classified by an algorithm based on these signal waveform patterns, and the cell counts are quantified by adaptive cluster analysis. See Figure 20-11 for an illustration of this process.



Automated Urinalysis Systems Images

Most automated instruments that classify and enumerate urine sediment display digital images for laboratory personnel to evaluate.

Figures 20-12 to 20-23 show digital images, produced by these instruments, of various form of urinary sediment. The classification of sediment can be readjusted as is needed as when sperm are classified as bacteria (Fig. 20-20). Some cells, such as squamous epithelial cells (Fig. 20-22), are identified as unclassified by instruments and do not need reclassification if they are not reported. Artifacts vary in size and may appear at any level of classification. Artifacts can falsely elevate the count and may be reclassified into their own category (Fig. 20-23), which is not reported.

Automated Body Fluid Analysis Systems

Automated cell analysis instruments are also available to do cell counts and WBC differentials on spinal fluid samples. With these instruments, cells are first mixed with reagent fixative and then counted. Differential counting enumerates numbers of neutrophils, lymphocytes, monocytes, and

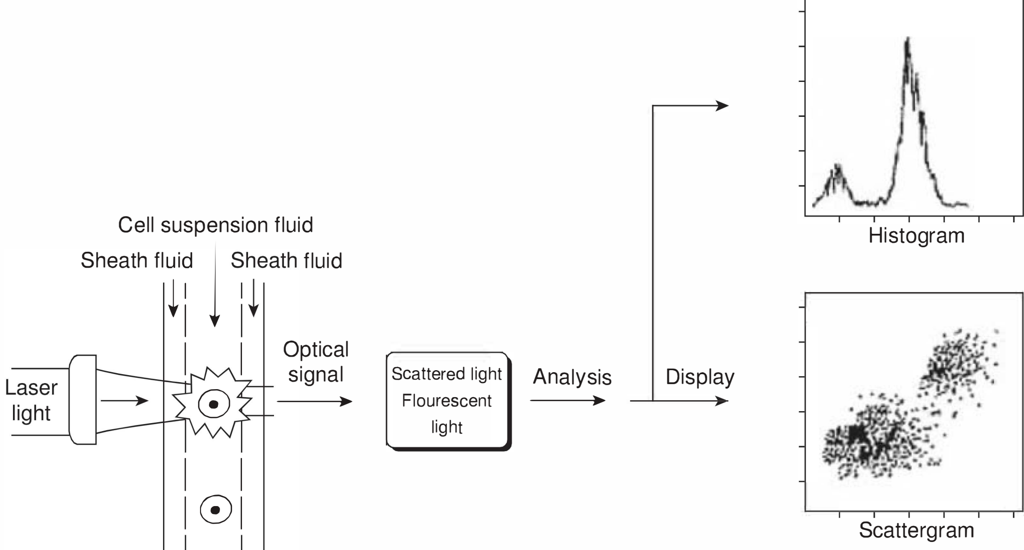


Figure 20-11. Illustration of UF-1000i flow cytometry technology. (Image courtesy of Sysmex America, Inc.)

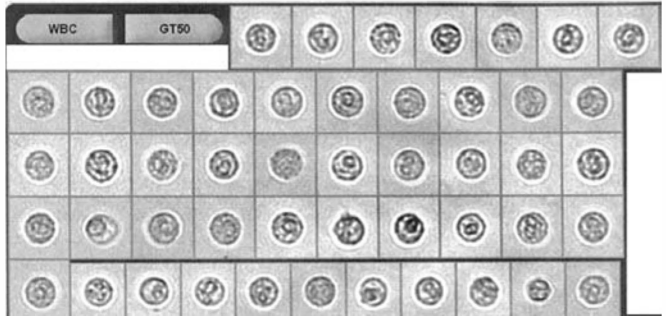


Figure 20-12. Digital images of urine sediment; white blood cells.

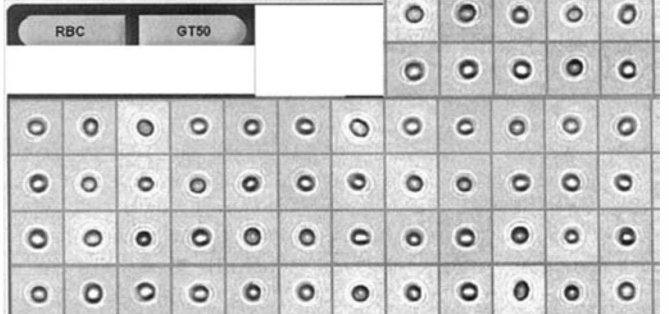


Figure 20-13. Digital images of urine sediment; red blood cells.

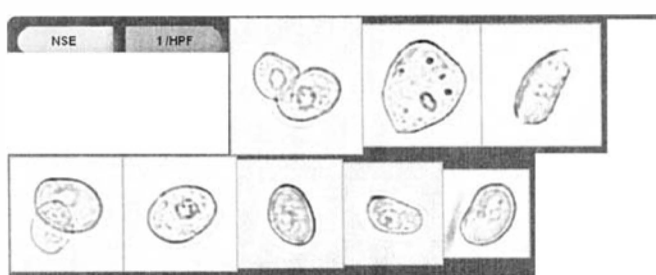


Figure 20-14. Digital images of urine sediment; transitional cells.

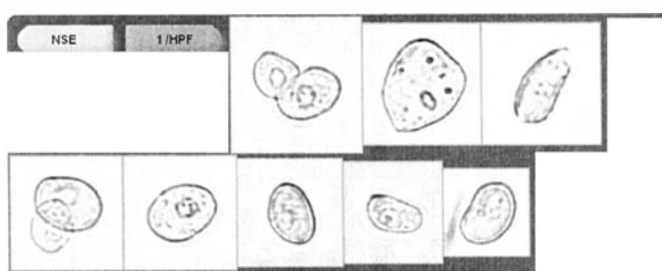


Figure 20-15. Digital images of urine sediment; hyaline casts.

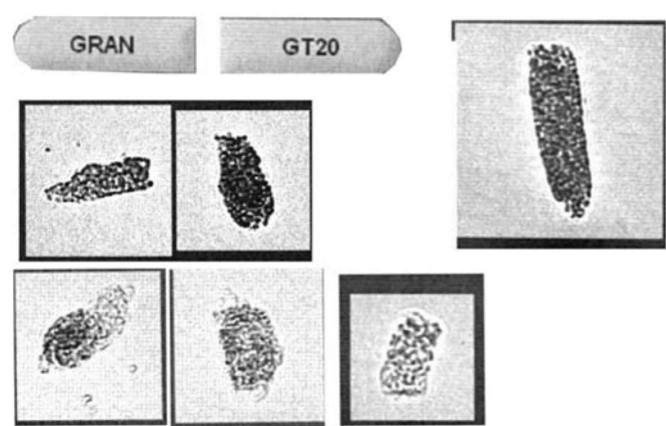


Figure 20-16. Digital images of urine sediment; granular casts.

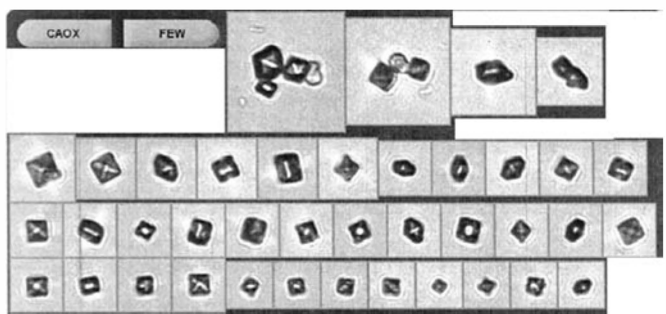


Figure 20-17. Digital images of urine sediment; calcium oxalate crystals.

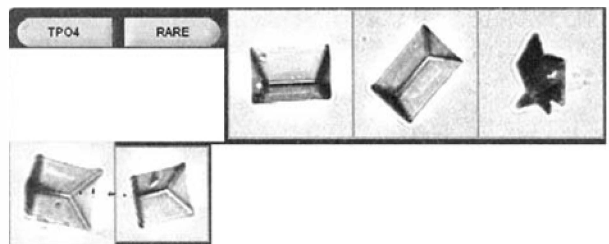


Figure 20-18. Digital images of urine sediment; triple phosphate crystals.

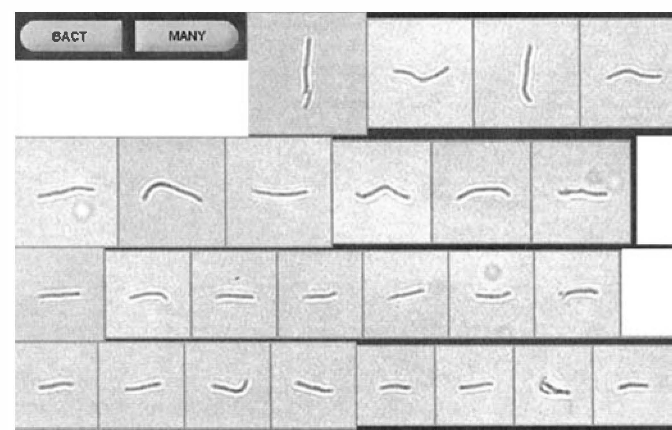


Figure 20-19. Digital images of urine sediment; bacteria.

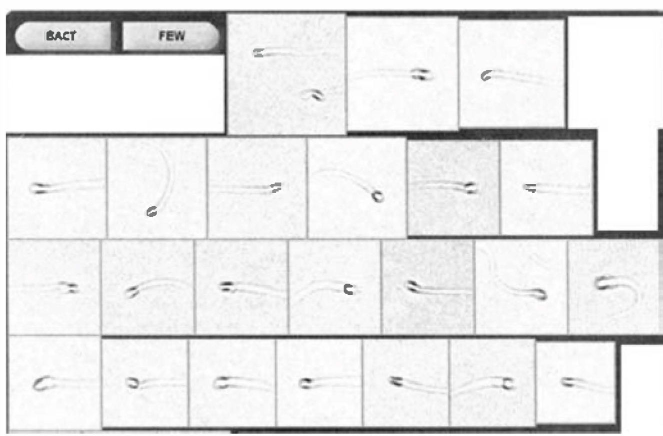


Figure 20-20. Digital images of urine sediment; these sperm were classified by the instrument at bacteria and must be reassigned to the correct category. Not all facilities report sperm in urine sediment.

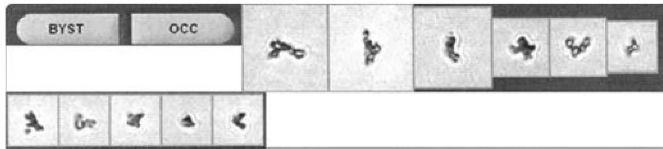


Figure 20-21. Digital images of urine sediment; yeast.

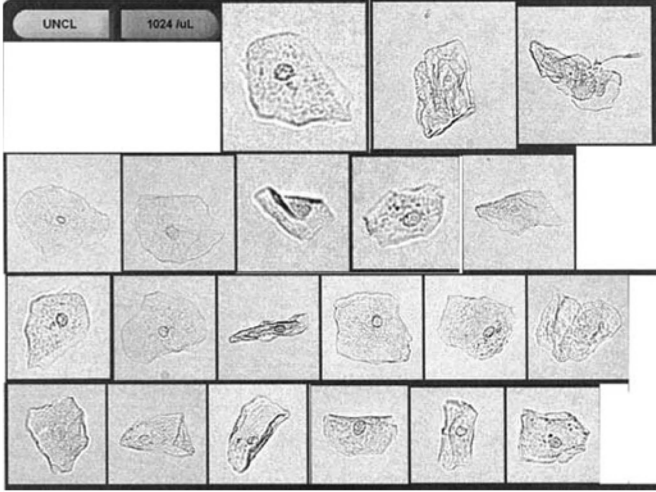


Figure 20-22. Squamous epithelial cells are not usually reported and are identified as "unclassified" urinary sediment.

eosinophils. Automated cell counters use larger numbers of cells, enhancing precision and accuracy. Table 20-2 lists automated systems available for body fluid cell counting.

IRIS INTERNATIONAL, INC.

The Iris iQ Body Fluid Module adapts the iQ200 (Fig. 20-24) for identification and enumeration of cells in most body fluids including cerebrospinal, pleural, peritoneal, peritoneal lavage, peritoneal dialysate, pericardial, general serous fluids, and synovial fluid.

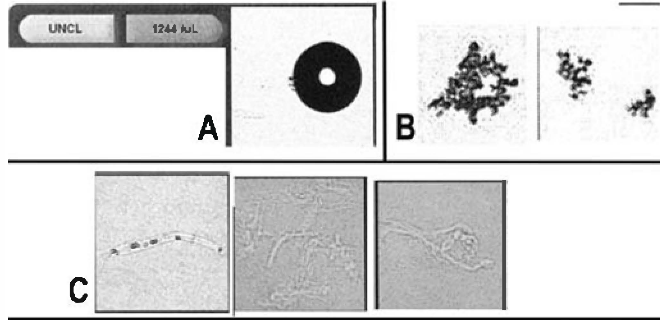


Figure 20-23. Compilation of urine sediment digital images; artifacts. **A:** Air bubble. **B:** Amorphous material/crystals. **C:** Mucous strands may be mistaken for casts, especially if they have debris or cells attached as seen in the one on the left.

SYSMEX

The Sysmex Hematology-XN-100 (Fig. 20-25) includes a body fluid–specific mode. This provides reportable WBC, RBC, and differential (polymorphonuclear and mononuclear) counts for cerebrospinal, synovial, and serous body fluids. The XN-100 analyzer uses fluorescent flow cytometry with hydrodynamic focusing technologies and a state-of-the-art diode laser bench to differentiate cell types in body fluid samples.

Table 20-2 Automated Body Fluid Analyzers

BODY FLUID ANALYZERS	FDA APPROVED FOR USE WITH THESE FLUIDS
Iris iQ Body Fluids Module	Cerebrospinal Cerebrospinal Peritoneal Peritoneal lavage Peritoneal dialysate Pleural Serous fluids in general Synovial
Siemens Medical Solutions Diagnostic ADVIA 120 and 2120	Cerebrospina
Sysmex XE-5000	Cerebrospinal
Automated Hematology System	Serous body fluids Synovial fluid
Medical Electronic Systems	Semen

Figure 20-24. Iris iQ Body Fluid Module. Product Images © 2009 IRIS International, Inc. All rights reserved.

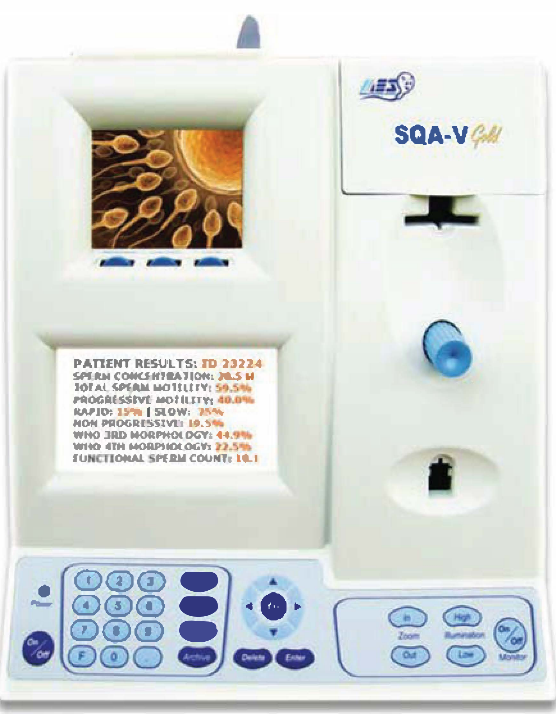


Figure 20-26. SQA-V *Gold* Sperm analyzer. (Image courtesy of Medical Electronic Systems.)

AUTOMATION OF SEMEN ANALYSIS

Medical Electronic Systems manufactures instruments that automate semen analysis. The SQA-V *Gold* (Fig. 20-26) has a two-channel measurement system that interacts with a specially designed testing capillary that contains the semen sample. Each channel consists of a light source, detector, and processor that interpret signals based on instructions from proprietary algorithms. Simply put, one channel "measures" light absorption and refraction in sperm cells and translates this into concentration. Another channel "counts" light interruptions (signals) caused by sperm cells moving across the field of light. In approximately 1 minute, thousands of signals are "read" resulting in exceptional accuracy and precision. Automating the motility analysis eliminates reader subjectivity and variance among technologists. In addition to sperm count and motility, the SQA-V *Gold* analysis includes sperm morphology and 13 other parameters. Acceptable specimens include fresh, washed, frozen, and post-vasectomy semen samples. The QwikCheck *Gold* (Fig. 20-27) is designed for smaller laboratories and is used primarily for fertility screening and sperm banking quality control. The SQAIIC-P (Fig. 20-28) is designed for small, remote laboratories and assesses sperm concentration, motility, and morphology.

Figure 20-25. The Sysmex Hematology-XN-100 includes a body fluid mode. (Image courtesy of Sysmex America, Inc.)

AUTOMATION OF URINE PREGNANCY

Special instruments have been developed that automate the interpretation of enzyme immunoassay tests. Once the test is set up and inserted into the reader, laboratory personnel can focus on other duties without worry of missing the read time. Quantitative human chorionic gonadotropin (HCG) is one such test that is interpreted by the VEDALAB Easy Reader. Immunochromatographic rapid test cards are read by the

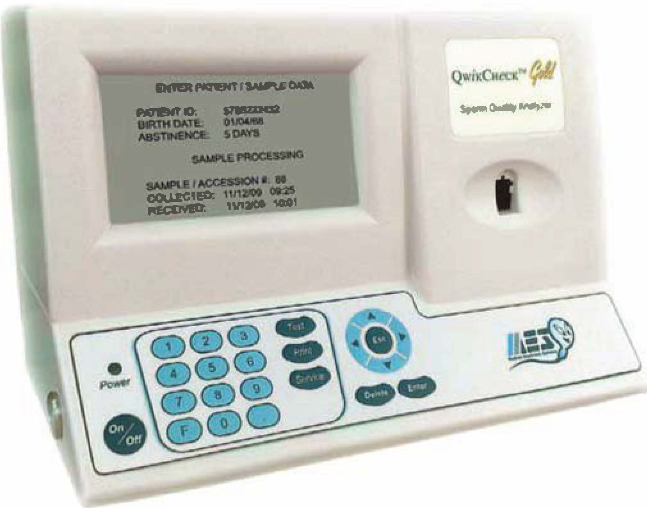


Figure 20-27. QwikCheck *Gold* Sperm analyzer. (Image courtesy of Medical Electronic Systems.) (Not currently available for the U.S. market.)

meter using a high-resolution CCD camera. Integrated software analyzes the images and records the results.

Siemens Medical Solutions Diagnostics offers a simple and more reliable method for performing qualitative urine pregnancy tests. The CLINITEST hCG test (Fig. 20-29) is designed for use with the CLINITEK Status family of analyzers providing fast, easy instrument-read-and-reported test results.

AUTOMATION OF FECAL OCCULT BLOOD

Most of the automated methods for the detection of blood in feces use an immunological test principle. One such instrument, the OC-Auto 80 by Polymedco (Fig. 20-30) uses a latex agglutination reaction with an optical measurement of the reaction.

Specimens for immunochemical fecal occult blood test (iFOBT or FIT) need to be transferred by the patient, a nurse, or the laboratory staff to special vials used for this test (Fig. 20-31) and labeled with appropriate patient identification.

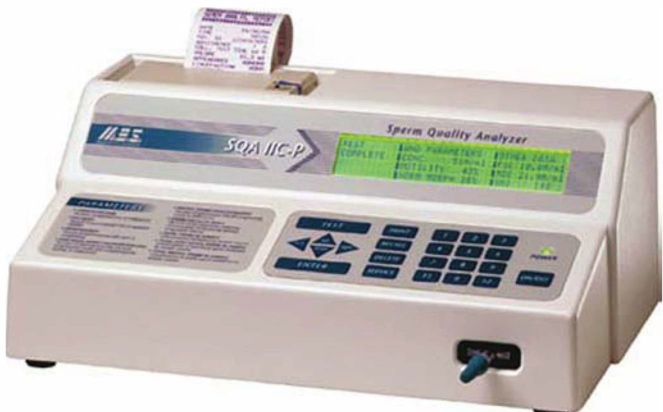


Figure 20-28. SQAIIC-P Sperm analyzer. (Image courtesy of Medical Electronic Systems.) (Not currently available for the U.S. market.)

Figure 20-29. CLINITEST hCG test for use with the CLINITEK Status family of analyzers. (Courtesy of Siemens Medical Solutions Diagnostics.)

Figure 20-30. Polymedco OC-Auto 80 FOBT analyzer; external view.

Figure 20-31. Polymedco FOBT sample collection vial/bottle.

Figure 20-32. Polymedco OC-Auto 80 FOBT analyzer; internal view.

The vial cap is equipped with an applicator that is "poked" into the specimen and then inserted into the vial.

Once loaded onto the instrument, along with latex reagent and reaction wells, the analysis takes only a few minutes (Fig. 20-32). The latex particles are sensitized with antihuman HbA0 antibodies. If hemoglobin is present in the sample, it reacts with the antihuman HbA0 antibodies thereby agglutinating the latex particles. The OC-Auto 80 detects a change in the optical density of the fluid in the reaction vial and provides a reading of positive. No agglutination produces a negative result.

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Answers to Study Guide Questions and Case Studies

APPENDIX

CHAPTER 1

Study Questions

1. The small blood vessels in the body are injured in diabetic patients due to elevated blood sugar. When the blood vessels in the kidneys are injured, your kidneys cannot clean your blood properly. You will retain more water and salt, which can result in weight gain and ankle swelling. You may have protein in your urine. Also, metabolic waste products will build up in your blood.

   Diabetes also may cause damage to nerves in your body. This can in turn cause difficulty in emptying your bladder. The pressure resulting from your full bladder can back up and injure the kidneys. Also, if urine remains in your bladder for a long time, you can develop an infection from rapid growth of bacteria in urine with an elevated sugar level.

   All of these factors often contribute to diabetic kidney disease and even kidney failure. A patient with kidney failure faces loss of vital kidney functions. One of the things the healthy kidney does is to produce erythropoietin and in a failing kidney this production is decreased, leading to anemia.
2. Label the parts of the kidney.
   a. See Figure 2-5.
3. Label the structures of the nephron that are involved in urine formation and excretion.
   a. See Figure 2-7.
4. Tubular reabsorption is a process for the body to recover needed water, ions, and nutrients from the ultrafiltrate, so they are not lost upon urine excretion. The reabsorbed substances are moved from the ultrafiltrate into the blood of the peritubular capillaries. Reabsorption can be active or passive.

   In tubular secretion, the direction of movement is opposite. Foreign substances, exogenous medicines, and toxins that were not filtered are moved from the blood into the tubular filtrate for excretion. Ions, such as hydrogen ions, are also secreted and this serves a key role in maintaining blood pH. Much of this process is active requiring energy input.
5. See Figure 1-16.
6. List the primary constituents of urine.
   a. The primary components are water, urea, uric acid, creatinine, sodium, potassium, chloride, calcium, magnesium, phosphates, sulfates, and ammonia.

7.	b	**10.**	a	**13.**	c	**16.**	a
8.	b	**11.**	d	**14.**	b		
9.	c	**12.**	c	**15.**	c		

Case Studies

1-1. The syndrome of inappropriate antidiuretic hormone (SIADH).

1-2. Diabetes insipidus.

1-3. SIADH can be a complication of brain injury, pneumonia, tumor growth, and certain medications such as SSRIs for depression.

1-4. When the body needs to conserve water, ADH is excreted and the walls of the distal and collecting tubules are made very permeable to water by ADH, thereby allowing water to be reabsorbed back into the body. If the body has excess water, less ADH is produced, and the walls of the tubules become less permeable, and the volume of excreted urine increases.

2. Most laboratories are now reporting the GFR automatically. The MDRD equation has now largely replaced the Cockcroft–Gault equation in calculations done in the clinical laboratory. These equations for estimating the glomerular filtration rate (GFR) facilitate earlier detection of kidney disease. However, the estimated GFR must be interpreted in the context of the individual patient. The diagnostic criteria and staging of chronic kidney disease must be understood so that it can be recognized and managed at the earliest possible stage. In this way, primary care physicians and nephrologists can better manage the care of their patients.

1-2-1. SFR decreases normally with age although the amount of the decrease can vary due to various conditions such as atherosclerosis.

1-2-2. Both measure glomerular filtration, but while creatinine clearance approximates GFR, it overestimates due to the fact that creatinine is secreted by the proximal tubule as well as filtered by the glomerulus. GFR can be measured from serum creatinine and creatinine excretion or estimated from serum creatinine using estimating equations. Measurement of creatinine clearance requires collection serum and of a timed urine sample, which is inconvenient and frequently inaccurate as well. Creatinine clearance levels are normally slightly higher than the GFR.

1-2-3. No, this patient's GFR is in stage 3A, indicating moderate chronic kidney disease.

CHAPTER 2

Study Questions

1. d
2. b
3. d
4. c
5. c
6. e
7. c
8. b
9. c
10. c

Case Studies

2-1-1. A
2-1-2. B
2-1-3. C
2-2-1. Acute.
2-2-2. Rapidly progressive (crescentic) glomerulonephritis.
2-2-3. Acute poststreptococcal glomerulonephritis, Goodpasture syndrome.
2-2-4. Look at the appearance of glomeruli in biopsy.
2-3-1. Chronic.
2-3-2. Immunoglobulin A nephropathy (Berger Disease).
2-3-3. Any of the chronic glomerulonephritis diseases with an immunological component.
2-3-4. It is not changed from that of the ultrafiltrate, suggesting loss of the ability to concentrate or dilute urine.
2-3-5. Tubular disease is also present.
2-3-6. Broad casts indicate severe disease with the involvement of many nephrons as they are formed in the collecting duct and many nephrons are fed into each collecting duct. Waxy casts are an indication of chronic disease.
2-3-7. From her chronic kidney disease, she has developed tubular dysfunction as well, and cannot reabsorb the glucose.
2-4-1. Chronic.
2-4-2. Nephrotic syndrome.
2-4-3. It is not changed from that of the ultrafiltrate, which if it stays that way despite varying hydration conditions, suggests loss of the ability to concentrate or dilute urine.
2-4-4. Tubular disease is also present.
2-4-5. Chronic, severe kidney disease.
2-4-6. Yes, in nephrotic syndrome the renal tubular epithelial cells fill with lipids and slough off.
2-4-7. Oval fat bodies are cells (thought to be either renal tubular epithelial cells or foam cell macrophages), with birefringent fat droplets inside their cytoplasm. True oval fat bodies show a characteristic "maltese cross" formation when viewed under polarized light. Under low-power magnification, oval fat bodies are typically seen as dark spots depending upon the intensity of the microscopic illumination. This coloration is due to the yellowish brown pigmented fat making droplets. These fat laden cells are usually seen along with lipiduria and heavy proteinuria.
2-4-8. Polarized microscopy.
2-4-9. Tubular damage prevents reabsorption of glucose.
2-5-1. Acute pyelonephritis.
2-5-2. Dipstick: 2+ protein, 1+ blood, 4+ leukocyte esterase, 4+ nitrate (of these, the leukocyte esterase and the nitrate especially correlate with a bacterial UTI). Blood and protein commonly accompany these findings as well.
2-5-3. The past history of urinary tract infections goes along with pyelonephritis.
2-5-4. Yes, vitamin C, a powerful reducing substance, can interfere with many urinalysis dipstick pad results, causing either false-negative or false-positive results. For this reason, the physician may ask that the patient temporarily refrain from taking vitamin C supplements while testing is needed.

CHAPTER 3

Study Questions

1. a
2. c
3. d
4. d
5. b
6. a
7. c
8. c
9. c
10. c
11. b
12. a, c
13. b
14. d
15. b
16. d

Case Studies

3-1-1. Cystinuria.
3-1-2. The patient history fits with kidney stones even though cystine stones are not as common as calcium stones.
3-1-3. The patient cannot reabsorb cystine and other amino acids such as arginine, lysine, and ornithine; and excretes these substances at high levels. This is an autosomal recessive disorder.
3-1-4. Cystinosis.
3-1-5. **a.** Aminoaciduria in cystinuria is limited to arginine, cysteine, lysine, and ornithine; aminoaciduria in cystinosis is generalized.

b. Cystine calculi/stones may be found in cystinuria; but rarely in cystinosis.
c. Cystine crystals are deposited in bone marrow and body tissues in cytinosis but not in cystinuria.

3-1-6. Cyanide nitroprusside, silver nitroprusside.

3-2-1. Acquired porphyria due to lead intoxication.

3-2-2. A common method for separating the individual porphyrin is high performance liquid chromatography which may be performed in the chemistry department. ALA will be increased, porphobilinogen will be normal or slightly increased, coproporphyrins will be increased, and uroporphyrins will be normal or slightly increased.

3-2-3. Persons with cutaneous porphyria demonstrate photosenstivity. This patient's condition is acquired and not an inherited disorder, therefore photosensitivity is not experienced.

3-3-1. Symptoms of failure to thrive, diarrhea, vomiting, jaundice, and possible liver and kidney failure in this infant along with the presence of tyrosine crystals in the urine point to tyrosinemia.

3-3-2. The most common form of tyrosinemia, Type I, is caused by decreased levels of the enzyme fumarylacetoacetate hydrolase needed to break down tyrosine.

3-3-3. Urinary aminoacid studies should indicate that there is a generalized aminoaciduria with a predominance of tyrosine. In addition, tests should be performed to rule out the possibility of these crystals been caused by drug metabolites.

CHAPTER 4

Study Questions

1. d
2. c
3. c
4. b
5. d

6.

Quadrant	Color	Meaning
Upper	Red	Flammability
Left	Blue	Health hazard
Right	Yellow	Reactivity
Bottom	White	Specific hazard

7.

Bar	Color	Meaning
Top	Blue	Health hazard
Second	Red	Flammability
Third	Yellow	Reactivity
Bottom	White	Protective equipment

Case Studies

4-1-1. Compressed gas tanks must be chained against the wall so that they remain upright and secure. Protection caps must be kept on the stored tanks to avoid valve damage as the valve is the weakest area of most cylinders.

4-1-2. Compressed gas tanks must be chained to a transport hand truck during transit and caution must be taken not to knock them over or to damage the valve stem.

4-1-3. Fire, explosion, asphyxiation, and mechanical injury can result from improper handling or storage of compressed gas cylinders. Do not handle these tanks so that they could fall over, resulting in a sudden release of pressure, making the tank like a torpedo as gas is released under pressure. Do not bang on the stem of the tank with a hammer as these activities could also cause a sudden release of gas under pressure or a slow leak of gas. Some of these gases, such as nitrogen, can be asphyxiating gases.

4-2-1. There may be a variety of infectious agents present in a sample sent for urinalysis testing. While the normal forming urine in the body is sterile, the organism can become contaminated after excretion. Also, it is more likely that urine sent for urinalysis testing comes from a patient with kidney disease such as infections. Bacterial, viral, and parasitic organisms may be present ranging from bacteria such as *Escherichia coli* or *Mycobacterium tuberculosis* to cytomegalovirus or a hepatitis virus to a wide variety of other organisms. Care must always be taken to avoid splashes or aerosolization of these specimens.

4-2-2. The technologist should immediately flush and, if appropriate, wash all affected areas, report the incident to a supervisor, and report to employee health for postexposure treatment.

4-2-3. The technologist should have been using PPEs such as a face shield or should have worked behind a specimen shield.

4-2-4. The technologist had an obligation to inform the student of the splash and guide her through the proper safety measures.

CHAPTER 5

Study Questions

1. Ocular (eyepiece)
2. Body tube (eyepiece tube)
3. Objective
4. Stage
5. Aperture diaphragm and sub-stage condenser
6. Field diaphragm
7. Light source

8. A
9. C
10. B
11. E
12. G
13. F
14. D
15. A
16. B
17. B
18. A
19. A
20. A
21. B
22. c
23. b
24. a and d
25. b and c
26. 7
27. 3
28. 8
29. 6
30. 4
31. 9
32. 5
33. 2
34. 1
35. 10

CHAPTER 6

Study Questions

1. E
2. H
3. G
4. A
5. D
6. F
7. B
8. J
9. I
10. C
11. F
12. D
13. B
14. A
15. C
16. E
17. d
18. d
19. c

20. Specimens for molecular testing for STDs.
21. Specimens for urine culture
22. Tests requiring nonpreserved specimens
23. Chemical screening tests for up to 72 hours

Case Studies

6-1-1. The specimen for this young female may have to be obtained by catheterization.

6-2-1. The specimen may have falsely decreased levels of glucose, ketones, bilirubin, urobilinogen, and cellular sediment. Falsely elevated pH, nitrite, and bacteria may be present. Other changes may include a change in urine color, clarity, and formation of crystals.

6-3-1. When transferring specimens from primary container to aliquot tubes you should wear appropriate PPEs, label the aliquot with the same patient identifiers as the primary container, and transfer specimens using sterile technique.

CHAPTER 7

Study Questions

1. Eyepiece
2. Objective lens
3. Liquid prism
4. Lens adjustment
5. Main prism
6. Bubble trap
7. Path of light

8. J
9. B
10. F
11. A
12. G
13. I
14. C
15. H
16. E
17. D
18. F
19. D
20. D
21. A
22. B
23. E
24. C
25. b,c
26. a, b
27. a
28. a

Case Studies

7-1-1. Although yellow is a normal color seen in urine, a cloudy urine indicates the presence of cells, crystals, bacteria, or fats.

7-2-1. Genetic predisposition to the pigment found in beets may have caused the urine to be red and clear.

7-2-2. Porphyrins and free hemoglobin can make the urine appear red and clear.

7-2-3. Cloudy red urine indicates the presence of intact red blood cells, many conditions can contribute red blood cells to urine. The cause for the presence of red blood cells in urine should be investigated by a physician.

7-3-1. A pH of greater than 7.0 usually lowers the reading of specific gravity on reagent strips. 0.005 should be added to the specific gravity result for alkaline urines tested by reagent strip methods.

7-4-1. Some laboratories report this type of finding as greater than 1.035. However, other laboratories require that a definitive value be reported. Therefore, diluting the specimen by combining one part of urine with one part distilled water and repeating the refractometer analysis will yield more accurate results.

For example, if a dilution is made with equal parts of urine and water and the refractometer reading is 1.020 the actual result is 1.040 $(1.000 + (0.020 \times 2))$. If an extremely concentrated urine requires a higher dilution, the calculation should reflect that. For example, if one part of urine is mixed with two parts of water and the refractometer reading is 1.025, the actual result is 1.075 $(1.000 + (0.025 \times 3))$.

CHAPTER 8

Study Questions

1. f
2. g
3. b
4. a
5. i
6. e
7. g
8. c
9. d
10. h
11. h
12. f
13. b
14. b
15. c
16. d
17. d
18. d
19. b
20. a
21. b
22. d
23. c
24. d
25. a, b

Case Studies

8-1-1. Reagent pads should not be discolored immediately upon removal from the container. Discoloration in any of the pads indicates that the reagent strips are either outdated or have not been stored properly. The urobilinogen reagent pad is particularly sensitive to moisture and may have turned brown. All discolored reagent strips should be discarded and not used for diagnostic testing.

8-2-1. A pink color may indicate an interfering substance. An Ictotest should be performed to rule in or rule out the presence of bilirubin. The test is reported as either negative if no purple color is observed or positive if there is a purple color.

8-3-1. Leukocytes may have lysed if the urine is hypotonic. False-positive leukocyte esterase results can occur because of the presence of strong oxidizing agents, contamination of the urine with vaginal discharge, and preservatives such as formalin. Nitrofurantoin contributes a color to urine that may cause misinterpretation of this test. Drugs that contain imipenem, meropenem, and clavulanic acid may cause false-positive leukocyte esterase results.

8-4-1. The oxidase test is specific for glucose and should be reported as negative for glucose. The copper reduction test (Clinitest) is positive when any reducing substance is present. Because this test is not specific, it should be reported as positive for reducing substance.

8-5-1. The test may be repeated if requested by the physician. However, the laboratory can also serve to educate healthcare providers about the causes for false-positive and false-negative results in laboratory testing. A negative nitrite test should never be interpreted as indicating the absence of bacterial infection. For nitrite, false-negative results can occur in urine with a high specific gravity or elevated level of ascorbic acid. In addition, infection may be present even if the nitrite test is negative because:

1. there may be pathogens present in the urine that do not form nitrite;
2. the urine may not have remained in the bladder long enough for the nitrate to be converted to nitrite;
3. there are cases in which the urine does not contain any nitrate, so bacteria may be present but the dipstick will be negative;
4. under certain circumstances, the bacterial enzymes may have reduced nitrate to nitrite and then converted nitrite to nitrogen, which will give a negative nitrite result.

8-6-1. If a urine specimen produces all negative results along with a very low specific gravity, it may not be urine. Testing for urea will verify that the specimen is urine. In addition, a creatinine test can be helpful with this same determination. The reagent strip method is available for the creatinine test.

8-7-1. A positive ascorbate test indicates large amount of vitamin C are present, which can interfere with urine chemical screening tests.

1. false-negative blood by some reagent strips
2. false-negative glucose by reagent strip methods
3. positive reducing substance
4. decreased sensitivity for bilirubin
5. false-negative leukocyte esterase

8-7-2. This patient has a high level of vitamin C intake.

8-7-3. If ascorbic acid is a constant finding in this patient's urine, they can develop oxalate kidney stones.

CHAPTER 9

Study Questions

1.	b	**6.**	d	**11.**	B	**16.**	A
2.	b, c, d, f	**7.**	b	**12.**	B	**17.**	A
3.	b	**8.**	a	**13.**	A	**18.**	A
4.	d	**9.**	a	**14.**	B	**19.**	A
5.	a, b, d, e	**10.**	d	**15.**	A	**20.**	A

21. a. Maltese cross; b. polarized light; c. cholesterol, leucine, starch

22. a. red blood cells; b. hematuria; c. hemoglobin

Case Studies

9-1-1. Tyrosine.

9-1-2. Needle form or uric acid crystals.

9-1-3. Solubility properties.

9-1-4. The presence of bilirubin and urobilinogen indicate the possibility of liver dysfunction as do tyrosine crystals.

9-1-5. Liver.

9-2-1. Hyaline casts and amorphous urates.

9-2-2. The presence of casts is usually accompanied by positive protein findings. Urate crystals can be seen in acidic urine. Trace blood and leukocyte esterase correlates with small numbers of RBCs and WBCs present in the sediment.

9-2-3. Although bacteria are present, nitrite is negative. This may be because bacteria are not nitrate reducers, the urine was not in the bladder long enough for the conversion of nitrate to nitrite, or the patient's diet did not contain nitrates.

9-3-1. Calcium carbonate (notice the dumbbell shape).

9-3-2. Carbonate crystals require an alkaline environment and are of no clinical significance. The color and turbidity correlate with the presence of RBCs, because blood contributes a brown color to urine as hemoglobin oxidizes.

9-3-3. The presence of blood is not unusual in patients who have undergone urological procedures such as cystoscopy.

9-4-1. Oval fat bodies.

9-4-2. No reagent strip tests detect the presence of fats in urine.

9-4-3. Renal tubule necrosis or any other condition resulting in renal tubule cell damage.

9-5-1. Air bubble and hair strand.

9-5-2. Inexperienced persons may identify these structures as a cast and an oval fat body.

CHAPTER 10

This chapter does not have study questions or case studies.

CHAPTER 11

Study Questions

1. A
2. B
3. B
4. A
5. d
6. a
7. C
8. A
9. B
10. D
11. D
12. C
13. E
14. A
15. B
16. F
17. a
18. c
19. C
20. A
21. B
22. D

Case Studies

11-1-1. RBCs = [190 × 1 × 10]/9 = 211 mm^3
Nucleated cells = [840 × 1 × 10]/9 = 933 mm^3

11-1-2. A few drops of 22% albumin can be added to the cytocentrifuge cuvette along with the specimen prior to centrifugation.

CHAPTER 12

Study Questions

1. c
2. d
3. a, c, and d
4. c
5. b and c
6. c
7. b
8. c
9. d
10. c
11. b and c
12. a
13. c
14. d
15. d
16. d
17. c
18. d
19. b
20. d

Case Studies

12-1-1. WBCs = 956 mm^3; RBCs = 67 mm^3

12-1-2. 90% neutrophils, 10% lymphocytes

12-1-3. Cocci-shaped bacteria.

12-1-4. Determining genus and species of bacteria cannot be made on Wright stain. A Gram stain is needed for the initial identification of gram positive or negative, followed by culture and sensitivity.

12-2-1. The presence of xanthochromia in each of the three specimen tubes rules out a traumatic tap. A traumatic tap would show pink to red coloration in the first tube collected with decreasing intensity of color in subsequent tubes. In addition, xanthochromia indicates that hemoglobin has had time to oxidize. Therefore, the hemorrhage is probably not fresh. The hemorrhage, and perhaps the fall, occurred approximately 1 to 2 days back as indicated by the presence of erythrophagocytosis on the Wright stain smear. Furthermore, red blood cells are still present in the specimen and there are no siderophages seen, suggesting that the hemorrhage did not occur any longer than a few days ago. See Table 12-2.

12-2-2. Testing for the presence of D-dimers can help distinguish between CNS hemorrhage and traumatic tap. If D-dimers are not present, then the appearance of RBCs in the CSF is probably due to a traumatic tap.

12-3-1. The tubes of CSF in Figure 12-28 show decreasing amounts of red color. This disappearance of color as CSF collection progress is suggestive of a traumatic tap. The nucleated red blood cells in Figure 12-29 confirms the occurrence of traumatic tap with nicking of a vertebrae.

12-4-1. The presence of tau transferrin band on protein electrophoresis of the fluid from the ear confirms that the fluid is primarily from the CNS.

CHAPTER 13

Study Questions

1. B
2. A
3. B
4. B
5. A
6. A
7. A
8. B
9. c
10. a
11. b
12. c
13. a
14. c
15. a, b, c, d

Case Studies

13-1-1. Peritoneum or abdomen.

13-1-2. Exudate based on cell count.

13-1-3. Escherichia coli.

13-1-4. Alkaline phosphatase.

13-2-1. Cholesterol crystals.

13-2-2. A milky appearance occurs when many WBCs are present. However, in this case the WBC count is low. Another cause for milky pleural fluid is cholesterol. The crystalline form of cholesterol contributes to the shimmery appearance of the fluid as the crystals reflect light when floating in the specimen.

13-2-3. Tests such as color & clarity, cell counts, and glucose can be used to differentiate between transudates and exudates.

CHAPTER 14

Study Questions

1. b
2. c
3. a
4. c
5. c
6. d
7. A
8. E
9. F
10. C
11. D
12. F
13. C
14. D
15. A
16. B
17. D
18. C
19. A
20. B

Case Studies

14-1-1. Red and cloudy.

14-1-2. Mixture of cells from peripheral blood: RBCs, neutrophils, lymphocytes.

14-1-3. Group V: hemorrhagic.

14-1-4. Hemorrhagic effusion due to trauma.

14-2-1. White and cloudy.

14-2-2. Monosodium urate.

14-2-3. Group IV: crystal induced.

14-2-4. Gout.

CHAPTER 15

Study Questions

1.	c	**5.**	a	**9.**	d	**13.**	a
2.	c	**6.**	b	**10.**	b	**14.**	b
3.	b	**7.**	d	**11.**	b	**15.**	d
4.	d	**8.**	c	**12.**	b		

Case Studies

15-1-1. The physician is concerned that the baby might have diarrhea associated with inflammatory necrotizing enterocolitis. Necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal disease in preterm neonates and will likely soon overtake respiratory disease as the leading cause of death overall in these patients. This enterocolitis is diagnosed in between 0.9 and 2.4 per 1,000 births, and the increase in survival rates of premature infants have led to an overall increase in the incidence of this disease. NEC is both an acute and a chronic disorder, characterized initially by intestinal inflammation which may progress to intestinal necrosis. Intestinal perforation is a great concern in this condition. In its most serious states, NEC may lead to severe acute infection and overwhelming multisystem organ failure and death from systemic sepsis or chronic infection and failure to thrive. This condition is more common in low-weight premies and it is thought to be helped by breast feeding rather than bottle feeding. Another concern the physician might have is that the child may have a congenital disaccharidase deficiency.

15-1-2. Infant fecal samples typically have a pH between 7 and 8. The 5.0 pH is definitely decreased because of bacterial overgrowth with acid byproducts from the bacterial metabolism of the increased carbohydrates.

15-2-1. The bleeding into the toilet may indicate hemorrhoids, which when bleeding cause a false-positive FOBT.

15-2-2. Other conditions that can cause a false-positive FOBT include gastrointestinal infection and inflammation, ulcers, and even bleeding gums.

15-2-3. False-positive FOBT can result from ingesting red meat, fish, bananas, cantaloupe, pars, plums, turnips, horseradish, broccoli, cauliflower, or aspirin. False-negative FOBT can occur with ingestion of vitamin C.

CHAPTER 16

Study Questions

1. A private, comfortable room should be provided for specimen collection that allows for quick delivery of the specimen to the laboratory. The preferred method of semen collection is by masturbation.
2. Specimen collection containers should be clean glass or plastic and have a wide opening.
3. Specimens should not be collected in a condom as these often contain spermicidal compounds and lubricants that may interfere with laboratory tests. If the specimen must be transported from a site distant to the laboratory, it must be kept near body temperature and extremes in temperature must be avoided.

4.	c	**8.**	b, c, d	**12.**	d	**16.**	b
5.	d	**9.**	c	**13.**	b	**17.**	a, b, c
6.	a	**10.**	c	**14.**	d		
7.	d	**11.**	c	**15.**	b		

18.
- **A.** double tail
- **B.** double head
- **C.** normal
- **D.** coiled tail
- **E.** flat head
- **F.** constriction
- **G.** bent neck
- **H.** excessive membrane
- **I.** pinhead

Case Studies

16-1-1. Liquefaction, viscosity, and concentration.

16-1-2. Congenital absence of seminal vesicles and vas deferens.

16-1-3. Fructose. Absent.

16-2-1. Liquefaction normally occurs in less than 60 minutes

16-2-2. Sperm that are motile are viable. Sperm that are nonmotile may also be motile. Viable sperm may or may not be motile. The percent motile sperm cannot be higher than the percent of viable sperm, but the percent of viable sperm can be higher than the percent of motile sperm.

16-2-3. A count of 150 million sperm per milliliter is consistent with a fertile semen specimen. The motility is abnormally low which may indicate infertility. However, because the sample was collected in a condom, these results are NOT valid. The condom may have contained a spermicidal compound that will alter the motility and viability results. The semen analysis should be repeated on a sample that is collected without the use of a condom and delivered to the laboratory in a timely fashion.

16-3-1. Volume and concentration.

16-3-2. Incomplete collection may be responsible for the low volume and the decreased count. The highest concentration of sperm is usually found in the first part of the ejaculate. The sperm count could be low if the patient was sexually active within 3 days of this specimen collection.

16-3-3. A semen with low sperm concentration that show normal motility and morphology is classified as oligozoospermic.

16-4-1. The presence of semen in a specimen can be verified by testing for acid phosphatase, prostate-specific antigen, and/or semenogelin.

CHAPTER 17

Study Questions

1. b
2. c
3. d
4. c
5. b
6. a
7. a
8. b
9. b
10. b
11. b
12. d

Case Studies

17-1-1. Yes, the bilirubin is elevated.

17-1-2. The amount that the curve deviates from a straight line at 450 nm (the A_{450}) is directly proportional to the amount of bilirubin in the amniotic fluid. The A_{410} corresponds to oxyhemoglobin, which is the major contaminant of concern.

17-1-3. Severely affected with intervention required (zone III).

17-1-4. Yes, an lecithin:sphingomyelin (L/S) ratio of 2.0 or greater is associated with fetal pulmonary system maturity.

CHAPTER 18

Study Questions

1. a, b, d
2. a, b
3. c
4. d
5. a
6. a
7. a, c, d
8. b
9. b
10. a

Case Studies

18-1-1. False-positive fetal fibronectin results may be obtained on specimens from patients in whom the cervix has been disrupted during sexual intercourse, digital cervical examination, vaginal probe ultrasound, or other disruptive events.

18-1-2. Traditional procedures for the diagnosis of rupture of fetal membranes (ROM) include pooling of amniotic fluid observed during speculum examination, nitrazine test, and a ferning test. Besides fetal fibronectin (fFN), other tests to detect ROM are AmniSure, which tests for placental alpha microglobulin-1 (PAMG-1) and the Actim PROM, which tests for insulin-like Growth Factor Binding Protein-1 (IGFBP-1).

18-2-1. Trichomonas vaginalis.

18-2-2. In pregnant women, Trichomonas is a risk factor for preterm rupture of membranes and preterm labor and delivery.

18-2-3. The wet mount is helpful to detect the majority of cases of Trichomonas, but culture or DNA probe for Trichomonas is useful when the wet preparation is negative but trichomoniasis is strongly suspected.

18-2-4. In Trichomonas, the bacterial flora is also altered and the pH is abnormally elevated to 5.0 or 6.0. The amine or "whiff" test may also be positive with Trichomonas as well as with vaginosis due to the altered bacterial flora and vaginal pH.

CHAPTER 19

Study Questions

1. b, c
2. a, b, c, d
3. a
4. a
5. a
6. a, b, c, d
7. c
8. a, b, c, d

Case Studies

19-1-1. *Pneumocystis jiroveci,* Aspergillus species, or other lower respiratory pathogens.

19-1-2. Fungal hyphae. These hyphae are suggestive of Aspergillus species as they are septate and branched at a 45-degree angle.

19-1-3. This organism is one of the opportunistic fungi that more often affect the immunocompromised host.

19-2-1. Bronchoscopy is used to examine the tracheobronchial tree and can help detect obstructions, pneumonia, carcinoma, hemoptysis, foreign bodies, or abscesses. Bronchial washings and bronchoalveolar lavage are used for cytological studies and for culture and stains for detecting infectious organisms.

19-2-2. It is definitely a fungus as yeast cells were seen. The morphology of the intracellular yeast is and the stain findings are suggestive of *Histoplasma capsulatum*.

CHAPTER 20

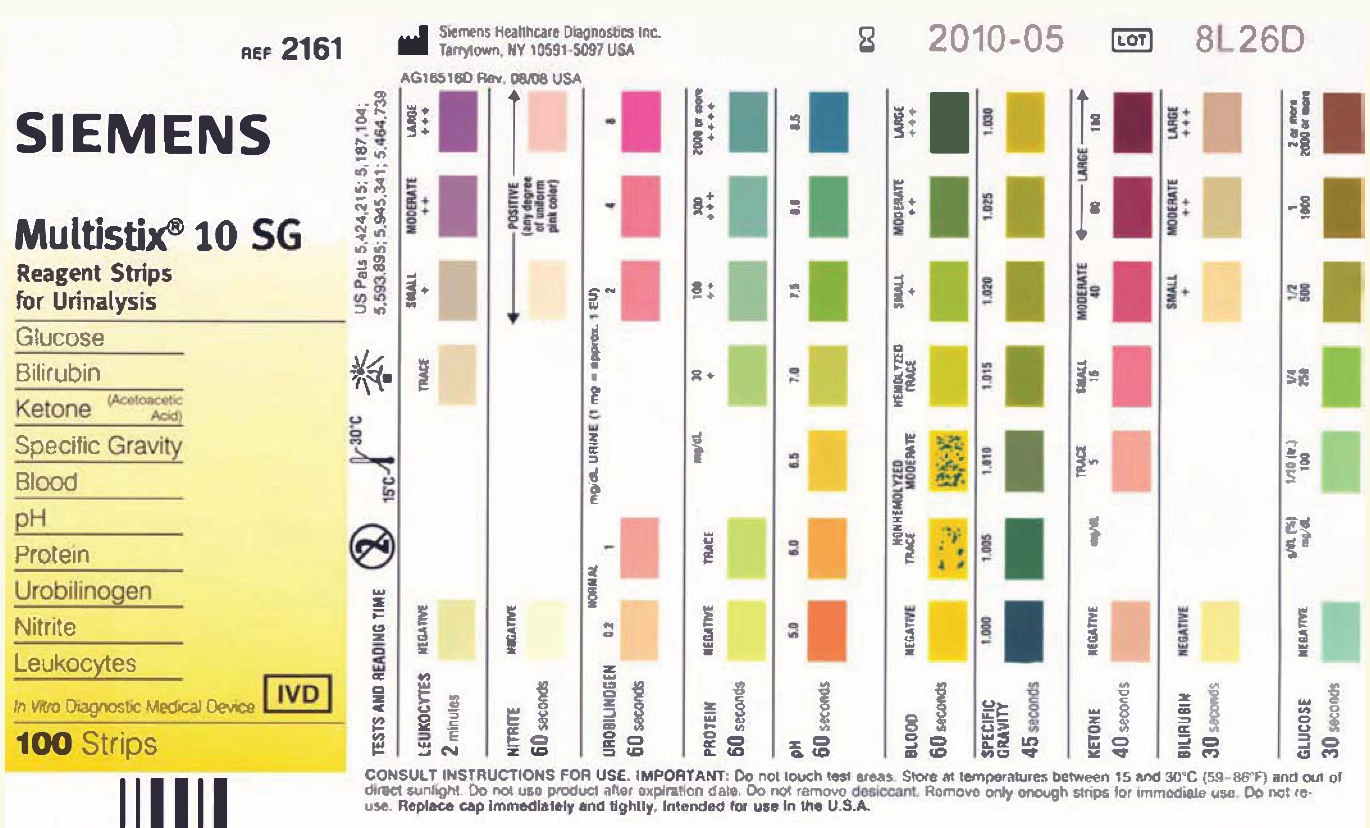
This chapter does not have study questions or case studies.

APPENDIX

B

Reagent Strip Color Chart

This appendix includes a scan of one of the urine testing strips most commonly used in the United States. This chart for the Multistix 10 SG is used with permission of Siemens and should not be used for diagnostic testing. This reproduction, as well as the representative color charts in Chapters 7 and 8, is for educational purposes only. Information regarding reagents, sensitivities, and specificities for these reagent strips can be found in Chapter 8.



Multistix®10 SG color chart. Do not use for diagnostic testing. (Courtesy of Siemens.)

APPENDIX

Hemocytometer Cell Counts

The hemocytometer (Fig. C-1) is a thick glass slide, specially designed for counting cells under the microscope. The most common hemocytometers contain counting chambers that use "improved Neubauer rulings," and must display the initials "N.S.B." to indicate it meets the specifications of the National Bureau of Standards. Manual cell counts are performed on body fluids and occasionally blood (when circumstances require manual methods). Therefore, laboratory personnel must be proficient in performing cell counts manually and understand their calculations in order to provide the most accurate results possible.

Macroscopic Appearance

The center section of the hemocytometer has three components: platforms with grids, moat, and bridges (Fig. C-2). There are two platforms, one on either side of the hemocytometer. Each platform is surrounded by a moat that separates it from the rest of the hemocytometer. Some hemocytometers have a groove at the outside edge of the platform that is used as a guide when loading the hemocytometer.

Beside each moat is a raised bridge, which is slightly higher than the counting platforms. The bridges provide support for the coverglass and create a uniform depth of 0.1 mm between the coverglass and the platform. This space is filled with fluid when the hemocytometer is loaded with the sample to be counted. Specially designed coverglasses that must be used that are optically flat to ensure the depth remains uniform over the entire platform at 0.1 mm (Fig. C-3).

MICROSCOPIC APPEARANCE

Each platform of the Neubauer hemocytometer is etched with a large grid measuring 3 mm on each side or 9 mm^2, ruled with triple lines to indicate 1 mm^2 that are further subdivided for ease of counting (Fig. C-4). The four corner square millimeters are typically used for white blood cell

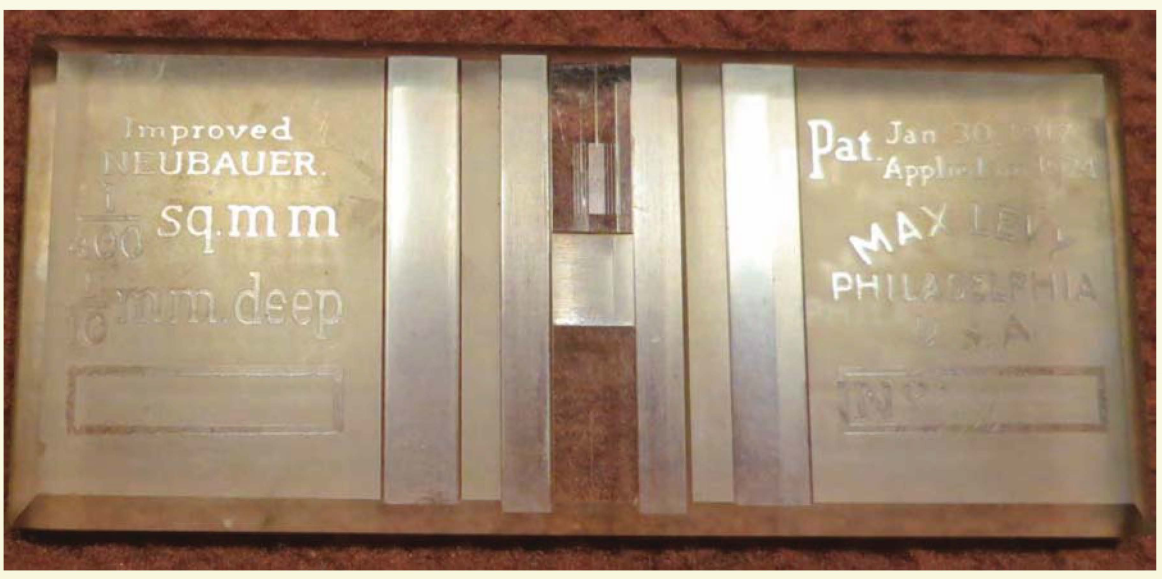


Figure C-1. Neubauer hemocytometer.

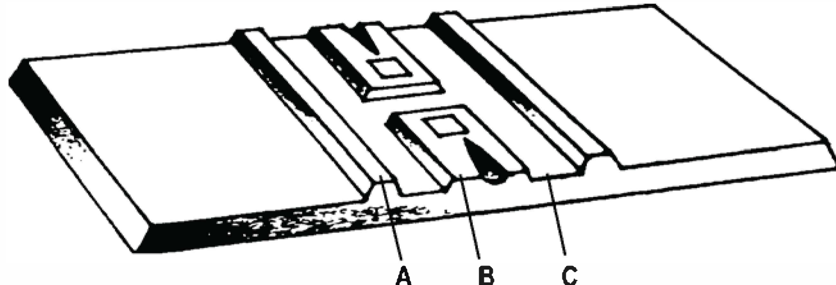


Figure C-2. Neubauer hemocytometer illustration. **A:** Bridge, **B:** Platform, **C:** Moat. (Educational Materials for Health Professionals.)

counts and are ruled into 16 medium-sized squares. The center square millimeter is ruled into units of 25 medium-sized squares each being further ruled in to 16 small squares. Five of these medium-sized squares (or 1/5 of a square millimeter) are used to count red blood cells. The area used for counting platelets and sperm is typically the entire center square millimeter. When counts are low, as in the case of nearly acellular fluids, the entire area of 9 mm^2 is counted.

Specimen Dilutions

The degree of dilution and the type of diluent depends upon the cellularity of the body fluid. Dilutions may be made using specially designed red blood cell and white blood cell diluting pipets. These pipets are becoming less common in the clinical setting and therefore not explained here. Follow manufacturer directions for proper use of these pipets. Dilutions can also be made using premeasure dilution vials and pipets. Follow manufacturer directions for proper use of these systems.

A simple method for diluting specimen samples to be counting is by using micropipets, which are available from many different manufacturers. After confirming specimen identification and labeling an aliquot tube with the same information, diluent is dispensed into the tube. A determined amount of sample is added to the diluent and thoroughly mixed without the introduction of bubbles. The dilution is calculated by using specific volumes of diluent and sample. For example, 10 mL of a cellular body fluid is added to 90 mL of saline to achieve a 1:10 dilution; one part sample out of a total volume of 10. If a highly cellular fluid requires a manual count 10 mL of sample can be added to 990 mL of saline for a 1:100 dilution. If an excessive amount of red blood cells are present, there may need to be two dilutions prepared, one using saline and another using a weak acid to lyse the red blood cells.

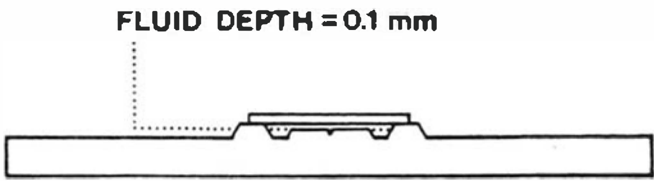


Figure C-3. Neubauer hemocytometer diagram side view showing 0.1 mm depth between platform and coverslip. (Educational Materials for Health Professionals.)

Loading the Hemocytometer

Delivering the specimen onto the hemocytometer counting surface is called "loading the hemocytometer" or "charging the chambers." Before loading the hemocytometer it must be checked for acceptability:

1. The counting platforms must be free from scratches.
2. The coverslip be a hemocytometer coverslip, free from scratches or chips.
3. Both the hemocytometer and coverslip be clean and greasefree. They may be cleaned with water and alcohol or lens cleaning solution with a lint-free cloth.
4. The coverslip is only held by its edges and set on the bridges over the center section of the hemocytometer, so that it covers both platforms equally.

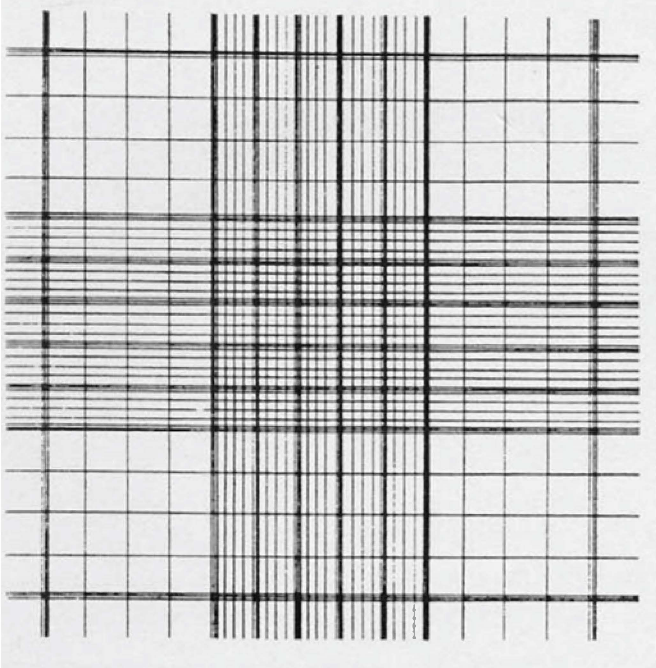


Figure C-4. Neubauer hemocytometer grid rulings; 9 mm^2.

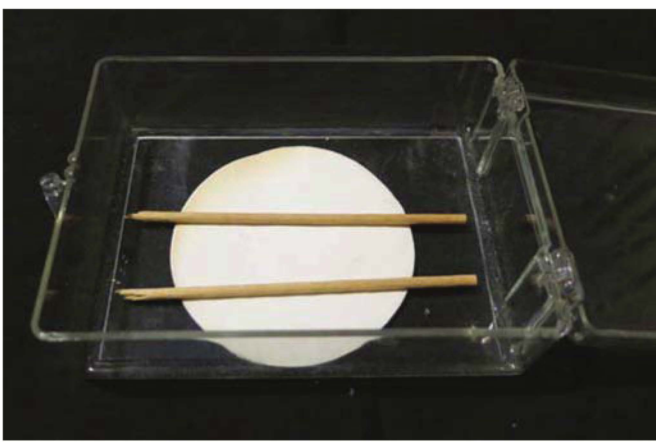


Figure C-5. Humidity chamber for a hemocytometer consists of a container with lid, moistened filter paper, gauze, or paper towel strips, and two applicator sticks to keep the bottom of the hemocytometer dry.

5. A humidity chamber (Fig. C-5) should be made ready to hold the hemocytometer either during loading or once loaded.

   a. Enclosure such as a petri dish or a plastic box.
   b. Moistened filter paper or strip of paper towel.
   c. Applicator stick cut into two pieces.

Once the hemocytometer is made ready and appropriate dilution of the sample is prepared, both chambers of the hemocytometer are loaded with the following requirements:

1. Mix the dilution completely.
2. Draw up a portion of the dilution into a pipet.
3. Place the tip of the pipet in the filling grove on the platform and at the edge of the coverglass (if no filling groove is present, gently touch the pipet to the edge of the coverglass) (Fig. C-6).

Figure C-6. A pipet is used to load the hemocytometer. Care must be taken to maintain a steady flow of fluid until the chamber is fully charged but not overflowing into the moat.

   a. The tip of the pipet should be at approximately 45 degrees.
   b. The coverglass should not be disturbed.

4. Slowly allow the fluid in the pipet to run out in one continuous motion until the area over the platform on that side is completely but just filled. Repeat for platform on the other side.

   a. Do not underfill the chamber. This may cause the cells not to lie over the counting grid.
   b. Do not overfill the chamber. If the chamber is filled to the point at which fluid is spilling over into the moat, cells will be pulled away from the counting grid. This will result in erroneous counts.
   c. Ensure that there are no bubbles present in the fluid under the coverglass.
   d. Place hemocytometer into the humidity chamber (if not loading with it in the chamber).
   e. The hemocytometer must rest on a level surface so that cell settling can occur evenly.

After the hemocytometer is filled, the cells are allowed enough time to settle to the same focal plane as the counting grid. A humid environment should be provided so that the fluid does not begin to evaporate from the chamber. If evaporation occurs, cells will be "pulled" closer together and falsely elevate counts.

Hemocytometer Counting Principles

Once the cells in the hemocytometer have settled, place the hemocytometer on the microscope stage and use the low-power objective to focus on the hemocytometer grid. Evaluate the distribution of cells as being even across both platforms. Select the appropriate area for the count being performed (described earlier). Select the appropriate power for the cells being counted (10× for white blood cells; 40× for red blood cells, platelets, and sperm). Using a systematic zig-zag approach to performing the count will ensure accurate results, with all cells lying in the prescribed area being counted only once. Figure C-7 shows cells to be counted as black, while those to be omitted from the count as white with black outline. Duplicate counts should agree within 20% of each other. If this criterion is not met, the counts must be repeated using a new dilution.

1. Begin at the upper left most corner of the square millimeter being counted.
2. Count all cells that lie within a square or touch **any** of the boundary lines (single, double, or triple) at the left and upper/top sides of that square, even if they are at the outer edge but still touch the line.
3. Omit counting cells that lie outside or touch **any** of the boundary lines (single, double, or triple) at the right and

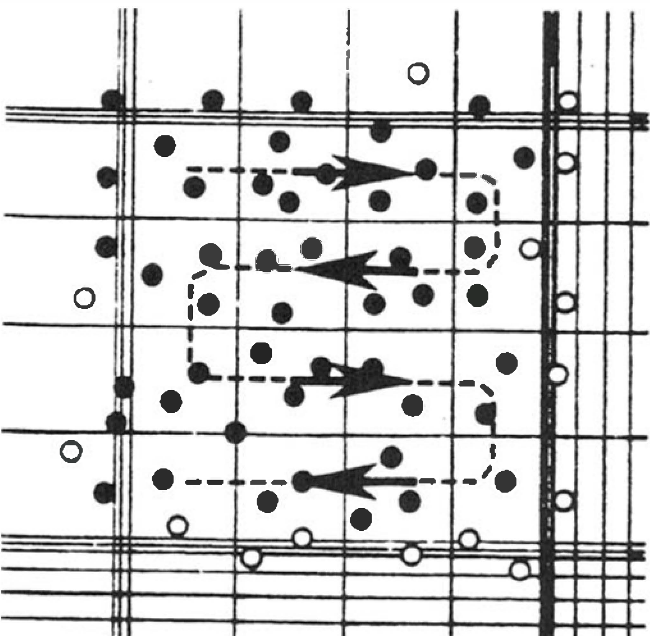


Figure C-7. Hemocytometer zig-zag counting pattern. Count cells touching out left and top lines and cells completely inside the ruled area. Omit cells touching any line on the right or bottom lines. (Educational Materials for Health Professionals.)

lower/bottom sides of that square, even if they appear to be inside the square but touch the line.

4. Move the field of view to the next horizontal square in the millimeter being counted and count as before.
5. When the entire square millimeter has been counted, move on to the next one in order from left to right, until the all required areas are counted.

Calculating Hemocytometer Count Results

Three pieces of information are necessary in order to calculate a cell count correctly: the number or cells counted, the dilution factor, and the volume counted.

CELLS COUNTED

The number of cells counted is specific for the type of cell counted. When counting both chambers of the hemocytometer, use the average of the two sides.

DILUTION FACTOR

The dilution factor is the degree to which the sample was diluted. As described earlier, this may be undiluted or factor of 1, 1:10 or factor of 10, 1:20 or factor of 20, 1:100 or factor of 100, and so on.

VOLUME COUNTED

The volume of fluid counted using a Neubauer hemocytometer is defined as the area multiplied by the depth. The volume for one counting chamber is the area of 9 square millimeters (mm^2) × the depth of 0.1 millimeter (mm) = 0.9 cubic millimeters (mm^3). The volume over the center square millimeter is 0.1 mm^3.

APPLYING THE FORMULA

The standard formula is:

$$(\text{cells counted/volume}) \times \text{dilution} = \text{count.}$$

For example, 48 cells are counted in 1 mm^2 as shown in Figure C-7. If the dilution is 1:20, the calculation for this count is:

$$(48/0.1) \times 20 = 9{,}600 \text{ cells per mm}^3$$

A simplified version of this formula does not require that the volume be calculated separately but incorporates the calculation into the formula:

cells counted × dilution factor × 10 (depth factor)/area counted in square millimeters

For example, this formula is applied to the same data as above:

$$48 \times 20 \times 10/1 = 9{,}6000 \text{ cells per mm}^3$$

In another example, 352 cells are counted in RBC counting area, or five of the medium-sized squares in the center millimeter of one side of the hemocytometer (one-fifth of a square millimeter) on a sample that was diluted 1:10. The calculation for this count is:

$$(352/0.02) \times 10 = 176{,}000 \text{ cells per mm}^3$$

The simplified calculation for this example is:

$352 \times 10 \times 10/(1/5)$ or $352 \times 10 \times 10 \times 5 = 176{,}000$ cells per mm^3

BIBLIOGRAPHY

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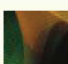
Historically Relevant Urine and Body Fluid Analysis Information

APPENDIX D

This appendix is intended to serve as an archive of procedures that are no longer routinely used in the clinical laboratory. With a few exceptions, these tests were originally included in the first edition of Sister Laurine Graff's text, *A Handbook of Routine Urinalysis.*



Bilirubin

Several screening tests for bilirubin were used prior to the Ictotest and subsequent development of reagent strip technology.

SMITH IODINE TEST

Place 5 mL of acidic urine in a test tube. Overlay the urine with 2 mL of 0.7% iodine in 95% ethyl alcohol. When bile is present, an emerald green ring will develop at the junction of the two liquids. The test is sensitive to 0.3 to 1.0 mg/dL of bilirubin. Report the results as positive or negative.

HARRISON SPOT TEST

In the Harrison spot test, barium chloride combines with sulfate radicals in the urine forming a precipitate of barium sulfate. Any bile pigments present will adhere to these large molecules. Ferric chloride in the presence of trichloroacetic acid will then oxidize yellow bilirubin to green biliverdin. This procedure is very sensitive and is said to detect 0.005 to 0.1 mg/dL of bilirubin.

Reagents

1. Fouchet reagent—combine the following:
   Trichloroacetic acid: 25 g
   Ferric chloride 10% solution: 10 mL
   Distilled water: 100 mL
2. Barium chloride 10% solution

Procedure

1. Add 5 mL of 10% barium chloride solution to 10 mL of acidified urine.
2. Shake well and filter to remove the precipitate.
3. Spread the precipitate out on another filter paper and allow to dry.
4. Add one drop of Fouchet reagent to the precipitate.

If bilirubin is present, a green or blue-green color will develop. Report as positive or negative. Barium chloride—impregnated strips of heavy filter paper can also be used. Moisten a strip of barium chloride paper with urine and add one drop of Fouchet reagent to the wet area.

Blood

Before the development of reagent strip technology, several methods using various reagents were used to detect the presence of occult blood in urine. Similar procedures may still be in use for detection of occult blood in stool.

HEMATEST

Hematest tablets are used to test urine although they are usually used for detecting occult blood in stool specimens. The reagents in the tablet are tartaric acid, calcium acetate, strontium peroxide, and the chromogen orthotolidine. When the Hematest tablet is moistened with water, the reagents are washed down onto the filter paper containing the sample. The tartaric acid and calcium acetate react with strontium peroxide to form hydrogen peroxide. The hemoglobin in the urine decomposes hydrogen peroxide with the liberation of oxygen, which then oxidizes orthotolidine to a blue-colored derivative. This procedure is very insensitive when used f or detecting occult blood in urine. It will not reliably detect less than 200 RBC/HPF (high-power field) unless some of the cells have hemolyzed. It is more sensitive to free hemoglobin, detecting amounts produced by the hemolysis of 25 to 30 RBC/HPF.

Procedure

1. Place one drop of urine on the filter paper.
2. Place a tablet in the center of the moistened portion of the filter paper.
3. Place one drop of water on top of the tablet, wait 5 to 10 seconds, then flow a second drop on the tablet so that it runs down the side and onto the filter paper.
4. If the test is positive, a blue color will appear on the filter paper around the tablet within 2 minutes. (The color of the tablet is of no significance.) The intensity of the color is proportional to the amount of red blood cells, hemoglobin, or myoglobin that is present, but it is difficult to try to semiquantitate the results. Report as positive or negative.

False-positive results: The contamination of the urine with hypochlorites or with large amounts of bacteria that have peroxidase activity can give false-positive results.

False-negative results: Because of the sensitivity of the procedure, urines containing less than 200 RBC/HPF or the hemoglobin contained in less than 25 RBC/HPF may appear as being negative for occult blood.

AMMONIUM SULFATE TEST

This procedure may be used to differentiate between hemoglobinuria and myoglobinuria after a test for occult blood is positive, but few or no red cells are seen in the microscopic findings.

Procedure

Prepare an 80% saturated urine solution of ammonium sulfate by adding 2.8 g of ammonium sulfate to 5 mL of urine in a test tube. Mix to dissolve. Filter or centrifuge. This procedure will precipitate out hemoglobin, but myoglobin will stay in solution. So, if the supernatant is a normal color, then the precipitated pigment is hemoglobin; if the supernatant is colored, then the pigment is myoglobin.

Ketone

Nitroprusside has been used to screen for ketones prior to the development of the Acetest or reagent strip technology. Other substances have also been used to detect the presence of ketones, although less specific. Some of these procedures are outline below.

ROTHERA TEST

Rothera test is a nitroprusside ring test which is very sensitive to diacetic acid but less sensitive to acetone; 13,-3-hydroxybutyric acid is not detected. This method can detect about 1 to 5 mg/dL of diacetic acid and 10 to 25 mg/dL of acetone.

Reagents

1. Rothera reagent—Pulverize and mix 7.5 g sodium nitroprusside and 200 g ammonium sulfate.
2. Concentrated ammonium hydroxide.

Procedure

1. Add about 1 g of Rothera reagent to 5 mL of urine in a test tube and mix well.
2. Overlay with 1 mL of concentrated ammonium hydroxide.
3. If positive, a red to purple ring will develop within 90 seconds at the point of contact. Report as follows:
   Negative—no ring or a brown ring
   Trace—faint pinkish purple ring
   1+—narrow lavender-purple ring
   2+—narrow dark purple ring
   4+—wide dark purple ring

This procedure has, for the most part, been replaced by the reagent dipsticks and Acetest.

GERHARDT TEST

Gerhardt test is based on the reaction of ferric chloride with diacetic acid to form a port wine or Bordeaux red color. Neither ace tone nor β-hydroxybutyric acid is detected by this method. It is not a very sensitive test because it can only detect about 25 to 50 mg/dL of diacetic acid.

Reagent

10% ferric chloride—10 g of ferric chloride; q.s. to 100 mL with distilled water

Procedure

1. Place from 3 to 5 mL of urine into a test tube.
2. Add 10% ferric chloride solution drop by drop until all phosphates are precipitated and then add a slight excess of ferric chloride. If diacetic acid is present, a Bordeaux red color will develop.
   Colors are produced by substances other than diacetic acid, such as blue to red-violet by salicylates, green by phenylpyruvic acid, dark red by aminopyrine, and gray by melanin. Phenothiazine drugs also give false-positive reactions.
3. To confirm the presence of diacetic acid, boil another portion of urine for 15 minutes; this will decompose diacetic acid to acetone, which is not detected by ferric chloride.
4. Repeat the test on the boiled sample and if the test is still positive, then diacetic acid is not present but the color is from an interfering substance.

If the repeated test is negative, then the color in the original test was due to diacetic acid. Gerhardt test is a qualitative

procedure and is reported as either positive or negative. Because of the sensitivity of this method, a positive result implies a significant level of ketonuria.

HART TEST

Hart test is an indirect method for the detection of 13-hydroxybutyric acid in the urine. The first part of the procedure uses boiling to break down the diacetic acid that is present into acetone, and then the acetone is removed by evaporation. Next, the β-hydroxybutyric acid is oxidized to diacetic acid and acetone by the use of peroxide. (Ferric ions or dichromate could also be used.) The diacetic acid and acetone can then be detected by any of the nitroprusside procedures.

Procedure

1. Place 20 mL of urine in a beaker.
2. Add 20 mL of distilled water and a few drops of acetic acid.
3. Boil until the volume is reduced to 10 mL. These steps will remove the diacetic acid and acetone.
4. Dilute to 20 mL with distilled water, mix and divide the contents into two equal portions.
5. To one of the portions add 1 mL of hydrogen peroxide, warm gently, and then let cool. This will change the β-hydroxybutyric acid into diacetic acid and some of this will become acetone.
6. Test both portions f or diacetic acid and acetone by using any nitroprusside method.
7. If β-hydroxybutyric acid is present, the tube containing the hydrogen peroxide will show a positive reaction. The other tube will show no reaction.

This procedure can be performed on less than 20 mL of urine. If, for instance, 15 mL is used, then add 15 mL of distilled water, evaporate to 7.5 mL, and dilute back up to 15 mL.

Protein

Early tests for protein used the principle of acid precipitation of proteins, with or without the application of heat. Proteins are more susceptible to precipitating agents when at the pH of their isoelectric point, which is usually low. Most commonly used acids to precipitate protein included sulfosalicylic, trichloroacetic, and nitric and acetic acid. Sulfosalicylic is the most frequently used acid test because it does not necessarily require the use of heat.

SULFOSALICYLIC ACID TEST

Different concentrations and proportions of sulfosalicylic acid have been used, each producing a different range of results. The procedure discussed here uses the solution known as Exton reagent, which is 5% sulfosalicylic acid in a solution of sodium sulfate. Exton (1925) found that adding sodium sulfate to the sulfosalicylic acid causes a more uniform precipitate to be formed.

This procedure is more sensitive than the dipstick, and it is specific for all proteins including albumin, globulins, glycoproteins, and Bence-Jones protein.

Exton Reagent

Dissolve 88 g of sodium sulfate in 600 mL of distilled water with the aid of heat.

Cool. Add 50 g of sulfosalicylic acid and dilute to 1,000 mL.

Procedure

1. Centrifuge an aliquot of urine and use the supernatant.
2. Mix equal volumes of supernatant and Exton reagent.
3. Grade for cloudiness as follows:
   - Negative—no cloudiness
   - Trace—cloudiness is just perceptible against a black background
   - 1+—cloudiness is distinct but not granular
   - 2+—cloudiness is distinct and granular
   - 3+—cloudiness is heavy with distinct clumping
   - 4+—cloud is dense with large clumps that may solidify

Comparing the results of the test with a set of graded standards provides for more accurate readings.

False-positive results: False-positive results can occur during therapy with tolbutamide, massive doses of penicillin, sulfonamides, and for up to 3 days following the administration of radiographic dyes.

False-negative results: A highly buffered alkaline urine can result in a false-negative reaction. A false negative can also occur in a very dilute sample.

Exton Standards

Standards used in the SSA test can be prepared by diluting blood bank albumin, which is 22%.

Make a 1 in 22 dilution of blood bank albumin with distilled water. This equals 1% = 4+

From the 1% solution make a 1 in 2 dilution. This equals 0.5% = 3+

From the 0.5% solution make a 1 in 2 dilution. This equals 0.25% = 2+

From the 0.5% solution make a 1 in 10 dilution. This equals 0.05% = 1+

From the .05% solution make a 1 in 100 dilution. This equals 0.005% = Trace

HEAT AND ACETIC ACID TEST

In addition to the acid precipitation principle, this method uses the fact that heat renders protein insoluble and causes it to coagulate.

Procedure

1. Centrifuge or filter about 10 mL of urine and decant the supernatant into a heat-resistant tube. The tube should be about two-thirds full.
2. Hold the bottom of the tube with a test tube holder and boil the upper portion of the tube for about 2 minutes. (The tube should be held at an angle over the flame and aimed away from the body.) If cloudiness appears, it may be due to protein, phosphates, or carbonates.
3. Add three to five drops of 5% or 10% acetic acid and boil again. The acid will dissolve any phosphates or carbonates which may be causing the cloudiness. It will also lower the pH, bringing it closer to the isoelectric point of proteins; therefore, the cloudiness may increase after addition of the acid due to increased precipitation of proteins.
4. Read the degree of cloudiness of the upper portion of the tube and report according to the same scale used for the Exton test.

Some urines remain clear when boiled but develop cloudiness when the acid is added and the sample is boiled again. This is because metaprotein in alkaline solution is uncoagulable, but when the solution becomes slightly acid or neutral, the protein is precipitated.

This procedure detects albumin, globulin, and mucoproteins; Bence-Jones protein can be detected if the tube is watched closely during heating. The test is very sensitive and can detect as little as 5 mg/dL of protein, but hemoglobin and myoglobin are not precipitated by this method.

False-positive results: Tolbutamide, massive doses of penicillin, and radiographic dyes can result in false-positive reactions.

False-negative results: As mentioned previously, hemoglobin and myoglobin are not detected by this method. Highly buffered alkaline urines and very dilute specimens can give false-negative results.

HELLER RING TEST

This method may be useful when only a small quantity of urine is available, but the test is not as sensitive as the other precipitation tests. It is also very difficult to attempt to semi-quantitate the results.

Procedure

1. Place a few milliliters of concentrated nitric acid in the bottom of a test tube.
2. Overlay the acid with centrifuged urine by allowing the urine to run slowly down the side of the tube, thus forming two layers of fluid.
3. A white precipitate forming at the junction of the liquids within 3 minutes indicates the presence of protein. An attempt may be made to quantitate the density of the ring that is formed.

False-positive results: This test is affected by the same interfering drugs as the heat and acid test. High concentrations of uric acid and urea may give false-positive reactions but these may be overcome by diluting the urine and repeating the test.

False-negative results: Since this test is not very sensitive, dilute urines may give false-negative results.

The routine use of concentrated nitric acid may be a disadvantage of this test. The procedure for the Robert ring test is identical to Heller test, except that the reagent for the former consists of one part concentrated nitric acid and five parts saturated magnesium sulfate.

BENCE-JONES PROTEIN

Bence-Jones protein consists of dimers of either kappa or lambda light chains from immunoglobulins. This protein was first recognized by Henry Bence-Jones in 1847 because of its unusual solubility properties: it precipitates when heated to 40° to 60°C but becomes soluble again when boiled. The molecular weight of the protein is small, around 44,000, and is easily filtered through a healthy glomerulus.

To understand the process whereby free light chains are excreted in the urine, it is necessary to trace the source of the production of these chains. In certain diseases, a malignant clone of immunoglobulin-producing immunocytes is formed. All of the cells in the clone are a result of the proliferation of a single cell, and therefore they have identical properties. These cells will produce a homogeneous immunoglobulin (e.g., all IgG or all IgA) and/or a single type of free light chain, either kappa or lambda. An imbalance in the production rates of the subunits (light and heavy chains), which make up the immunoglobulin molecule, can result in the overproduction of light chains which will be filtered at the glomerulus and excreted in the urine (Bence-Jones protein). But this all depends upon the relative quantities of light and heavy chains which the clone produces.

Three types of abnormalities can occur. First, the clone can produce equal amounts of one type of light chain and one type of heavy chain. These will combine to form a homogeneous immunoglobulin which can be detected as a monoclonal spike on the serum electrophoretic pattern. Since no excess light chains are produced, none will be present in the urine (no Bence-Jones protein). Second, the clone may produce more light chains than heavy chains. The light chains will combine with all of the available heavy chains and the resulting immunoglobulin can again be detected by serum electrophoresis. The excess light chains will be excreted in the urine (Bence-Jones protein). In the third type, the clone produces only the homogeneous light chains without any heavy chains. Serum electrophoresis will show no monoclonal spike since no homogeneous immunoglobulin molecules can be formed. All of the light chains will be excreted in the urine unless there is renal insufficiency. The urine will therefore contain large quantities of Bence-Jones protein and this can best be identified by a spike on the urine electrophoretic pattern.

Multiple myeloma, a disease in which there is a malignant proliferation of plasma cells, usually in the bone marrow, is

the disease most frequently associated with Bence-Jones protein. It is estimated that 50% to 80% of patients with multiple myeloma will have Bence-Jones protein in their urine. The remaining cases can be diagnosed by serum electrophoresis or immunoelectrophoresis which can detect the monoclonal immunoglobulin.

Bence-Jones proteinuria is not specific for multiple myeloma but can also be found in cases of lymphoma, macroglobulinemia, leukemia, osteogenic sarcoma, amyloidosis, and other malignancies. The daily urinary excretion of light chains may vary from less than 1 g/day to 15 to 20 g/day. With multiple myeloma, however, it is characteristic that if Bence-Jones protein is present, it will appear in large quantities. After prolonged Bence-Jones proteinuria the glomerular membrane may become more permeable to larger proteins, and because of the large demand for protein reabsorption, the tubule cells degenerate, so normal serum proteins, albumin, and globulin will also appear in the urine.

Testing for Bence-Jones proteinuria is not part of the routine urinalysis but this protein may be accidentally recognized in the heat and acid test. If a request is made for Bence-Jones protein, the sulfosalicylic acid test may be performed first as a screening test for all proteins. If the results are negative, then no Bence-Jones protein is present, but if positive results are obtained, then further testing is required to determine whether the precipitation is due to Bence-Jones or other proteins. The best method for detecting the presence of these light chains is by protein electrophoresis and immunoelectrophoresis using specific antisera on a urine specimen that has been well concentrated, usually by dialysis. There are two other screening procedures that can be used, but they are not as reliable as electrophoresis. One method is based on the protein's unusual solubility properties, whereas the other is a precipitation test using toluene sulfonic acid (TSA).

HEAT PRECIPITATION TEST

Bence-Jones protein precipitates at temperatures between 40° and 60°C (56°C optimum), but redissolves again at 100°C. Upon cooling, the precipitate will reappear around 60°C and will dissolve again below 40°C.

Procedure

1. Place several milliliters of centrifuged urine in a test tube and acidify to pH 5.0 to 5.5 using 10% acetic acid.
2. Heat for 15 minutes in a 56°C water bath. If a precipitate forms, it is indicative of Bence-Jones protein.
3. If precipitation occurs, place the tube in a boiling water bath and allow to boil for 3 minutes. A decrease in precipitation is due to the presence of Bence-Jones protein, whereas an increase in precipitation is due to other proteins.
4. If an increase in precipitation occurs at 100°C, filter the urine while it is hot to remove the interfering proteins. The Bence-Jones protein will be in solution at that temperature and will, therefore, remain in the filtrate.
5. Upon cooling, the Bence-Jones protein will reappear in the filtrate at approximately 60°C and will dissolve again below 40°C.

False-negative results: A very heavy precipitation of Bence-Jones protein at 56°C may not redissolve on boiling, so the procedure should be repeated on diluted urine. If the sample needs to be filtered in step no. 4, it must remain above 70°C during filtration or else the Bence-Jones protein will begin to precipitate out and will remain in the filter.

TOLUENE SULFONIC ACID TEST

Toluene sulfonic acid reagent precipitates Bence-Jones protein and can detect as little as 0.03 mg/mL. It will not precipitate albumin, but globulins will give a positive test if present at concentrations greater than 500 mg/l00 mL.

TSA Reagent

p-Toluene sulfonic acid—12 g
Glacial acetic acid—q.s. to 100 mL

Procedure

1. Place 2 mL of clear urine in a test tube.
2. Add 1 mL TSA reagent by allowing it to flow slowly down the side of the tube. (Take 15 to 30 seconds to add the reagent.)
3. Flick the tube with a finger to mix.
4. A precipitate forming within 5 minutes indicates the presence of free light chains.

Reducing Substances

Many sugars and medication metabolites function as reducing substances. Reagent strips test specifically for glucose. The Clinitest is the method currently used for detecting other reducing substances. The Clinitest is based on the principles employed by the classic Benedict Test.

BENEDICT QUALITATIVE TEST

Benedict test had long been the standard method for detecting glycosuria although it is not specific f or glucose. The reaction is very similar to that of Clinitest, with a blue alkaline copper sulfate reagent being reduced to red cuprous oxide precipitate.

Reagent

Copper sulfate—17.3 g
Sodium or potassium citrate—173 g

Sodium carbonate crystals—200 g, or anhydrous sodium carbonate—100 g
Distilled water to make 1,000 mL
Dissolve the citrate and carbonate in about 700 mL of water with the aid of heat. Filter. Dissolve the copper sulfate in approximately 100 mL of hot water and pour into the citrate–carbonate solution with stirring. Allow to cool before diluting up to 1,000 mL with water.

Procedure

1. Place 5 mL of reagent in a test tube.
2. Add eight drops of urine and mix well.
3. Place in a boiling water bath for 5 minutes or boil over a flame for 1 to 2 minutes.
4. Allow to cool slowly.

The test is usually graded in intensity according to the following:

Negative—clear blue color, blue precipitate may form
Trace—bluish-green color
1+—green color, green or yellow precipitate
2+—yellow to green color, yellow precipitate
3+—yellow-orange color, yellow-orange precipitate
4+—reddish-yellow color, brick red or red precipitate

This procedure is very sensitive and may be capable of detecting as little as 0.02% or 0.05% of reducing substances and as high as 4%. Because of this extreme sensitivity, healthy individuals may show a "trace" reaction.

False-positive results: Benedict reagent is also reduced by glucuronides and homogentisic acid. Massive doses of various drugs including penicillin, streptomycin, salicylates, oxytetracycline, polyvinylpyrrolidone, dextran, and *p*-aminosalicylic acid may also cause a false-positive Benedict test. Urinary preservatives formalin and formaldehyde are reducing substances and may result in a false positive. Prolonged boiling during the procedure may also give false-positive results. Heavy proteinuria and heavy urate deposits can also interfere with the test, giving false positives. The protein may be removed by precipitating out the protein and then filtering the urine before performing the procedure.

False-negative results: Failure to follow the procedure correctly is the only cause of false negatives.

Porphobilinogen and Urobilinogen

WATSON–SCHWARTZ TEST

The Watson–Schwartz test detects porphobilinogen and urobilinogen and differentiates them by means of their solubility properties. The principle of the test is that porphobilinogen and urobilinogen react with Ehrlich reagent to form red-colored aldehyde. Sodium acetate is added to increase the pH and to intensify color development. Extractions are performed to separate urobilinogen aldehyde from porphobilinogen aldehyde. Urobilinogen aldehyde is soluble in both chloroform and butanol. Porphobilinogen aldehyde is insoluble in both chloroform and butanol but is soluble in water.

The test should be performed on freshly voided urine which has been allowed to reach room temperature. When urine is allowed to stand before testing, the porphobilinogen may be oxidized to porphobilin which is not detected by this procedure. Also, if the urine is warm, a false-positive reaction may occur due to the "warm benzaldehyde reaction."

Reagents

1. Modified Ehrlich reagent:
   0.7 g p-dimethylaminobenzaldehyde
   150 ml concentrated HCl
   100 ml distilled deionized H_2O
   Mix and store in a brown bottle.
2. Saturated sodium acetate
   Dissolve 1 kg of sodium acetate in 1 L of water at 60°C.
3. Chloroform
4. Butanol

Procedure

1. In a large test tube, combine 3 mL of urine and 3 mL of modified Ehrlich reagent and mix.
2. Add 6 mL of saturated sodium acetate and mix well. A pink to red color indicates porphobilinogen, urobilinogen, or other Ehrlich-reacting substances (e.g., indole).
3. Add 3 mL of chloroform, shake well, and centrifuge briefly or allow the layers to settle out.
4. Porphobilinogen aldehyde is insoluble in chloroform and will remain in the aqueous or top layer, giving it a pink or red color. Urobilinogen aldehyde and other Ehrlich-reacting compounds are soluble in chloroform and so these colored compounds will be present in the chloroform (bottom) layer (Fig. D-1).
5. If both layers have a pink color, extract with chloroform again.

Interpretation:
Pink or red aqueous (top) layer = porphobilinogen
Pink or red chloroform (bottom) layer = urobilinogen or other Ehrlich-reacting substances (Fig. D-1).
If the top layer is pink or red, the following confirmatory step for porphobilinogen can be used.

6. Remove some of the supernatant or aqueous layer and add an equal amount of butanol. Mix well and allow to separate. The butanol layer will be on the top and the aqueous layer on the bottom. All known Ehrlich aldehyde compounds, except for porphobilinogen, will be extracted into the butanol layer. Porphobilinogen will remain in the aqueous (bottom) layer.

(*Note:* The aldehyde derivatives of melanogen, serotonin, and some indoles are insoluble in chloroform but soluble in butanol.)

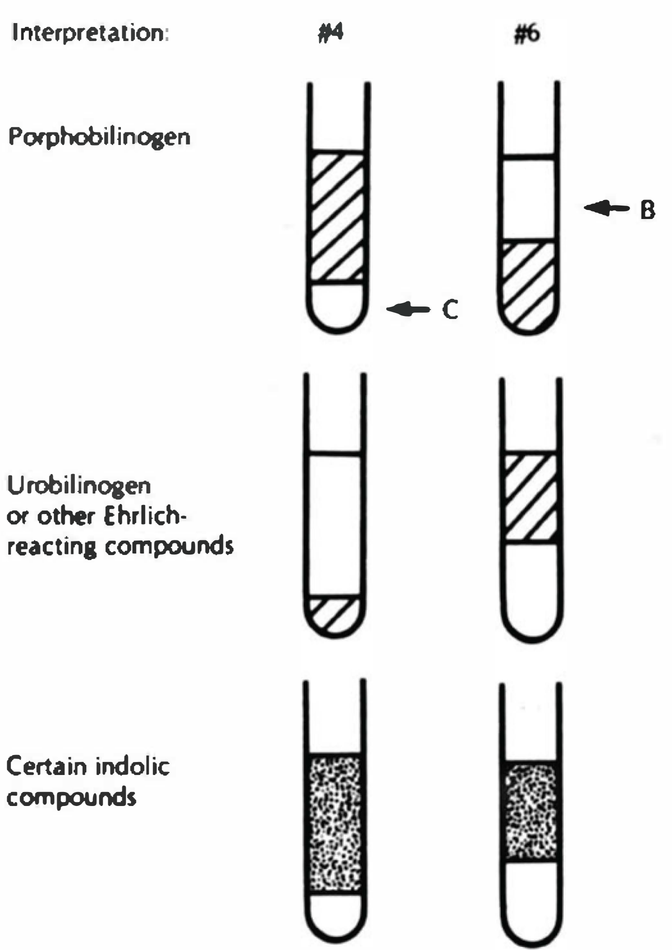


Figure D-1. If porphobilinogen is present, the Watson–Schwartz test will show a red or pink aqueous layer (upper layer) when extracted with chloroform (*arrow* C) and will follow the aqueous layer (lower layer) when further extracted with butanol (*arrow* B). Urobilinogen and other Ehrlich reacting compounds extract into the solvent layers (lower layer with chloroform, and upper layer with butanol). Certain indolic compounds will demonstrate color in both aqueous and solvent layers.

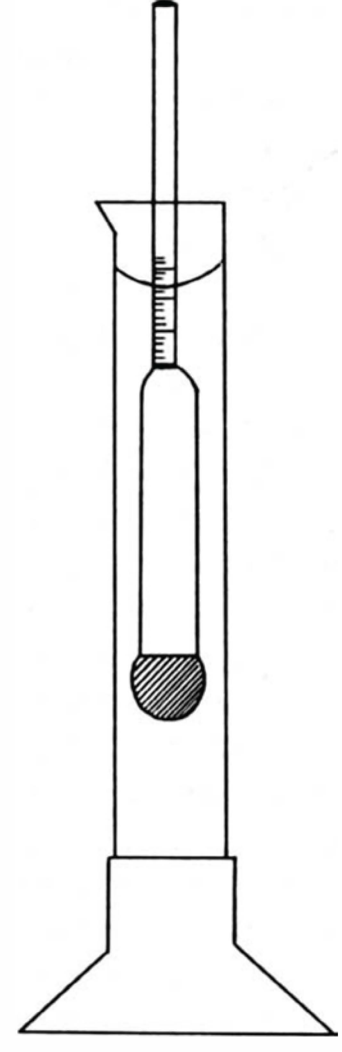


Figure D-2. Urinometer for measuring specific gravity placed in an appropriate cylinder filled partway with liquid.

There are some occasions in which both porphobilinogen and urobilinogen are present, but this is very rare.

HOESCH TEST FOR PORPHOBILINOGEN

The Hoesch test uses Ehrlich original reagent but is based on the inverse Ehrlich reaction (i.e., of maintaining an acid solution by adding a small amount of urine to a relatively large volume of reagent). The procedure is specific for porphobilinogen; urobilinogen is not detected. As with all procedures for porphobilinogen, this test must be performed on fresh urine.

Ehrlich Reagent

20 g p-dimethylaminobenzaldehyde
HCl (6 mol/L)—q.s. to 1,000 mL

First, make 1,000 mL of 6 M HCl by mixing 500 mL of concentrated HCl and 500 mL of water. Then place 20 g of p-dimethylaminobenzaldehyde in a liter volumetric flask and q.s. to 1,000 mL with the 6 M HCl. Reagent can be kept in a clear glass container for up to 9 months.

Procedure

1. Place 2 to 3 mL of the Ehrlich reagent in a test tube, and add two drops of fresh urine.
2. If porphobilinogen is present, a cherry-red color will instantly appear at the top of the solution and will spread throughout when the tube is agitated. Report as positive or negative for porphobilinogen.

The Hoesch test does not detect the low concentrations of porphobilinogen that are present in normal urine.

Specific Gravity

Current methods for measuring specific gravity (explained in Chapter 7) are easier to perform and use lesser amounts of specimen than originally developed methods. Newer methods also measure parameters that are not truly specific gravity but correlate with specific gravity measurements performed by older methods.

URINOMETER

The urinometer is a hydrometer that is calibrated to measure specific gravity of urine at a specific temperature, usually 20°C. It is based on the principle of buoyancy, so the urinometer will float higher in urine and in water, because urine is denser. A urinometer is a blown-glass float that has a weight at the bottom and a graduated scale enclosed or etched at the top. When placed in a cylinder of liquid, the urinometer displaces a volume of liquid equal to its weight (Fig. D-2).

This weighted float is calibrated to register a specific gravity of 1.000 when it is placed in distilled water. Dissolved substances add additional mass and cause the float to displace less liquid than distilled water, thereby registering a higher specific gravity on the scale. To use the urinometer, about 15 mL of mixed urine is placed in a glass cylinder. Any foam or bubbles must be removed, because they will interfere with proper reading of the meniscus. The float must not come in contact with the bottom or sides of the cylinder. To ensure the hydrometer floats freely, spin it as it is placed in the urine. Read the bottom of the meniscus while looking at the scale at eye level.

The markings on the scale usually range from 0 to 50, with lines in between units of 10 to indicate single units. This scale is understood to be read as 1.000 plus the scale reading. For example, if the meniscus of the displaced liquid is at the 27 mark, the specific gravity is 1.027. If the specific gravity is too high to get a reading, then it is necessary to make a 1:2 (one in two) dilution of urine using distilled water. Multiply only the last two digits of the reading by 2 to get the true specific gravity. For example, if the dilution reads 1.026, then the specific gravity is 1.052. A correction to the specific gravity reading is necessary when measuring urine above or below room temperature.

HARMONIC RESONANCE

Similar to urinometry, harmonic resonance or harmonic oscillation densitometry (HOD) is not commonly used in the clinical laboratory. An older model of the IRIS automated system used HOD. Urine enters a glass tube with an electromagnetic coil at one end. Sonic oscillation is generated when an electric current is applied to the coil. The oscillation detected is proportional to the density of the urine. A microprocessor corrects sample temperature. Result is valid up to a specific gravity of 1.080.

Sperm Penetration

Penetrak was used in the laboratory setting to determine the ability of sperm to penetrate bovine cervical mucus. Bovine mucus was frozen in flat glass capillary tubes, which was scored at one end. These tubes were thawed upright with the scored end up to assist with the elimination of air bubbles from the test area. Once thawed and opened at the score marks, the opened ends of the tubes were placed in a sample cup containing 0.2 mL of fresh semen. This setup was incubated at room temperature for 90 minutes. Placing the penetration test setup inside a closed cabinet kept it free from drafts that may alter its temperature. After incubation, the capillary tubes are removed from the specimen, placed on a ruled slide, and observed microscopically. The distance obtained by the vanguard sperm (the sperm that traveled the greatest distance) is recorded f or both tubes and the average calculated. Normal sperm should be able to penetrate bovine cervical mucus to at least a distance of 30 mm.

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Glossary

Aberration: Image error caused by color or spherical errors.

Accessory digestive organs: Glands that support the alimentary canal.

Accreditation: Process through which a program or institution establishes that it has met required guidelines.

Acetest: Trademarked name for tablet test used to detect ketones and cystine.

Acetylcholinesterase: Enzyme specific for neural tube disorders.

Achromat: Lens that spherically corrects for green light, and chromatically corrects for red and blue-green light.

Acute interstitial nephritis (AIN): Drug-induced acute renal failure.

Acute kidney injury (acute renal failure): Abrupt loss of kidney function that results in retention of urea and other nitrogenous waste products and also results in impaired regulation of extracellular volume and electrolytes.

Acute poststreptococcal glomerulonephritis: An autoimmune condition that may arise after a *Streptococcus pyogenes* (group A) infection of the throat or skin.

Acute tubular necrosis: A kidney disorder involving damage to the tubule cells of the kidneys, resulting in acute kidney failure.

Adrenal gland: Endocrine glands that sit on top of the kidneys that are chiefly responsible for regulating the stress response through the synthesis of corticosteroids and catecholamines.

Afferent arteriole: A branch of the interlobular renal artery that becomes the glomerular tuft within the glomerulus.

Agglutination: Clumping of cells.

Albinism: A congenital disorder that results in the absence of melanin.

Alcoholic stool: Pale stool, without normal fecal color.

Aldosterone: A steroid hormone from the adrenal gland that stimulates the absorption of sodium and the excretion of potassium in the distal tubules and is regulated by the renin–angiotensin–aldosterone system.

Alimentary canal: The gastrointestinal tract.

Alkaptonuria: A rare disease characterized by the excretion of homogentisic acid in the urine.

Alpha-fetoprotein (AFP): Most abundant fetal plasma protein and is secreted in the fetal urine.

Alport syndrome: Inherited form of kidney inflammation that can cause symptoms ranging from mild hematuria to gross hematuria to nephrosis and end-stage kidney disease.

Amine or Whiff test: A test used to detect a foul-smelling trimethylamine odor when KOH is added to vaginal secretions due to bacterial vaginosis.

Aminoacidurias: Disorders that cause increased amino acids in the urine.

Amniocentesis: The procedure for collecting amniotic fluid.

Amniostat-FLM: A commercial product that uses antibodies to phosphatidyl glycerol.

Angiotensin-converting enzyme (ACE): ACE is actually found all over the body but has its highest density in the lung due to the high density of capillary beds there. ACE is a target for inactivation by ACE inhibitor drugs, which decrease the rate of angiotensin II production. ACE inhibitor drugs are major drugs against hypertension.

Angiotensin I: Angiotensin I is formed by the action of renin on angiotensinogen and appears to have no biological activity but serves as a precursor to angiotensin II. Angiotensin I is converted to angiotensin II by the enzyme angiotensin-converting enzyme (ACE), which is found predominantly in the capillaries of the lung.

Angiotensin II: Acts as an endocrine, autocrine/paracrine, and intracrine hormone and increases blood pressure by stimulating vascular smooth muscle cell contraction.

Anthocyanins: Complex pigments present in beets resulting in the excretion of red urine in persons with an inherited metabolic sensitivity.

Antidiuretic hormone (ADH): Also known as vasopressin, this hormone is produced in the hypothalamus and is released by the posterior pituitary and causes the collecting duct and tubule to reabsorb more water.

Antinuclear antibody: An antibody that is directed against nuclear material of cells.

Antisperm antibodies: Antibodies formed against sperm; may be autoantibodies or antibodies produced by female partner.

Anuria: The absence of or cessation of urine excretion.

Apochromat: Lens that has better correction of chromatic and spherical aberration than achromat lenses.

Arachnoid: The middle layer of membrane covering the brain. It is also called arachnoidea.

Arachnoid villi: Arachnoid membrane that herniates into the lumen of the dural sinuses.

Arthrocentesis: Procedure for collecting synovial fluid from a joint capsule.

Ascites: An accumulation of fluid in the peritoneal cavity.

Ascorbate (ascorbic acid): Also known as vitamin C, ascorbate may affect the reading of several reagent strip pads.

Asthenozoospermic: Normal sperm count with less than normal motility, and morphology.

Atrophic vaginitis: Condition caused by physiologic and structural changes that occur to the vulvovaginal mucosa primarily as a result of a decrease in estrogen levels at menopause.

Automated urine sediment analyzers: Instruments that can perform laboratory tests with minimum human interaction.

Azoospermia: The absence of sperm in a semen sample.

Azotemia: Elevation of nitrogen waste byproducts.

Bacterial vaginosis: Most common vaginal infection in women due to alteration of the vaginal flora.

Barcode-labeled specimen: Specimen label that displays a series of light and dark bars containing patient and specimen information, which can be read by a computerized scanner.

Beta-human chorion gonadotropin (ß-hCG): A hormone that is secreted in urine within 2 to 3 days after implantation of an embryo; and is the analyte which pregnancy tests detect.

Bilirubin: A breakdown product of hemoglobin degradation.

Biliverdin: Oxidized product of bilirubin producing a green color.

Birefringence: The ability to refract light in two directions, one following the original light path, the other rotated 90 degrees to the original.

Blood–brain barrier: Consists of choroidal cells and capillary endothelial cells.

Blood Urea Nitrogen (BUN): The nitrogen in your blood that comes from the waste product urea. Urea is made when protein is broken down in your body. Urea is made in the liver and passed out of your body in the urine. The BUN level relates to the level of kidney function.

Boric acid: Preservative used for specimen collection for urine cultures.

Bowman capsule: Portion of the nephron that contains the glomerulus.

Brightfield: Microscopy in which the image of the specimen appears dark against a bright background.

Bronchial washings: Washings that are collected to assess the cellular composition and to detect any infectious agents present in the lower respiratory tract.

Bronchoaveolar lavage (BAL): Procedure used to collect cellular alveolar material from more distal sites in the lower parts of the respiratory tract.

Bronchoscopy: Procedure used to examine the tracheobronchial tree.

Bulbourethral glands: Two small exocrine glands present in the reproductive system of human males.

Bulge test: A noninvasive procedure to determine the existence of excess fluid around a joint.

Calcofluor white: Fluorescent stain that has increased sensitivity in the detection of fungi.

Calyces, major and minor: Funnel-like extensions from the renal pyramids into the renal pelvis, where collecting ducts empty their developing urine into the renal pelvis.

Candidiasis: A common vaginal fungal infection in women caused by *Candida albicans*.

Carbohydrate disorders of metabolism: Disorders involving glucose and other sugar metabolism.

Carbonic anhydrase: A zinc-containing metalloenzyme that catalyzes the rapid conversion of carbon dioxide to bicarbonate and hydrogen ion, a reaction that occurs rather slowly in the absence of a catalyst.

Casts: See urinary sediment casts.

Catheterization: Insertion of a sterile tubing.

Centers for Disease Control and Prevention (CDC): The agency that implements public health regulations and reporting requirements and categorizes developed laboratory tests.

Centers for Medicare & Medicaid Services (CMS): US federal agency which administers Medicare, Medicaid, and the State Children's Health Insurance Program.

Central nervous system: The brain and the spinal column.

Cerebral ventricle: An area of the brain that forms a continuum with the central canal of the spinal cord.

Cerebrospinal fluid: Provides the cushion for the brain and the spinal column by occupying the space between the arachnoid and the pia mater.

Chain of custody: The process of documentation of each step of patient test analysis, from specimen collection to reporting of results. Persons involved at each stage must be documented and the custody specimen must be maintained by keeping it in a secure storage area.

Chemical hygiene plan: Laboratory plan which addresses the policies, procedures, etc. that ensure that employees are protected from harm due to chemicals.

Chlorhexidine: Preservative used to stabilize urine for routine testing for up to 72 hours.

Chloroform: Preservative used for inhibiting bacterial growth, but it is not recommended for the routine specimen because it causes changes in the characteristics of the cellular sediment.

Chorion: Fetal sac.

Choroidal cells: Cells that line the choroid plexus.

Choroid plexus: Area of the brain where cerebrospinal fluid is produced.

Chromogens: Substances used in chemical testing requiring the development of a color during the reaction.

Chronic glomerulonephritis: Advanced stage of several kidney disorders, resulting in inflammation and slowly worsening destruction of glomeruli, with progressive loss of kidney function.

Chyle: A milky appearing body fluid that consists of lymph and emulsified fats.

Chyme: The semifluid mass of partly digested food expelled by the stomach into the duodenum.

Clarity: The degree of urine transparency, often reported as clear, hazy, or cloudy.

Clean-catch: Clean-voided midstream urine specimen obtained after special cleansing procedure.

CLIA'88: The Centers for Medicare & Medicaid Services regulates all laboratory testing other than research performed on humans in the United States through the Clinical Laboratory Improvement Amendments to ensure laboratory-testing quality.

Clinical Laboratory Standards Institute (CLSI): A nonprofit organization that utilizes laboratory volunteers to establish voluntary laboratory consensus standards to ensure the highest laboratory quality.

Clinitest: Trademarked name for tablet test used to detect reducing substances such as various sugars.

Clue cells: Sloughed off squamous epithelial cells covered with numerous small thin, curved gram-variable bacilli.

Collecting duct: The end of the nephron, following the distal convoluted tubules of several nephrons that attach to it. This duct transports the developing urine through the cortex and medulla to the renal papilla and into the renal calyces of the kidney.

College of American Pathologists (CAP): An organization of board-certified pathologists that the Centers for Medicare & Medicaid Services also grants laboratory accreditation deeming authority.

Colligative property: Changes in characteristic of a solution depending on its concentration.

Color: Appearance of urine determined by type and amount of pigment present.

Colorectal cancer: Relatively common cancer of the gastrointestinal tract.

Commission on Office Laboratory Accreditation (COLA): A clinical laboratory education, consultation, and accreditation organization.

Compensated light microscopy: Microscopy in which a filter composed of highly linear polymers is placed between the two crossed filters of a polarizer.

Compliance: Adherence to established laws.

Condenser: Part of a microscope that evenly distributes light coming from the primary source.

Confidential information: All medical personnel involved in testing and procedures need to maintain the patient's privacy and not divulge their medical information to unauthorized persons.

Confocal microscopy: Microscopy in which both the illumination and detection optics are focused down on a single volume element of the specimen.

Control: Specimen that is similar in composition to those patient specimens being tested. Control specimens are used to monitor a test or tests because their value ranges have been previously established.

Cortex: The outermost part of the kidney that has a granular appearance and contains the glomeruli and the convoluted tubules.

Countercurrent multiplication: A process occurring in the loop of Henle that maintains the osmotic gradient in the medullary interstitium while allowing the body to further concentrate urine.

Creatinine clearance: A test used to assess glomerular filtration by assessing serum creatinine.

Creatorrhea: Undigested muscle fibers in the feces due to deficiency of pancreatic trypsin.

Critical values: Laboratory test values that indicate a critical situation for the patient requiring phoning or otherwise rapidly communicating results to the physician for immediate intervention for the patient.

Crystal-induced arthritis: Caused by the accumulation of crystals in the joints of persons with gout or pseudogout.

Cylindroids: Urine sediment that resemble casts but have one end which tapers out like a strand of mucus.

Cystatin C: A protein encoded by the CST3 gene, is mainly used as a biomarker of kidney function.

Cystic fibrosis: A hereditary disease affecting mucous secretion in the pancreas and lungs.

Cystinuria: An inherited autosomal recessive metabolic disorder characterized by the formation of cystine stones in the kidneys, ureter, and bladder. Excessive concentration of cystine is due to inadequate reabsorption, transport, and metabolism of this amino acid.

Cystinosis: Generalized aminoaciduria not limited to arginine, cysteine, lysine, and ornithine.

Cystitis: An inflammation of the bladder.

Cytocentrifuge: A centrifuge specifically designed to separate cells from fluids and deposit them onto slides.

Cytogenetic studies: Analysis of chromosomes and DNA.

Darkfield microscopy: Microscopy which produces a bright specimen image against a dark or black background.

Decidua: Uterine lining of the placenta.

Delta check: A system of monitoring test results to assess whether a biological unlikely change in values has occurred that might indicate an error or that the wrong patient may have inadvertently been tested.

Department of Health and Human Services (HHS): The United States government's principal agency for protecting the health of all Americans and providing essential human services.

Diabetes insipidus: A rare form of diabetes caused by a deficiency of the pituitary hormone vasopressin, which regulates kidney function.

Diabetes mellitus: A chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three types: Type 1, Type 2 and gestational.

Diabetic nephropathy (Kimmelstiel–Wilson disease): Continual damage to the glomerular membrane due to thickening and increased cellular proliferation and an accumulation of solid substances around the vascular tuft.

Diarrhea: An increase in the frequency of bowel movements or a decrease in the form of stool (greater looseness of stool). Although changes in frequency of bowel movements and looseness of stools can vary independently, changes often occur in both.

Diarthotic: Movable articulating joints.

Differential contrast microscopy: Microscopy in which intensity differences in the specimen image are produced through the use of birefringent crystal prisms as beam splitters.

Distal convoluted tubule: The portion of the nephron tubule between the loop of Henle and the collecting duct.

Diurnal variation: Changes in concentration based on the time of day.

Dry preparations: Smears of specimens are fixed and stained.

Dura mater: The outermost membrane covering the brain.

Dural sinuses: Venous channels in the dura mater that drain blood from veins in the brain and cerebrospinal fluid from the subarachnoid space into the internal jugular vein.

Dysentery: A disorder of the digestive system that results in severe diarrhea containing mucus and/or blood in the feces which if untreated is often fatal.

Edema: An abnormal accumulation of fluid beneath the skin, in the tissues.

Efferent arteriole: This arteriole exits the glomerulus and is smaller than the afferent arteriole, creating a higher glomerular filtration pressure.

Effusions: Accumulations of fluid into body cavities.

Endogenous substance: Chemicals normally found in the body.

Ependymal cells: Line the ventricles of the brain and spinal cord.

Epididymis: Connects the testicle to its vas deferens and is the site of sperm maturation and storage.

Erythroblastosis fetalis: Diseases of the newborn.

Erythrophagocytosis: Term used to indicate that macrophages have engulfed red blood cells.

Estimated glomerular filtration rate: An estimate of actual creatinine clearance calculated using the Cockcroft–Gault formula that employs creatinine measurements and a patient's weight to predict the creatinine clearance.

Examination errors: Formerly called analytical errors are errors that occur during testing.

Exogenous substance: Chemicals not normally found in the body.

Exposure control plan: A laboratory plan that sets forth the requirements for personnel and environmental protection from pathogens that may contaminate human blood and blood-associated materials with the assumption that all specimens may contain pathogens.

Exudates: Effusions that filter from the circulatory system into areas of inflammation.

Fanconi syndrome: Occurs when the functions of cells in renal tubules are impaired, leading to abnormal amounts of carbohydrates and amino acids in the urine, excessive urination, and low blood levels of potassium and phosphates.

Fatty acid oxidation disorders: A group of inherited metabolic conditions that lead to an accumulation of fatty acids, and a decrease in cell energy metabolism.

Fecal leukocytes: White blood cells in the stool especially neutrophils, are commonly associated with dysentery or invasion of the intestine.

Fecal occult blood: Nonvisible blood in the feces.

Fern test: Test used differentiate amniotic fluid from maternal urine.

Fetal fibronectin (fFN): An adhesive glycoprotein produced by fetal cells and is found in the space between the chorion and the decidua.

Fetal lung maturity: Determined by testing amniotic fluid prior to preterm delivery and can be a factor in determining the timing of delivery in these cases.

Fetal lung surfactant: A surface-active lipoprotein complex (phospholipoprotein) formed by type II alveolar cells.

Fetal neural tube defects: Disorders such as anencephaly and spina bifida.

Field diaphragm: Part of a microscope that limits diameter of the light beam entering the substage condenser as well as reduces stray light.

Filtration pressure: Also called capillary hydrostatic pressure, is one of the pressures that regulate the outward flow of fluid from the capillary.

First morning urine: The most concentrated urine and preferred specimen of choice.

Flavins: Compounds derived from B vitamins that contribute a yellow color to fluids, mainly semen.

Fluorescent light: Microscopy in which a selected wavelength of light is presented to the specimen, absorbed by a fluorescent substance (if present), and upon excitation emits light at a different, longer wavelength, which is transmitted to the eyepiece for viewing.

Foam stability test: A screening test for fetal lung surfactant in amniotic fluid.

Focal segmental glomerulosclerosis (focal glomerular sclerosis; focal nodular glomerulosclerosis): Causes nephrotic syndrome in children and adolescents, and kidney failure in adults.

Formalin: Preservative for urinary sediment and other tissues.

Freezing point depression: Method that measures osmolality by determining colligative properties using freezing point depression do so by super cooling a measured amount of sample.

Galactosuria: Presence of galactose in urine to do hereditary causes

Gastrin: A peptide hormone that stimulates gastric acid (HCl) secretion by the stomach's perietal cells and aids in gastric motility. The release of gastrin is stimulated by peptides found in the lumen of the stomach.

Gastric occult blood: Non-visible blood present in gastric fluid.

Glioma: A type of brain tumor that arises form glial cells.

Glitter cells: WBCs (neutrophils) that demonstrate brownian movement of their granules in dilute or hypotonic urine.

Globally Harmonized System (GHS): Globalizes system for chemical or reagent labeling.

Glomerular disease: Disorders of the glomerulus that present with nephritic or nephrotic syndrome.

Glomerular filtrate: The beginning of urine formation; the fluid leaving the glomerular capillary and going into Bowman capsule and then into the proximal convoluted tubule.

Glomerular filtration barrier: Structure within the glomerulus that determines the composition of the ultrafiltrate based on molecular size and electrical charge.

Glomerular filtration rate: The rate of plasma volume cleared by the glomeruli per unit of time (milliliters per minute). This rate is determined using substances that are known to be cleared exclusively by glomerular filtration (not reabsorbed or secreted by the nephron).

Glomerulonephritis: Diseases characterized by inflammation and damage of the glomeruli due to immunologic, metabolic, and hereditary causes.

Glomerulus: A tuft of capillaries involved in blood filtration and urine formation that is located in Bowman capsule where the blood filtrate becomes the ultrafiltrate.

Glycosuria: Presence of significant amounts of glucose in the urine.

Goodpasteur syndrome (Goodpatur's disease): An uncommon autoimmune disease caused by anti-glomerular basement antibody (anti-GBM).

Ground pepper: Appearance of ochronotic shards when present in synovial fluid.

Hartnup syndrome: A rare disorder that results in general difficulty for the body to absorb a variety of amino acids.

Hazardous Materials Identification System (HMIS): System of chemical labeling that conveys broad health warning information but is not intended for emergency handling information.

Hazard statement: Describes the nature and degree of the chemical's hazard(s).

HAZMATs: Potentially hazardous materials that pose a chemical or biological hazard.

Health Insurance Portability and Accountability Act (HIPAA): Law enacted by the US Congress in 1996 to protect health insurance coverage for workers and their families when they change or lose their jobs, and the privacy of electronic healthcare transactions, national identifiers for providers, health insurance plans, and employers.

Hematin crystals: Heme breakdown products that accumulate and crystalize in macrophages that have digested red blood cells.

Hematochezia: Bright red blood in feces.

Hematuria: The presence of blood or intact red blood cells in the urine.

Hemocytometer: Specialized slide with a grid for counting cells in a dilution of a fluid.

Hemoglobinuria: The presence of free hemoglobin in the urine as a result of intravascular hemolysis.

Hemolytic disease of the newborn or erythroblastosis fetalis: Caused when mother developed antibodies to an antigen on the fetal erythrocytes cross the placenta and destroy fetal red blood cells.

Hemorrhage: The leaking of blood leaks from blood vessels to spaces inside the body.

High-complexity testing: Tests that are categorized as of high complexity and are regulated under CLIA and are subject to accreditation, personnel regulations, quality management, and inspection regulations.

Hilus: Opening in the middle of the concave medial border of the kidney for nerves and blood vessels to pass into the renal sinus.

Homocystinuria: Inherited autosomal recessive disorder of amino acid metabolism due to the lack of the enzyme cystathionine β-synthase necessary for metabolism of methionine.

Homogentisic acid: Also known as melanic acid, accumulation of excess homogentisic acid is a result of the failure of the enzyme homogentisic acid 1,2-dioxygenase due to a genetic mutation in this enzyme and is associated with alkaptonuria.

Hyaluronate: A mucopolysaccharide synthesized by the synovium and other connective tissues comprising extracellular matrix found in joint fluid connective tissue, epithelium, and neural tissues.

Hydramnios: An increase in amniotic fluid volume.

Hydronephrosis: Dilation of the pelvis and calyces of one or both kidneys.

5-Hydroxyindoleacetic acid: The main metabolite of serotonin in the human body.

Hypermotility: Excessive movement of the involuntary muscles, particularly in the GI tract resulting in frequent bowel movements.

Hypersthenuria: The excretion of urine of unusually high specific gravity.

Hyposthenuria: Urine with a consistently low specific gravity.

Ictotest: Trademarked name for tablet test used to detect bilirubin.

Immunoglobulm a nephropathy (Berger's Disease): A glomerular inflammatory kidney disease caused by IgA antibodies. Up to 50% of cases progress to end stage renal disease or kidney failure.

Inborn errors of metabolism: Congenital disease with a metabolic defect, enzyme deficiency, that causes a buildup of metabolites in the blood and/or urine.

Infertility: Refers to the biological inability of a male or a female to contribute to conception.

Informed consent: The right of a patient to know and agree or disagree to what is going to be done in a medical test and what will be done with the results of the test.

Interference contrast: Microscopy in which differences in the optical path through the specimen are converted to intensity differences.

Interstitial fluid pressure: Also called tissue colloidal pressure, is one of the pressures that regulate the outward flow of fluid from the capillary.

Iris diaphragm: Also called an aperture diaphragm, controls the angle of light passing from the lamp through the aperture.

Isotheuria: Fixed urine specific gravity of 1.010, which indicates poor tubular reabsorption.

Juxtaglomerular apparatus: A group of cells from the afferent arteriole that works together with the efferent arteriole, the macula densa of the distal tubule, and the mesangium; and releases the hormone renin.

Ketones: Acetone, beta hydroxyl butyric acid, and diacetic acid are breakdown products formed during the catabolism of fatty acids.

Kohlar illumination: Adjustment made to lighting so the lamp filament is not visible in the field of view.

KOH preparation: Procedure, used in the amine or "whiff" test slide prepared for examination vaginal secretions to detect bacterial vaginosis. Kohler illumination adjustments made so that the image of the lamp filament does not appear in the field of view but is actually focused in the plane of the aperture diaphragm.

KOVA system: Commercially available system used to standardize the procedure for microscopic examination of urinary sediment.

Lamellar bodies: Storage forms of lung phospholipids counts provide a reliable estimate of fetal lung maturity.

Lesch–Nyhan disease: An inherited disorder of purine metabolism that results in excessive uric acid in urine.

Leukocyte esterase: Enzyme present in neutrophils.

Liley graph: Graph used to classify fetal risk.

Lipophage: Macrophage containing lipids (fats) from cellular degeneration.

Liquefaction (of coagulated semen): Occurs as a result of enzymatic action.

Loop of Henle: A structure of the nephron that is located in the medulla, at the end of the proximal tubule composed of a thin descending limb, a "U" bottom, and a thicker ascending limb.

Lumbar puncture: Procedure that is performed to collect a sample of cerebrospinal fluid.

Lypophage: Lipid-laden/fat-filled macrophage.

Macula densa: A specialized area of cells of the distal convoluted tubule that interact with the juxtaglomerular apparatus.

Major calyx: Formed by several minor calyces joining together.

Malabsorption: Inadequate or defective absorption of nutrients from the GI tract.

Maldigestion: Incomplete digestion that occurs as a result of pancreatic exocrine or bile salt deficiency.

Maple syrup urine disease (MSUD): An accumulation of branched chain amino acids such as valine, leucine, and isoleucine, which spill over into the urine producing a sweet odor.

Meconium: A newborn's first fecal bowel movements.

Medical negligence: Medical negligence occurs when a hospital, physician, or other healthcare provider does not treat a patient with the professionally recognized medical standard of care, resulting in injury, harm, or death.

Medulla: The inner, hypertonic portion of the kidney that microscopically contains the loop of Henle and the collecting ducts of the nephron and macroscopically contains the renal pyramid structures.

Medulloblastoma: A brain tumor that originates in the cerebellum.

Melanin: Dark pigment that provides coloration to the skin.

Melena: Black, tarry feces associated with gastrointestinal hemorrhage.

Meninges: The layers of membrane covering the brain.

Meningiomas: Brain tumors that arise from the arachnoid cells.

Microalbuminuria: A condition in which the glomerulus leaks a small amount of albumin into the urine. Microalbumin screening is used to detect early signs of kidney damage in people who have a risk of kidney disease.

Middle ear effusion (MME): Excess fluid in the tympanic cavity.

Minor calyx: A funnel-shaped space in the kidney formed by the tips of the renal pyramids, the papillae.

Moderate complexity testing: Tests that are categorized of medium complexity and are regulated under Clinical Laboratory Improvement Amendments of 1988 (CLIA) and require meeting accreditation, personnel regulations, quality management, and inspection regulations.

Modulation contrast: Microscopy in which light intensity varies above and below an average value.

Morphology of cells: The observance and description of cell size and shape (e.g., sperm).

Motility evaluation: Describes the degree of forward motion of sperm.

Mucin: A glycosylated protein that combines with hyaluronate to form the lubricant in synovial fluid.

Myoglobin: The heme protein of striated muscle.

Nelson illumination (critical illumination): Produces an image of the lamp filament (or the ground glass in front of the lamp condenser) superimposed on the image of the specimen.

Nephritis: Inflammation of the nephrons (kidney).

Nephron: The major functional unit of the kidney filters waste products from the blood and forms urine. Each kidney contains more than 1 million nephrons. It is a microscopic structure composed of the blood supply to and around the nephron, the glomerulus, Bowman capsule, proximal tubules, distal tubules, loop of Henle, and the collecting duct.

Nephrosis (nephrotic syndrome): A noninfectious nephropathy characterized by edema and large amounts of protein and lipids in the urine and usually increased blood cholesterol, often involving degeneration of renal tubular epithelium. It often follows a glomerulonephritis or a systemic disease.

Neutrophils ragocytes: Neutrophils in synovial fluid appearing to have dark cytoplasmic granules and are sometimes called RA cells.

Newborn screening: Blood tests for newborn disorders collected onto special filter paper which is then sent to the state or regional health department for testing.

Nitrite: A substance formed by the reduction of nitrate by nitrate-reducing bacteria.

Normozoospermic: Normal sperm count, motility, and morphology.

Objective lens: Lens that produces the magnified primary image of the specimen.

Occult blood: Hidden blood not readily seen macroscopically.

Occupational Safety and Health Administration (OSHA): US federal agency charged with protection of employees from potential hazards in the workplace through regulation and workplace monitoring.

Ochronotic shards: Debris from metal and plastic joint prosthesis.

Ocular: Eyepiece.

Oligohydramnios: Abnormally decreased amounts of amniotic fluid.

Oligospermia: Term for low sperm count.

Oligozoospermic: Less than normal sperm count with normal sperm motility, and morphology.

Oliguria: A significant decrease in urine production or excretion (<400 mL/day).

Optical tube: Microscope part that provides a light-tight support and correct spacing for the objective lenses and the eyepieces.

Osmolality: A measure of concentration that is directly proportional to the amount of solute regardless of the type of solute.

Otitis media with effusion (OME): Inflammation of the middle ear with collection of fluid in the tympanic cavity.

Otorrhea: A significant amount of fluid discharge from the ears such as occurs from leaking of cerebrospinal fluid into the ear.

Oval fat bodies: Renal tubular epithelial cells or macrophages filled with lipids to the point where they may not be recognizable as cells.

Pancreatic insufficiency: A less than normal amount of digestive enzymes are being secreted by the pancreas into the intestine.

Parabasal cells: Immature epithelial cells of the vagina that are smaller than squamous cells and are usually round in appearance.

Paracentesis: General term used for puncture of any body cavity.

Parafocality: A state in which the distance between the sample and the intermediate image is made the same for each member of the objective lens set.

Penetration: The ability of sperm to penetrate the egg's zona pellucida. This is measured in vitro by observing the distance traveled by sperm in a thin tube of cervical mucus.

Pericardiocentesis: A procedure in which fluid is aspirated from the pericardium.

Pericardium(ial): A double-walled sac that contains the heart and the roots of the great vessels.

Peritoneal lavage: A procedure performed using a syringe to infuse saline into the abdomen and aspirate fluid for analysis.

Peritoneum(al): The serous membrane forming the lining of the abdominal cavity.

Peritubular capillaries: The capillaries that surround the renal tubules in the renal cortex.

Personal protection equipment (PPE): Equipment used to protect workers from chemical or biological hazards and may include face shield, goggles, gloves, gown, laboratory apron, mask, and respirator.

pH: Hydrogen ion concentration.

Phase contrast: Microscopy in which subtle differences in refractive index and the subtle changes in phase these produce are converted into clear-cut variations of light intensity and contrast.

Phenazopyridine (Pyridium): Acts as an analgesic in the bladder and gives an orange color to the urine.

Phenylketonuria: A gene tic disorder in which the body lacks the enzyme necessary to metabolize phenylalanine to tyrosine. Left untreated, the disorder can cause brain damage and progressive mental retardation as a result of the accumulation of phenylalanine and its breakdown products.

Physician office laboratories (POLs): Clinical laboratory associated with physician office laboratories.

Physician-performed microscopy: Microscopic examinations that physicians are allowed to perform as regulated by CLIA '88.

Pia-arachnoid mesothelial cells: Epithelial cells that originate from the mesoderm and line the pia and the arachnoid.

Pia mater: Innermost layer of membrane covering the brain.

Pictogram: A symbol that conveys specific information about the hazards of a chemical.

Placenta: Organ surrounding the fetus and that forms amniotic fluid.

Placental alpha microglobulin-1 (PAMG-1): A protein expressed by the cells of the decidual part of placenta.

Plan achromat: Objective lens corrected for curvature of field

Pleocytosis: Term used to describe an increased cell count in blood or body fluids.

Pleura: Referring to the space between the parietal and visceral layers of the cavity surrounding the lungs.

Podocytes: The specialized epithelial cells that line the inner space of Bowman capsule that is a crucial component of the glomerular filtration barrier.

Point-of-care testing (POCT): Diagnostic testing performed at or very near the point of patient care.

Polarizing microscopy: Microscopy in which a polarizer permits light vibrating in an east-west direction perpendicular to the light path to pass to the specimen, while the other filter permits the passage of light that is vibrating in a north-south direction perpendicular to the light path. When the two filters are "crossed," the field appears black unless an optically active material is present to rotate the plane of the polarized light.

Polyuria: Excess urine production.

Porphobilinogen (PBG): A pyrrole in porphyrin metabolism generated by aminolevulinate (ALA) and the enzyme ALA dehydratase.

Porphyria: An inherited disorder of pigment metabolism with excretion of porphyrins in the urine and dangerous sensitivity to sunlight.

Porphyrins: Organic compounds containing four pyrrole rings, occurring universally in protoplasm, and functioning as a metal-binding cofactor in hemoglobin, chlorophyll, and certain enzymes.

Post-examination errors: Formerly called post-analytical errors are errors that occur after the testing process is completing in recording or communicating the results.

Postprandial: Specimens collected 2 to 3 hours after eating.

Potter complex: Pulmonary hypoplasia, contractures of the limbs, and nodular appearance of the amnion.

Precautionary statement: Explains how to prevent adverse effects due to exposure to a hazardous chemical.

Pre-examination errors: Formerly called pre-analytical errors are errors whose source is prior to testing.

Proficiency testing: Quality control is established between clinical laboratories by these unknown samples that are sent to a group of laboratories for analysis with comparison of results between laboratories.

Prostate gland: Male endocrine gland that contributes approximately 20% to the volume of semen. Prostatic fluid contains many proteolytic enzymes.

Protein error of indicators: A color-change phenomenon occurring because proteins act as hydrogen ion acceptors at a constant pH.

Proteinuria: The presence of protein in urine.

Proximal convoluted tubule: Portion of the nephron tubule between Bowman capsule and the loop of Henle that contains brush border cells on its luminal surface.

Pseudochylous: Describes the appearance of an effusion that resembles chyle.

Purines and pyrimidines: Molecules that are found naturally occurring in all living things and provide the basic building blocks for DNA and RNA.

Pyelonephritis (acute and chronic): Infection or inflammation of the nephrons of the kidney.

Pyuria: The presence of pus in the urine.

Quality assessment: A variety of methods and measures to ensure quality patient care.

Quality assurance and process improvement (QAPI): The discovery of problems in a process and the means by which they are corrected.

Quality control: Methods utilized to ensure the accuracy and precision of laboratory test procedures.

Ragocyte: Neutrophils containing inclusions of immune complexes.

Random specimen: Sample collected at any time convenient for the patient and is usually sufficient for performance of most urinary screening tests.

Rapidly progressive (crescentic) glomerulonephritis: A form of acute kidney disease that causes damage to the glomeruli and progressive loss of kidney function over weeks to months. This condition is also called necrotizing or crescentic glomerulonephritis (due to its pathologic appearance with crescent-shaped glomeruli).

Reabsorption: Movement of substances through either active or passive transport from the tubular ultrafiltrate back into the peritubular blood and body.

Reagent strip: A plastic strip containing absorbent reagent pads that are impregnated with substances that generate specific chemical reactions for the detection of various urine analytes.

Reducing substance: A substance that removes oxygen from a compound.

Refractive index: The ratio of the velocity of light in air to the velocity of light in solution.

Refractometer: Instrument that measures total solids (TS) of a solution.

Renal columns: A medullary extension of the renal cortex in between the renal pyramids anchoring the cortex. Each column consists of blood vessels and fibrous material.

Renal failure: A situation in which the kidneys fail to function adequately. It occurs as acute and chronic form and either form may be due to a large number of other medical problems. It is typically detected by an elevated serum creatinine or the estimated glomerular filtration rate.

Renal glycosuria: A rare condition in which glucose is excreted in the urine despite normal or low blood glucose levels due to improper functioning of the renal tubules causing glycosuria.

Renal pelvis: Funnel-shaped structure adjacent to the indented area of the kidney that collects urine from the renal calyces and conveys the urine to the ureters.

Renal pyramids: Cone-shaped tissues of the renal medulla with the broad base of each pyramid facing the renal cortex and its apex, or papilla, pointing internally.

Renal sinus: A cavity within the kidney occupied by the renal pelvis, renal calyces, blood vessels, nerves, and fat.

Renin: An enzyme produced and stored by the cells of the juxtaglomerular apparatus of the renal nephron that converts angiotensinogen into angiotensin and results in the secretion of aldosterone, thus increasing blood pressure.

Respiratory distress syndrome: A condition in newborns caused by a lack of fetal lung surfactant.

Rheumatoid arthritis: A chronic inflammatory disorder that primarily attacks joints, causing synovitis and destroys the articular cartilage of the bones.

Rheumatoid factor (RF): An antibody to immunoglobulins.

Rhinorrhea: A significant amount of nasal discharge. One of the causes for rhinorrhea is the leaking of cerebrospinal fluid into the nasal cavity.

Rice bodies: Free-floating aggregates of tissue.

Rope's test: A laboratory test that evaluates the integrity of the hyaluronate-mucin complex. A sample of synovial fluid is acidified and observed for the formation of a clot, which is manipulated to determine its strength.

Run-over effect: The spilling over of chemicals from one reagent pad to another causing misreadings of color reactions.

Rupture of fetal membranes (ROM): Tearing of the amniotic sac with release of amniotic fluid.

Safety data sheets (SDS): Written notification, which is to be available to workers, with information on the hazards associated with and the precautions to be taken with specific chemicals.

Sanguineous: Term used to describe the color of body fluids appearing bloody.

Scanning electron microscopy: Microscopy in which images are produced by scanning specimens with a focused beam of electrons that interact with atoms in the specimen.

Secretagogue: An agent that promotes secretion such as acetylcholine, gastrin, or secretin.

Secretion: Movement of substances through either active or passive transport from the body via the peritubular blood into tubular ultrafiltrate to be excreted.

Semen analysis: Several laboratory tests performed on semen samples to evaluate fertility.

Semenogelin: A fibrinogen-like precursor substance that is produced by the seminal vesicles.

Seminal vesicles: Pair of tubular glands that lie behind and slightly below the bladder in males.

Seminiferous tubules: Located in the testes and are the site of sperm meiosis.

Septic arthritis: A type of arthritis that is caused by infection in the joint.

Serous: Term used to describe body fluids that are pale yellow and transparent, resembling serum.

Sertoli cells: Part of the germinal epithelium of the seminiferous tubules, give rise to spermatozoa.

Shield of negativity: Impediment, produced by negatively charged components of the glomerular filtration barrier that limits the filtration of negatively charged particles from the blood into the urinary space.

Siderophages: Macrophages containing digested erythrocytes, and hemosiderin (iron).

Signal word: Either "danger" or "warning" is used to indicate the level of severity of hazard.

Specific gravity: The ratio of the weight of a volume of urine to the weight of the same volume of distilled water at a constant temperature.

Spermatogenesis: The process of sperm formation and maturation involving the Sertoli cells of the seminiferous tubules of the testes.

Spermatozoa: The haploid cell that is the male gamete.

Spermiogenesis: The phase in which the gamete cell develops a flagellum and transforms from a spermatid into a spermatozoon.

Standard: Very pure substances of known concentration used to establish accurate standard curves or calculations of test analytes.

Standard of care: The standard of patient care that a reasonable person would take to prevent injury or harm.

Standard precautions: These guidelines stress safe work practices to prevent disease transmission that include the following as well as guidelines on handling biological waste.

Steatorrhea: Condition in which the feces contains increased levels of fat due to poor fat absorption in the GI tract.

Sterile pyuria: Increased urine leukocytes but no bacteria are present.

Sternheimer–Malbin: Stain consisting of crystal violet and safranin used to stain urinary sediment.

Suprapubic aspiration: Insertion of a needle directly into the distended bladder.

Supravital: Specimens stained in the living state.

Syndrome of Inappropriate Antidiuretic Hormone (SIADH): The excretion of ADH when it is not needed.

Synovial: Term that refers to the joints. Synovial fluid is a thick fluid that resembles (syn) egg whites (ovum) and is found in the cavities of synovial joints.

Systemic lupus erythematosus (SLE): A chronic autoimmune connective tissue disease that can affect any part of the body. SLE cells can be found in synovial fluid of a particular joint if it is affected by the disease.

Tamm–Horsfall protein: Secreted by the renal tubular cells in the ascending loop of Henle and forms the matrix of urinary casts.

Teratozoospermic: Normal sperm count and motility but less than normal morphology.

Testis: The male glands in which are located the seminiferous tubules, the site of sperm formation.

Thoracentesis: A procedure used to remove fluid or air from the pleural space around the lungs.

Three-glass collection: All portions of urine, beginning, middle, and final portion of the void, are collected in three separate containers.

Threshold substance: Any blood constituent (such as glucose) that is excreted in the urine only when its plasma concentration exceeds a certain value.

Thymol: An adequate but rarely used preservative for most urinary constituents.

Timed specimen: Specimens collected at a specific time of day to assess substances having diurnal variation.

Toluene: Preserves, ketones, proteins, and reducing substances, but it is not effective against bacteria already present in the urine though not often used due to flammability and problems with separation from specimens.

Transmission electron microscopy: Microscopy in which a beam of electrons interacts with a specimen as they are transmitted through the specimen.

Transudates: Effusions that result from increased fluid pressures or decreased osmotic pressures in the plasma.

Traumatic tap: A lumbar puncture that has nicked a blood vessel or vertebrae.

Trichomoniasis: Caused by *Trichomonas vaginalis,* is the most common parasitic cause of vaginosis.

Tubular disorders: Conditions can affect the tubules via a buildup of metabolic byproducts that exceed the ability of the kidney tubules to reabsorb them.

Tubulointerstitial disease: Infections and inflammatory conditions that affect both the interstitium and the tubules, which are in close proximity.

Tyrosinemia: Inherited disorders of tyrosine metabolism can result in three types of tyrosinemia, each caused by deficiency of a different enzyme.

Ultrafiltrate: A solution that has passed under pressure through the semipermeable membrane with very small pores in the glomerulus.

Upper gastrointestinal bleeding (UGIB): Bleeding in the esophagus, stomach, or duodenum.

Uremia: Increased levels of blood urea nitrogen (BUN) in the blood.

Ureter: Tube carrying urine from the renal pelvis of the kidney to the bladder.

Urethra: Tube connecting the bladder to the outside of the body that is shorter in females and longer in males where it runs through the penis.

Urinary sediment artifacts: Structures not arising from the urinary tract but may be present in the urine by accident and include air bubbles, cloth fibers, hair, glass fragments, starch or talc crystals, and contaminants such as fecal matter.

Urinary sediment casts: Structures formed in, and taking on the shape of, the lumen kidney tubules. Urinary casts, which are comprised of Tamm–Horsfall protein, may be hyaline or include any cells or crystals that may be present in the tubules.

Urinary sediment cells: Cells that may be present in the urine include red blood cells, white blood cells, renal tubule cells, squamous epithelial cells, bacteria, parasites, and yeast.

Urinary sediment crystals: Microscopic solid formations of chemicals that are normally or abnormally present in urine.

Urine collection bags: Soft, pliable plastic bags, suitable for collecting specimens from infants and small children by attaching them to the genitalia.

Urine eosinophils: Eosinophils seen in urine during acute interstitial nephritis, cystitis, glomerulonephritis, pyelonephritis, kidney transplant rejection, *Schistosoma haematobium* infection, and prostatitis.

Urinometer: A type of hydrometer that is an instrument that truly measures specific gravity.

Urobilin: A pyrrole resulting from the breakdown of heme produced when urobilinogen is oxidized by intestinal bacteria causing the brown pigment responsible for the normal color of stool.

Urobilinogen: A breakdown product of hemoglobin degradation formed from bilirubin by the action of intestinal bacteria.

Urochrome: Pigment that produces urine color varying from a pale yellow to dark amber.

Urolithiasis: The condition in which urinary calculi (kidney stones) are formed in the urinary tract.

Vapor pressure (dew point): Method of measuring colligative properties by determining the temperature at which water vapor in a sample condenses to its liquid state.

Varicocele: Dilation of pampinform venous plexus of the spermatic cord.

Vasa deferentia (vas deferens): Tubules that lead from the epididymis to transport sperm to the ejaculate.

Vasa recta: Long hair pin-shaped blood vessels that arise from the arteriole leading away from a renal glomerulus, descend into the renal medulla pyramids, reunite as they ascend, and play a role in the formation of urine.

Vasectomy: Excision of a segment of the vas deferens to produce sterility.

Vernix caseosa: Fine hair covering of fetal epidermis.

Viability: The parameter that is evaluated to determine whether sperm are dead or alive.

Viscosity: A term to describe the thick appearance of a fluid.

Vitreous fluid: Fluid of the vitreous body of the eye.

Waived tests: Simple laboratory tests and procedures that are cleared by the Food and Drug Administration for home use, that employ simple methodologies that are accurate and unlikely to cause error, and are tests that pose no reasonable risk of harm to the patient if the test is performed incorrectly.

Wet mount: Microscopic examination of a drop of freshly collected fluid.

Xanthochromia/Xanthochromic: These terms refer to a yellow appearance that is normal in serous body fluids but in fluids such as cerebrospinal fluid and synovial fluid indicates the presence of oxidized hemoglobin from lysed red blood cells.

Zollinger–Ellison syndrome (pancreatic tumor that secretes increased amounts of gastrin).

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