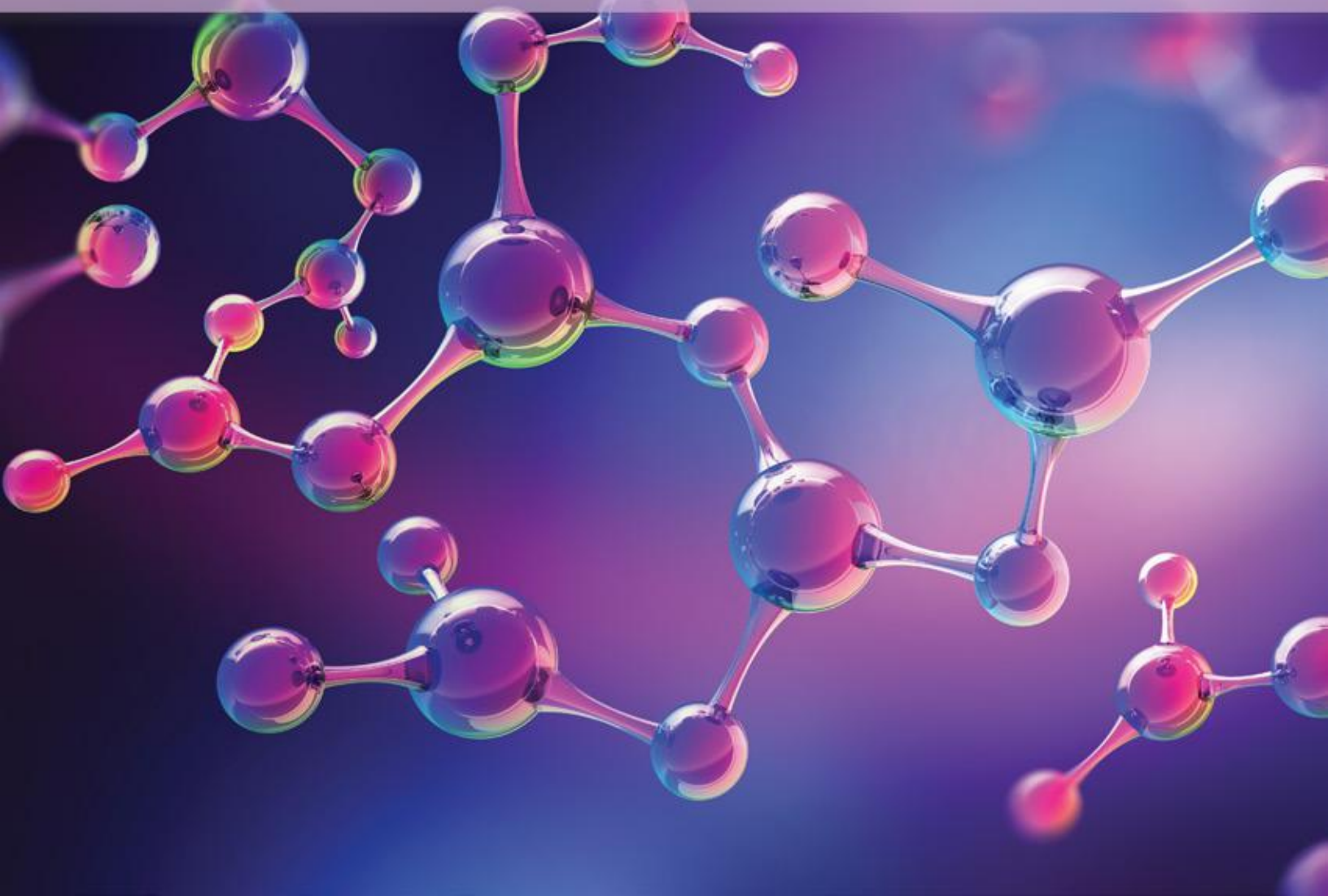
NINTH EDITION

CLINICAL CHEMISTRY

Principles, Techniques, and Correlations

Michael L. Bishop | Edward P. Fody
Carleen Van Siclen | James March Mistler | Michelle Moy



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Principles, Techniques, and Correlations

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The authors, editors, and publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accordance with the current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new or infrequently employed drug.

Some drugs and medical devices presented in this publication have Health Canada clearance for limited use in restricted research settings. It is the responsibility of the healthcare provider to ascertain the Health Canada status of each drug or device planned for use in his or her clinical practice.

Brief Contents

Contents

To all Clinical Laboratory practitioners, educators, and healthcare professionals for their previous and continuing extraordinary commitment, service, and professionalism during the COVID-19 pandemic.

MLB, EPF, CVS, JMM, MM

In memory of my mother and father, Betty Beck Bishop and William Stewart Bishop, Sr., for support, guidance, and encouragement.

To Sheila, Chris, and Carson for their support, patience, and inspiration.

MLB

To Nancy, my wife, for continuing support and dedication.

EPF

To Gary, my husband, for his support of my professional goals and to all the laboratory professionals, including my students, who have contributed to my knowledge and passion for lifelong learning.

CVS

To my husband, Keith, for everything.

JMM

To my college mentors: Pete Gebauer and Herb Miller, I thank you for believing in me.

In memory of my mother SG (1940–2021)

MM

Foreword

Many years ago, I wrote the Foreword to some earlier editions of this text. A ninth edition seems like an unbelievably long time until I reflect that this year is the 40th anniversary of the paper that introduced a multi-rule Shewhart control chart,[1] more commonly known as "Westgard Rules." That paper was written early in my career, but now in my retirement we have updated that approach to provide "Westgard Sigma Rules" in order to customize the QC design on the basis of the quality required by a test and the Sigma performance observed for a method.[2] Even well-established "standard" laboratory practices need periodic review and updating to keep current with the improvements in testing processes. Likewise, this 9th edition of the standard clinical chemistry text reflects the latest knowledge and improvements for laboratory science. That is a testament to the authors' commitment and dedication to providing an up-to-date knowledge base for the professionals in clinical laboratory science.

I am writing this on the one-year anniversary of the declaration of a global pandemic, a year during which over half a million Americans died of COVID-19. This pandemic has revealed the importance of laboratory testing for the health of the nation. Laboratory testing has often been viewed as a behind-the-scenes service in health care. During the pandemic, laboratory testing has been center stage as an essential service for assessing the state of disease, diagnosing those with infection, monitoring those under treatment, and monitoring the immunity and the health of the community.

Laboratory scientists were on the front line in introducing new diagnostic tests, validating their performance, and implementing testing in many diverse settings, including central laboratories, clinic laboratories, and point-of-care settings, including drive-through testing services. Understanding the performance of qualitative tests brought new importance to ideas such as clinical sensitivity, clinical specificity, and the predictive value of laboratory tests. That also meant new protocols for validating new tests to characterize test performance, including adaptations for the nature of molecular tests, such as the real-time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) methods that were critical in the early diagnosis and management of patients. Antibody tests flooded the market and required care and attention by laboratories, especially during the early phases when the FDA exercised very limited control of the companies introducing the new tests. Antigen tests emerged later and more slowly but were critical for providing more widespread diagnostic testing. All in all, this short time period has provided the lessons of a lifetime and demonstrated the importance of what you will be learning in your studies.

This new edition of *Clinical Chemistry: Principles, Techniques, and Correlations* continues its mission of addressing the formal educational needs of students in clinical laboratory science, as well as the ongoing needs of professionals in the field. It facilitates the educational process by identifying the learning objectives, focusing on key concepts and ideas, and applying the theory through case studies. It covers the basics of laboratory testing, as well as many special areas of testing. And it is still possible to carry this text with you to class, to the laboratory, to the office, or home to study!

Having personally worked with some of the editors and contributors, I know they have high standards both in the laboratory and in the classroom. Their interests and background provide an excellent balance between the academic and the practical, ensuring that students are exposed to a well-developed base of knowledge that has been carefully refined by experience.

For the many students for whom this book is intended, let me offer some advice from my close friend and mentor, Hagar the Horrible. It seems his young Viking son was embarking on a voyage to the real world of work. Needing advice, he asked "How do I get to the top?" Hagar's response, "You have to start at the bottom and work your way up." After pondering this for a moment, his son then asked, "How do I get to the bottom?" Hagar replied, "You have to

know somebody." The people you need to know are the authors of this book, as well as the instructors in your courses and your bench teachers in the laboratory. You need to seek them out to profit from their learning and experiences. They are the professionals who know the state of laboratory practice, possess the current knowledge of the field, and are dedicated to helping you become a successful laboratory scientist.

—James O. Westgard,
Madison, WI

References

1. Westgard JO, Barry PL, Hunt MR, Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 1981;27:493–501.
2. Westgard JO, Westgard SA. Establishing evidence-based statistical quality control practices. *Am J Clin Pathol* 2019;151:364–370.

Preface

The events with the worldwide pandemic have placed an extraordinary burden on our healthcare system. Facing staffing, PPE, and diagnostic supply shortages, healthcare professionals stepped up with effort, critical process evaluation, and extraordinary dedication to provide quality patient care with compassion and empathy. Initially, the nightly news became a presentation of CDC guidelines, mask mandates, business shutdowns, travel restrictions, metrics, trends, positivity rates, and hospitalization and death statistics. Months later, the metrics related to more positive information—initial results of vaccine clinical trials, emergency use authorizations, vaccine shipments, and "shots in arms." Through it all, the healthcare system functioned as effectively as possible due to individual efforts and interdisciplinary teamwork. Healthcare professionals have improved communication with each other, as well as with the patient and their families. Collaborative efforts between healthcare disciplines are emerging across the patient care spectrum landscape.

Since the initial idea for this textbook was discussed in a meeting of the Biochemistry/Urinalysis section of ASMT (now ASCLS) in the late 1970s, the only constant has been change and the never wavering commitment of the clinical laboratory professionals. Now almost 45 years since the initiation of this effort, the editors have had the privilege of completing the ninth edition with another diverse team of dedicated clinical laboratory professionals. In this era of focusing on metrics, the editors would like to share the following information. The 401 contributions in the 9 editions and supporting material represent 115 clinical laboratory science education programs, 83 clinical laboratories, 28 medical device companies, 4 government agencies, and 3 professional societies representing 40 states and territories. One hundred and sixty-four contributors were clinical laboratory scientists with advanced degrees. These contributors have produced 289 chapters citing 12,054 references for a total of 5,708 pages that included 2,158 figures and 691 case studies. With today's global focus, the previous editions of the text have been translated into at least six languages. By definition, a profession is a calling requiring specialized knowledge and intensive academic preparation to define its scope of practice and produce its own literature. The clinical laboratory science profession has evolved significantly over these past four-and-a-half decades.

Clinical chemistry continues to be one of the most rapidly advancing areas of laboratory medicine. New technologies and analytical techniques have been introduced, with a dramatic impact on the practice of clinical chemistry and laboratory medicine. In addition, the healthcare system itself is rapidly changing. There is ever-increasing emphasis on improving the quality of patient care, individualized medicine, patient outcomes, financial responsibility, and total quality management. Now, more than ever, clinical laboratorians need to be concerned with disease correlations, result interpretations, problem solving, quality assurance, and cost-effectiveness. Laboratory professionals need to know not only the *how* of tests but more importantly be able to communicate the *what, why*, and *when* to the patient and the healthcare team. The editors of *Clinical Chemistry: Principles, Techniques, and Correlations* have designed the ninth edition to be an even more valuable resource to both students and practitioners.

The ninth edition of *Clinical Chemistry: Principles, Techniques, and Correlations* is comprehensive, up-to-date, and easy to understand for students at all entry levels. It is also intended to be a practically organized resource for both instructors and practitioners. The editors have tried to maintain the book's readability and further improve its content while rearranging content and focusing on the scaffolding provided by the ASCLS MLT and MLS Entry Level Curriculum and the ASCP BOC guidelines. Because clinical laboratorians use their interpretative and analytic skills in the practice of clinical chemistry, an effort has been made to maintain an appropriate balance between analytic principles, techniques, and the correlation of results with disease states.

In this edition, the editors have maintained features in response to requests from our readers,

students, instructors, and practitioners. Ancillary materials have been updated and expanded. Chapters now include current, more frequently encountered case studies modelled after the nursing PICOT initiative in a structured, unfolding style. To provide a thorough, up-to-date study of clinical chemistry, all chapters have been updated and reviewed by professionals who practice clinical chemistry and laboratory medicine on a daily basis. The basic principles of the analytic procedures discussed in the chapters reflect the most recent or commonly performed techniques in the clinical chemistry laboratory. Detailed procedures have been omitted because of the variety of equipment and commercial kits used in today's clinical laboratories. Instrument manuals and analyte package inserts are the most reliable reference for detailed instructions on current analytic procedures. All chapter material has been updated, improved, and rearranged for better continuity and readability.

The **Navigate 2 Advantage** digital access contains additional case studies, review questions, teaching resources, teaching tips, student laboratory procedures, and teaching aids for instructors and students; it is included with the purchase of this textbook and is also available for separate purchase from the publisher.

One last piece of advice to make you successful in the field of clinical laboratory science:

Work with compassion, empathy, and professionalism until you no longer have to introduce yourself.*

Michael L. Bishop
Edward P. Fody
Carleen Van Siclen
James March Mistler
Michelle Moy

*Modified from Harvey Specter in *Suits*.

New to This Edition

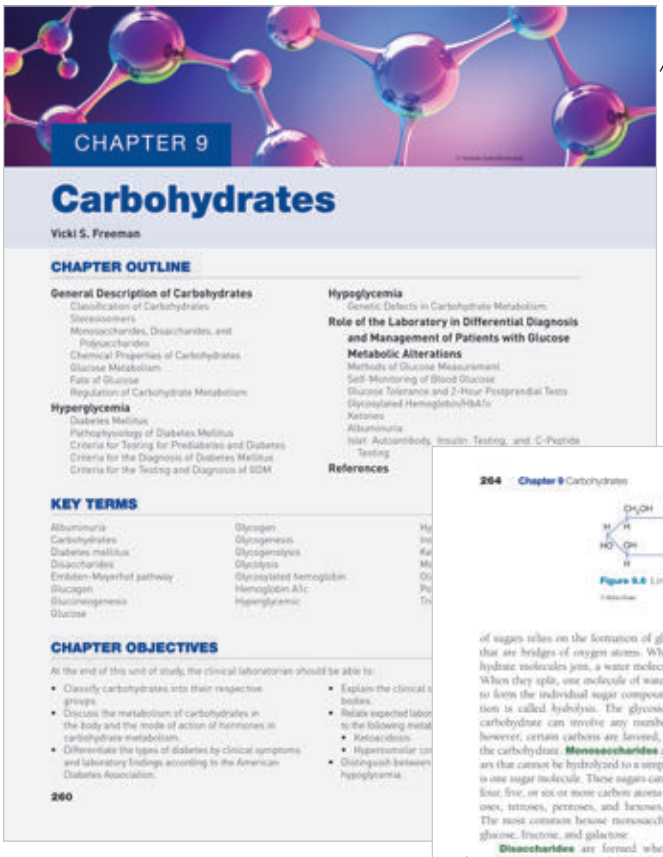
Medical laboratory science students need a strong foundation in applied chemistry to meet the requirements of certifying bodies and accreditation organizations that ensure students are prepared for employment.

This textbook provides clear explanations that balance analytic principles, techniques, and correlation of results with coverage of disease states, helping students develop interpretive and analytic skills for their future careers.

Updates to this edition include:

- Chapter content based on the ASCLS Entry Level Curriculum and current ASCP Content Guidelines
- Reorganization of chapter order to reflect clinical chemistry flow in most courses today
- Over 60 unique case studies that evolve throughout the chapters
- NEW Chapter 13: Basic Endocrinology
- NEW Chapter 24: Pregnancy and Prenatal Testing
- Reference range table is included as an Appendix in the printed book and online.

A map of how the textbook correlates to the ASCLS curriculum and ASCP guidelines is provided as an instructor resource.


CHAPTER 9

Carbohydrates

Vicki S. Freeman

CHAPTER OUTLINE

General Description of Carbohydrates
Classification of Carbohydrates
Stereoisomers
Monosaccharides, Disaccharides, and Polysaccharides
Chemical Properties of Carbohydrates
Glucose Metabolism
Fate of Glucose
Regulation of Carbohydrate Metabolism
Hyperglycemia
Diabetes Mellitus
Pathophysiology of Diabetes Mellitus
Criteria for Testing for Prediabetes and Diabetes
Criteria for the Diagnosis of Diabetes Mellitus
Criteria for the Testing and Diagnosis of GDM
Hypoglycemia
Genetic Defects in Carbohydrate Metabolism
Role of the Laboratory in Differential Diagnosis and Management of Patients with Glucose Metabolic Alterations
Methods of Glucose Measurement
Self-Monitoring of Blood Glucose
Glucose Tolerance and 2-Hour Postprandial Tests
Glycosylated Hemoglobin/HbA1c
Ketones
Albuminuria
Islet Autoantibody, Insulin Testing, and C-Peptide Testing
References

KEY TERMS

Albuminuria, Carbohydrates, Diabetes mellitus, Disaccharides, Embden-Meyerhof pathway, Glucagon, Gluconeogenesis, Glucose, Glycogen, Glycogenesis, Glycogenolysis, Glycolysis, Glycosylated hemoglobin, Hemoglobin A1c, Hyperglycemic

CHAPTER OBJECTIVES

At the end of this unit of study, the clinical laboratorian should be able to:

- Classify carbohydrates into their respective groups.
- Discuss the metabolism of carbohydrates in the body and the mode of action of hormones in carbohydrate metabolism.
- Differentiate the types of diabetes by clinical symptoms and laboratory findings according to the American Diabetes Association.
- Explain the clinical
bodies.
- Relate expected labor
to the following metab
- Ketoacidosis
- Hyperosmolar co
- Distinguish between
hypoglycemia

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Each chapter opens with a **Chapter Outline, Key Terms**, and **Chapter Objectives** that correlate to the ASCLS entry-level curriculum and current ASCP content guidelines.


264 Chapter 9 Carbohydrates

Figure 9.8 Linkage of monosaccharides.

of sugars relies on the formation of glycoside bonds that are bridges of oxygen atoms. When two carbohydrate molecules join, a water molecule is released. When they split, one molecule of water is consumed to form the individual sugar compounds. This reaction is called *hydrolysis*. The glycoside linkages of carbohydrate can involve any number of carbons; however, certain carbons are favored, depending on the carbohydrate. **Monosaccharides** are simple sugars that cannot be hydrolyzed to a simpler form; there is one sugar molecule. These sugars can contain three, four, five, or six or more carbon atoms (known as trioses, tetroses, pentoses, and hexoses, respectively). The most common hexose monosaccharides include glucose, fructose, and galactose.

Disaccharides are formed when two monosaccharide units are joined by a glycosidic linkage. On hydrolysis, disaccharides will be split into two monosaccharides by disaccharide enzyme
tase) located on the microvilli of the intes
monosaccharides are then actively abs
most common disaccharides are maltose
two d-glucose molecules in a 1 → 4 linka
and sucrose.

Oligosaccharides are the chain
10 sugar units, whereas **polysaccharides**
by the linkage of many monosaccharide
hydrolysis, polysaccharides will yield 1
monosaccharides. Amylase, an enzyme fou
and in the stomach, hydrolyzes starch to di
in the duodenum. The most common poly
are starch (plant based glucose molecule
cogen (animal based glucose molecules) (F

Chemical Properties of Carbohydrates

Some carbohydrates are reducing substa
carbohydrates can reduce other compou
they themselves are oxidized. To be a red
stance, the carbohydrate must contain
(available) ketone or an aldehyde group.
erty was used in many past laboratory t
the determination of carbohydrates.

Carbohydrates can form glycosidic bonds with other carbohydrates and with noncarbohydrates. Two sugar molecules can be joined in tandem, forming a glycosidic bond between the hemiacetal group of one molecule and the hydroxyl group on the other molecule. In forming the glycosidic bond, an acetal is generated on one sugar (at carbon 1) in place of the hemiacetal. If the bond forms with one of the other carbons on the carbohydrate other than the anomeric (reducing) carbon, the anomeric carbon is unaltered, and the resulting compound remains a reducing substance. Examples of common reducing sugars include glucose, maltose, fructose, lactose, and galactose. If a glycosidic bond is formed with the anomeric carbon on the other carbohydrate, the resulting compound is no longer a reducing substance. Nonreducing carbohydrates *do not* have an active ketone or aldehyde group and therefore will not reduce other

Key Terms are also highlighted within the chapter and defined in the book's Glossary.


Glossary

1,25-Dihydroxyvitamin D ([OH]$_2$D) (calcitriol) Active metabolite of vitamin D; reduces active absorption of calcium in the small intestine.

1$_{ss}$ rule A data quality control rule that indicates that one data point cannot exceed three SDs. The presence of a data point beyond 3 SDs would trigger a rejection of the analytic run.

25-hydroxyvitamin D Inactive precursor of 1,25 dihydroxyvitamin D.

5-Dihydrotestosterone (DHT) An endogenous androgen sex steroid and hormone. The enzyme 5α-reductase catalyzes the formation of DHT from testosterone in certain tissues including the prostate gland, seminal vesicles, epididymides, skin, hair follicles, liver, and brain.

A

Accuracy How close the measured value is to the true value due to systematic error, which can be either constant or proportional.

Acidemia A condition in which the pH of blood is below the lower limit of the reference range (<7.35), indicating that the hydrogen-ion concentration in the blood is increased.

Activation energy The excess energy needed to form the transition state of a reaction.

Activators Inorganic cofactors, such as metal ions, needed for enzyme activity.

Active transport Use of energy to move ions or substances across cell membranes.

Acute coronary syndrome (ACS) A progression of pathologic conditions involved in ischemic heart disease, including erosion and rupture of coronary artery plaques, activation of platelets, and thrombi. This progression ranges from unstable angina to extensive tissue necrosis in acute myocardial infarction.

Acute kidney injury (AKI) A sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys.

Adrenocorticotropin hormone (ACTH) A peptide hormone secreted by the anterior pituitary. It stimulates the cortex of the adrenal glands to produce adrenal cortical hormones.

Affinity Attraction or force causing two substances to unite.

Airborne pathogens Any infectious agent transmissible by air, e.g., tuberculosis, virus particle, etc.

Albuminuria The presence of albumin in the urine.

Aldosterone The main mineralocorticoid steroid hormone produced by the zona glomerulosa of the adrenal cortex in the adrenal gland. This hormone controls the sodium-potassium pump, the primary mechanism for sodium reabsorption in the kidney and regulator of the blood sodium and potassium levels.

Alkalemia A condition in which blood pH is greater than the uppermost limit of the reference range (>7.45), indicating that the hydrogen ion concentration in the blood is decreased.

Amenorrhea Temporary cessation of menstruation in a female who is past menarche but not yet in menopause.

Amines Hormones that are derived directly from amino acids.

Amino acid Simple organic compounds that serve as the building blocks of proteins; contain at least one amine functional group, one carboxyl function group, and a unique R group.

Aminoacidopathies Inborn errors of metabolism that inhibit the body's ability to metabolize specific amino acids.

Ammonia A compound consisting of nitrogen and hydrogen. Formula: NH_3 or H_3N.

Amniocentesis Puncture of the amniotic sac to obtain fluid for analysis.

Amniotic fluid (AF) A fluid in which the fetus is suspended; it provides a cushioning medium for the fetus and serves as a matrix for influx and efflux of constituents.

Amperometry The measurement of amperes. It is the unit of measure for electric current. The reduction of oxygen produces a current that is proportional to the amount of oxygen present in the sample.

Amphoteric A molecule that is both an acid and a base.

Analyte Substance of interest being measured.

Analytic Introduced during the phase of processing and assaying the specimen in the clinical laboratory.

Analytic measurement range (AMR) Also known as linear or dynamic range. Range of analyte concentrations that can be directly measured without dilution, concentration, or other pretreatment.

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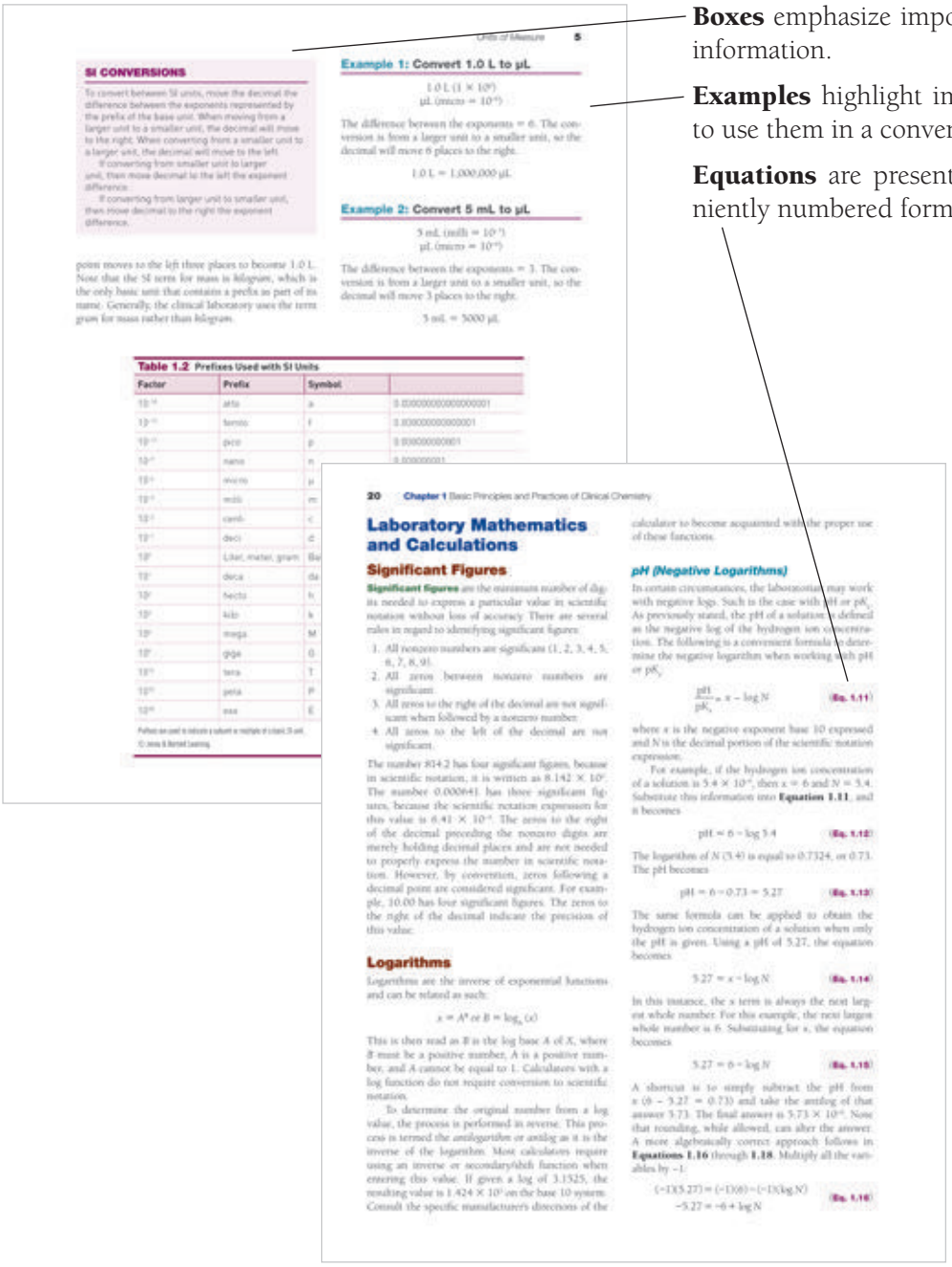
Case Studies with patient visuals progress through the chapter and pose critical-thinking questions, prompting students to synthesize and apply their new knowledge. A case study answer key is available to instructors.



Boxes emphasize important points and additional information.

Examples highlight important formulas and how to use them in a convenient, numbered format.

Equations are presented throughout in a conveniently numbered format.

Figures and **Tables** provide dynamic visuals and populate the new edition throughout, including illustrations, photos, charts, and graphs.

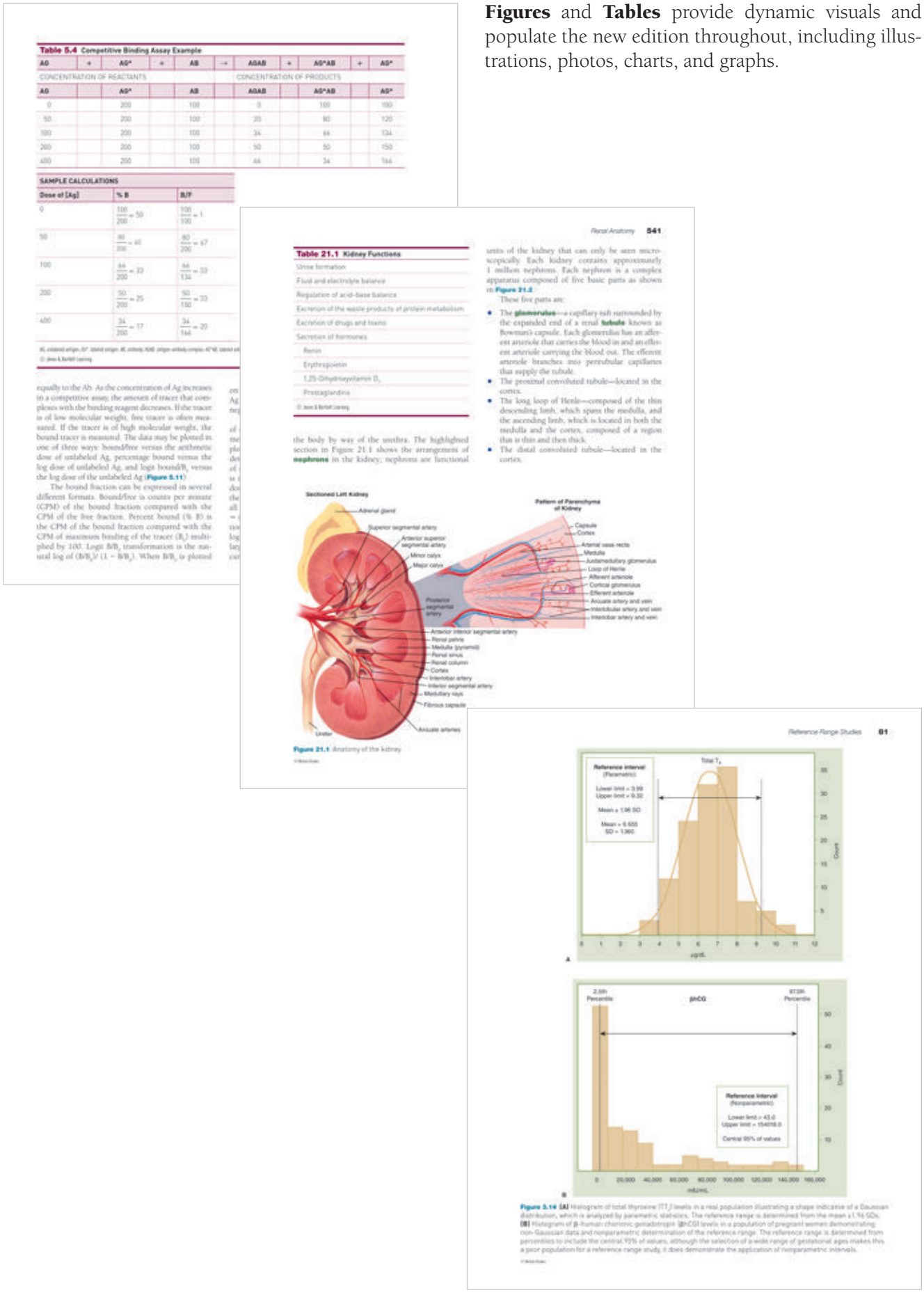


Table 5.4 Competitive Binding Assay Example

Ag	+	Ag*	+	Ab	→	AgAb	+	Ag*Ab	+	Ag*
CONCENTRATION OF REACTANTS						CONCENTRATION OF PRODUCTS				
Ag		Ag*		Ab		AgAb		Ag*Ab		Ag*
0		200		100		0		100		100
50		200		100		20		80		120
100		200		100		34		66		134
200		200		100		50		50		150
400		200		100		66		34		166

SAMPLE CALCULATIONS

Dose of [Ag]	% B	B/F
0	$\frac{100}{200} = 50$	$\frac{100}{100} = 1$
50	$\frac{80}{200} = 40$	$\frac{80}{200} = 67$
100	$\frac{66}{200} = 33$	$\frac{66}{134} = 33$
200	$\frac{50}{200} = 25$	$\frac{50}{150} = 33$
400	$\frac{34}{200} = 17$	$\frac{34}{166} = 20$

equally to the Ab. As the concentration of Ag increases in a competitive assay, the amount of tracer that complexes with the binding reagent decreases. If the tracer is of low molecular weight, free tracer is often measured. If the tracer is of high molecular weight, the bound tracer is measured. The data may be plotted in one of three ways: bound/free versus the arithmetic dose of unlabeled Ag, percentage bound versus the log dose of unlabeled Ag, and logit bound/B_0 versus the log dose of the unlabeled Ag (Figure 5.11).

The bound fraction can be expressed in several different formats. Bound/free is counts per minute (CPM) of the bound fraction compared with the CPM of the free fraction. Percent bound (% B) is the CPM of the bound fraction compared with the CPM of maximum binding of the tracer (B_0) multiplied by 100. Logit B/B_0 transformation is the natural log of $(B/B_0)/(1 - B/B_0)$. When B/B_0 is plotted

Renal Anatomy 541

Table 21.1 Kidney Functions

Urine formation
Fluid and electrolyte balance
Regulation of acid-base balance
Excretion of the waste products of protein metabolism
Excretion of drugs and toxins
Secretion of hormones
Renin
Erythropoietin
1,25-Dihydroxyvitamin D_3
Prostaglandins

the body by way of the urethra. The highlighted section in Figure 21.1 shows the arrangement of **nephrons** in the kidney; nephrons are functional units of the kidney that can only be seen microscopically. Each kidney contains approximately 1 million nephrons. Each nephron is a complex apparatus composed of five basic parts as shown in Figure 21.2.

These five parts are:

- The **glomerulus**—a capillary tuft surrounded by the expanded end of a renal **tubule** known as Bowman's capsule. Each glomerulus has an afferent arteriole that carries the blood in and an efferent arteriole carrying the blood out. The efferent arteriole branches into peritubular capillaries that supply the tubule.
- The proximal convoluted tubule—located in the cortex.
- The long loop of Henle—composed of the thin descending limb, which spans the medulla, and the ascending limb, which is located in both the medulla and the cortex, composed of a region that is thin and then thick.
- The distal convoluted tubule—located in the cortex.

Sectioned Left Kidney

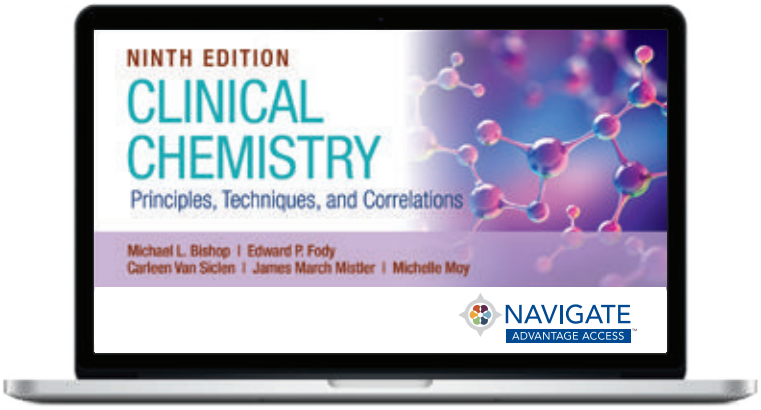
Pattern of Parenchyma of Kidney

Figure 21.1 Anatomy of the kidney.

Reference Range Studies 81

Figure 3.14 (A) Histogram of total thyroxine (TT_4) levels in a real population illustrating a shape indicative of a Gaussian distribution, which is analyzed by parametric statistics. The reference range is determined from the mean ± 1.96 SDs. (B) Histogram of β-human chorionic gonadotropin (βhCG) levels in a population of pregnant women demonstrating non-Gaussian data and nonparametric determination of the reference range. The reference range is determined from percentiles to include the central 95% of values, although the selection of a wide range of gestational ages makes this a poor population for a reference range study; it does demonstrate the application of nonparametric intervals.

Student Resources



To support your learning, review the chapter learning objectives and complete the online activities. The **Navigate 2 Advantage Access** included with each new print copy of this book offers a wealth of resources. These include practical learning activities and study tools such as flashcards, math practice, an eBook with interactive questions, and more!

- eBook with embedded assessments
- Case Studies
- Review Questions
- Flashcards
- Reference Range Table
- General Reference Tables
- Supplemental Chapter
  - Molecular Theory and Techniques

Instructor Resources

Instructor resources, available to qualified instructors, include the following:

- Learning Objectives mapped to:
  - ASCLS Entry-Level Curriculum (MLS and MLT)
  - Current ASCP Board of Certification Content Guidelines (MLS and MLT)
- Slides in PowerPoint format
- Teaching Resources
- Test Bank (Available in LMS-compatible formats)
- Student Lab Procedures
- Image Bank
- Answer Key to Case Studies
- Answer Key to Eighth Edition Case Studies
- Answer Key to Review Questions
- Sample Syllabus

Acknowledgments

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Basic Principles and Practice in Clinical Chemistry

Basic Principles and Practices of Clinical Chemistry

Kathryn Dugan and Elizabeth Warning

CHAPTER OUTLINE

KEY TERMS

Analyte
Anhydrous
Arterial blood
Beer's law
Buffer
Calibration
Centrifugation
Cerebrospinal fluid (CSF)
Colligative property
Conductivity
Deionized water
Delta absorbance
Density
Desiccant
Dilution
Distilled water
Equivalent weight
Erlenmeyer flasks
Filtration
Graduated cylinder
Griffin Beaker
Hemolysis
Henderson-Hasselbalch equation
Hydrate
Hygroscopic
Icterus
International unit
Ionic strength
Linearity
Lipemia
Molality
Molarity
Normality
One-point calibration
Osmotic pressure
Oxidized

Oxidizing agent
Percent solution
pH
Pipette
Primary standard
Reagent-grade water
Redox potential
Reduced
Reducing agent
Reverse osmosis
Serial dilution
Serum
Significant figures
Solute
Solution
Solvent
Specific gravity
Standard reference materials (SRMs)
Système International d'Unités (SI)
Thermistor
Valence
Volumetric
Whole blood

CHAPTER OBJECTIVES

Upon completion of this chapter, the clinical laboratorian should be able to do the following:

- Convert results from one unit format to another using the SI and traditional systems.
- Describe the classifications used for reagent-grade water.
- Identify the varying chemical grades used in reagent preparation and indicate their correct use.
- Define primary standard and standard reference materials.
- Describe the following terms that are associated with solutions and, when appropriate, provide the respective units: percent, molarity, normality, molality, saturation, colligative properties, redox potential, and conductivity.
- Compare and contrast osmolarity and osmolality.
- Define a buffer and give the formula for pH and p*K* calculations.
- Use the Henderson-Hasselbalch equation to determine the missing variable when given either the p*K* and pH or the p*K* and concentration of the weak acid and its conjugate base.
- List and describe the types of thermometers used in the clinical laboratory.
- Classify the type of pipette when given an actual pipette or its description.
- Demonstrate the proper use of a measuring and volumetric pipette.
- Describe two ways to calibrate a pipetting device.
- Define a desiccant and discuss how it is used in the clinical laboratory.
- Describe how to properly care for and balance a centrifuge.
- Correctly perform the laboratory mathematical calculations provided in this chapter.
- Identify and describe the types of samples used in clinical chemistry.
- Outline the general steps for processing blood samples.
- Apply Beer's law to determine the concentration of a sample when the absorbance or change in absorbance is provided.
- Identify the preanalytic variables that can adversely affect laboratory results as presented in this chapter.

CASE STUDY 1.1, PART 1

Meet Miles, a 25-year-old graduate who accepted his first job offer working in the chemistry department at a large medical center. Miles and Mía were classmates in college and often support each other on technical issues, even though they work at different facilities within the same health system.

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CASE STUDY 1.2, PART 1

Meet Mía, a 35-year-old graduate who is also newly hired and works as a generalist in a small community hospital. Mía received a rainbow of tubes from the emergency department. She handed her coworker the lavender- and blue-top tubes and placed the 8.0-mL plain red-top tube and the 3.5-mL plasma separator tube in the centrifuge. She placed the heparinized whole blood specimen on the mixer and logged in to the laboratory information system to receive the specimens. Once the specimens were accessioned, she ran a STAT profile on the Nova pHOx analyzer using the whole blood specimen, and the results were autoverified.

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The primary purpose of a clinical chemistry laboratory is to perform analytic procedures that yield accurate and precise information, aiding in patient diagnosis and treatment. The achievement of reliable results requires that the clinical laboratorian be able to correctly use basic supplies and equipment and possess an understanding of fundamental concepts critical to any analytic procedure. The topics in this chapter include units of measure, basic laboratory supplies, and introductory laboratory mathematics, plus a brief discussion of specimen collection, processing, and reporting.

Units of Measure

Any meaningful *quantitative* laboratory result consists of two components: the first component represents the number related to the actual test value, and the second is a label identifying the units. The unit defines the physical quantity or dimension, such as mass, length, time, or volume.[1] There are a few laboratory tests that do not have units, but whenever possible, units should be used.

The **Système International d'Unités (SI)** was adopted in 1960. It is preferred in scientific literature and clinical laboratories and is the only system employed in many countries. This system was devised to provide the global scientific community with a uniform method of describing physical quantities. The SI system units (referred to as *SI units*) are based on the metric system. Several subclassifications exist within the SI system, one of which is the *basic unit*. There are seven basic units (**Table 1.1**), with length (meter), mass (kilogram), and quantity of a substance (mole) being the units most frequently encountered. Derived units are another subclassification of the SI system. A derived unit is a mathematical function describing one of the basic units. An example of an SI-derived unit is meters per second (m/s), which is used to express velocity. Some non-SI units are so widely used that they have become acceptable for use within the SI system (Table 1.1). These include units such as hour, minute, day, gram, liter, and plane angles expressed as degrees. The SI system uses standard prefixes to indicate a decimal fraction or multiples of that basic unit (**Table 1.2**).[1] For example, 0.001 liter can be expressed using the prefix *milli*, or 10^{-3}, and since it requires moving the decimal point three places to the right, it can then be written as 1 milliliter, or abbreviated as 1 mL. It may also be written in scientific notation as 1×10^{-3} L. Likewise, 1000 liters would use the prefix of kilo (10^3) and could be written as 1 kiloliter or expressed in scientific notation as 1×10^3 L. Table 1.2 indicates prefixes that are frequently used in clinical laboratories. Prefixes smaller than the basic unit have a negative exponent (deci: 10^{-1}), and prefixes larger than the base unit have a positive exponent (kilo: 10^3). When converting between prefixes, note the relationship between the two prefixes based on whether you are changing to a smaller or larger prefix. When converting from a larger to smaller, the decimal will move to the right. For example, converting one liter (1.0×10^0 or 1.0) to milliliters (1.0×10^{-3} or 0.001), the starting unit (L) is larger than milliliters, by a factor of 1000, or 10^3. This means that the decimal place moves to the *right* three places, so 1.0 liter (L) equals 1000 milliliters (mL). The opposite is also true. When converting to a larger unit, the decimal place moves to the left. For example, converting 1000 milliliters (mL) to 1.0 liter (L), the decimal

Table 1.1 SI Units

Base Quantity	Name	Symbol
Length	Meter	m
Mass	Kilogram	kg
Time	Second	s
Electric current	Ampere	A
Thermodynamic temperature	Kelvin	K
Amount of substance	Mole	mol
Luminous intensity	Candela	cd
Selected Derived		
Frequency	Hertz	Hz
Force	Newton	N
Celsius temperature	Degree Celsius	°C
Catalytic activity	Katal	kat
Selected Accepted Non-SI		
Minute (time)	(60 s)	min
Hour	(3600 s)	h
Day	(86,400 s)	d
Liter (volume)	($1\ dm^3 = 10^{-3}\ m^3$)	L
Angstrom	($0.1\ nm = 10^{-10}\ m$)	Å

SI CONVERSIONS

To convert between SI units, move the decimal the difference between the exponents represented by the prefix of the base unit. When moving from a larger unit to a smaller unit, the decimal will move to the right. When converting from a smaller unit to a larger unit, the decimal will move to the left.

If converting from smaller unit to larger unit, then move decimal to the left the exponent difference.

If converting from larger unit to smaller unit, then move decimal to the right the exponent difference.

point moves to the *left* three places to become 1.0 L. Note that the SI term for mass is *kilogram*, which is the only basic unit that contains a prefix as part of its name. Generally, the clinical laboratory uses the term *gram* for mass rather than *kilogram*.

Example 1: Convert 1.0 L to µL

$$1.0\ \text{L}\ (1 \times 10^{0})$$
$$\mu\text{L}\ (\text{micro} = 10^{-6})$$

The difference between the exponents = 6. The conversion is from a larger unit to a smaller unit, so the decimal will move 6 places to the right.

$$1.0\ \text{L} = 1{,}000{,}000\ \mu\text{L}$$

Example 2: Convert 5 mL to µL

$$5\ \text{mL}\ (\text{milli} = 10^{-3})$$
$$\mu\text{L}\ (\text{micro} = 10^{-6})$$

The difference between the exponents = 3. The conversion is from a larger unit to a smaller unit, so the decimal will move 3 places to the right.

$$5\ \text{mL} = 5000\ \mu\text{L}$$

Table 1.2 Prefixes Used with SI Units

Factor	Prefix	Symbol	
10^{-18}	atto	a	0.000000000000000001
10^{-15}	femto	f	0.000000000000001
10^{-12}	pico	p	0.000000000001
10^{-9}	nano	n	0.000000001
10^{-6}	micro	µ	0.000001
10^{-3}	milli	m	0.001
10^{-2}	centi	c	0.01
10^{-1}	deci	d	0.1
10^{0}	Liter, meter, gram	Basic unit	1.0
10^{1}	deca	da	10
10^{2}	hecto	h	100
10^{3}	kilo	k	1000
10^{6}	mega	M	1,000,000
10^{9}	giga	G	1,000,000,000
10^{12}	tera	T	1,000,000,000,000
10^{15}	peta	P	1,000,000,000,000,000
10^{18}	exa	E	1,000,000,000,000,000,000

Prefixes are used to indicate a subunit or multiple of a basic SI unit.

Example 3: Convert 5.3 mL to dL

$$5.3 \text{ mL (milli} = 10^{-3})$$
$$\text{dL (deci} = 10^{-1})$$

The conversion is moving from a smaller unit to a larger unit, so the decimal place will move two places to the left.

$$5.3 \text{ mL} = 0.053 \text{ dL}$$

Reporting of laboratory results is often expressed in terms of substance concentration (e.g., moles) or the mass of a substance (e.g., mg/dL, g/dL, g/L, mmol/L, and IU) rather than in SI units. These traditional units can cause confusion during interpretation and conversion to SI units: examples of conversions can be found later in the chapter. As with other areas of industry, the laboratory and the rest of medicine are moving toward adopting universal standards promoted by the International Organization for Standardization, often referred to as ISO. This group develops standards of practice, definitions, and guidelines that can be adopted by everyone in a given field, providing for more uniform terminology. Many national initiatives have recommended common units for laboratory test results, but none have been widely adopted.[2] As with any transition, the clinical laboratorian should be familiar with all the terms currently used in their field and how to convert these to SI units.

Reagents

In today's highly automated laboratory, there is little need for reagent preparation by the laboratorian. Most instrument manufacturers make the reagents in a ready-to-use form or "kit" in which all necessary reagents and respective storage containers are prepackaged as a unit, requiring only the addition of water or buffer for reconstitution. A heightened awareness of the hazards of certain chemicals and the numerous regulatory agency requirements has caused clinical chemistry laboratories to eliminate massive stocks of chemicals and opt instead for the ease of using prepared reagents. Periodically, the laboratorian may still need to prepare reagents or solutions, especially in hospital laboratories involved in research and development, biotechnology applications, specialized analyses, or method validation.

Chemicals

Analytic chemicals exist in varying grades of purity: Reagent grade or analytic reagent (AR); ultrapure, chemically pure (CP); United States Pharmacopeia (USP); National Formulary (NF); and technical or commercial grade.[3] Chemicals with AR designation are suitable for use in most analytic laboratory procedures. A committee of the American Chemical Society (ACS) established specifications for AR grade chemicals, and chemical manufacturers must either meet or exceed these requirements. The labels on reagents should clearly state the actual impurities for each chemical lot or list the maximum allowable impurities. The label should also include one of the following designations: AR or ACS or *For laboratory use* or *ACS Standard-Grade Reference Materials*. Ultrapure chemicals have additional purification steps for use in specific procedures such as chromatography, immunoassays, molecular diagnostics, standardization, or other techniques that require extremely pure chemicals. These reagents may have designations of HPLC (high-performance liquid chromatography) or chromatographic on their labels.

Because USP- and NF-grade chemicals are used to manufacture drugs, the limitations established for this group of chemicals are based only on the criterion of not being injurious to individuals. Chemicals in this group may be pure enough for use in most chemical procedures, but the purity standards they meet are not based on the needs of the laboratory and may or may not meet all assay requirements.

Reagent designations of CP or ultrapure grade indicate that the impurity limitations are not stated, and preparation of these chemicals is not uniform. It is not recommended that clinical laboratories use these chemicals for reagent preparation unless further purification or a reagent blank is included. Technical or commercial grade reagents are used primarily in manufacturing and should never be used in the clinical laboratory.

Organic reagents also have varying grades of purity that differ from those used to classify inorganic reagents. These grades include a practical grade with some impurities; CP, which approaches the purity level of reagent-grade chemicals; spectroscopic (spectrally pure) and chromatographic grade organic reagents; and reagent grade (ACS), which is certified to contain impurities below established ACS levels. Other than the purity aspects of the chemicals, laws related to the Occupational Safety and Health Administration (OSHA)[4] require manufacturers to indicate any physical or biologic health

hazards and precautions needed for the safe use, storage, and disposal of any chemical. Manufacturers are required to provide a Safety Data Sheet (SDS). A copy of the SDS must be readily available to ensure the safety of laboratorians.

Reference Materials

Unlike other areas of chemistry, clinical chemistry is involved in the analysis of biochemical by-products found in *biological* fluids, such as serum, plasma, or urine. For this reason, traditionally defined standards used in analytical chemistry do not readily apply in clinical chemistry.

A **primary standard** is a highly purified chemical that can be measured directly to have an *exact* known concentration and purity. The ACS has purity tolerances for primary standards; because most biologic constituents are unavailable within these tolerance limitations, the National Institute of Standards and Technology (NIST) has certified **standard reference materials (SRMs)** that are used in place of ACS primary standard materials.[5–7]

These SRMs are assigned a value after analysis using state-of-the-art methods and equipment. The chemical composition of these substances is then certified; however, they may not have the purity of a primary standard. Because each substance has been characterized for certain chemical or physical properties, it can be used in place of an ACS primary standard in clinical work and is often used to verify **calibration** or accuracy/bias assessments. Many manufacturers use a NIST SRM when producing calibrator and standard materials. These materials are considered "traceable to NIST" and may meet certain accreditation requirements. Standard reference materials are used for **linearity** studies to determine the relationship between the standard's concentration and the instrument result. Linearity studies are required when a new test or new test methodology is introduced. There are SRMs for a number of routine analytes, hormones, drugs, and blood gases, with others being added.[5] Calibration of an instrument is a process that pairs an analytical signal with a concentration value of an analyte. When performing a calibration, a series of calibrators with known concentrations of a specific analyte are used. The instrument is programmed with the known concentrations and will adjust the analytic signal to match the given concentration. Calibrators can be purchased as a kit or made by diluting a known stock solution.

Water Specifications[8]

Water is the most frequently used reagent in the laboratory. Tap water is unsuitable for laboratory applications. Most procedures, including reagent and control preparation, require water that has been substantially purified, known as **reagent-grade water**. There are various water purification methods including distillation, ion exchange, reverse osmosis, ultrafiltration, ultraviolet light, sterilization, and ozone treatment. According to the Clinical and Laboratory Standards Institute (CLSI), reagent-grade water is classified into one of six categories based on the specifications needed for its use rather than the method of purification or preparation.[9] These categories include clinical laboratory reagent water (CLRW), special reagent water (SRW), instrument feed water, water supplied by method manufacturer, autoclave and wash water, and commercially bottled purified water. Each category has a specific acceptable limit. The College of American Pathologists requires laboratories to define the specific type of water required for each of its testing procedures and requires water quality testing at least annually. Water quality testing routinely includes monitoring microbial colony-forming units/mL and may also include other parameters.

Distilled water has been purified to remove almost all organic materials, using a technique of distillation where water is boiled and vaporized. Many impurities do not rise in the water vapor and will remain in the boiling apparatus so that the water collected after condensation has less contamination. Water may be distilled more than once, with each distillation cycle removing additional impurities. Ultrafiltration and nanofiltration, like distillation, are excellent in removing particulate matter, microorganisms, and any pyrogens or endotoxins.

Deionized water has some or all ions removed, although organic material may still be present, so it is neither pure nor sterile. Generally, deionized water is purified from previously treated water, such as prefiltered or distilled water. Deionized water is produced using either an anion- or a cation-exchange resin, followed by replacement of the removed ions with hydroxyl or hydrogen ions. A combination of several ion-exchange resins will produce different grades of deionized water. A two-bed system uses an anion resin followed by a cation resin. The different resins may be in separate columns or in the same column. This process is excellent at removing dissolved ionized solids and dissolved gases.

Reverse osmosis is a process that uses pressure to force water through a semipermeable membrane, producing a filtered product. Reverse osmosis may be used for the pretreatment of water, however, it does not remove dissolved gases.

Filtration can remove particulate matter from municipal water supplies before any additional treatments. Filtration cartridges can be composed of glass, cotton, or activated charcoal, which removes organic materials and chlorine. Some have submicron filters (≤0.2 μm), which remove any substances larger than the filter's pores, including bacteria. The use of these filters depends on the quality of the municipal water and the other purification methods used. For example, hard water (containing calcium, iron, and other dissolved elements) may require prefiltration with a glass or cotton filter rather than activated charcoal or submicron filters, which quickly become clogged and are expensive to use. The submicron filter may be better suited after distillation, deionization, or reverse osmosis treatment.

Ultraviolet oxidation, which removes some trace organic material or sterilization processes at specific wavelengths, can destroy bacteria when used as part of a system but may leave behind some residual products. This technique is often followed by other purification processes.

Reagent-grade water can be obtained by initially filtering to remove particulate matter, followed by reverse osmosis, deionization, and a 0.2-μm filter or more restrictive filtration process. Autoclave wash water is acceptable for glassware washing but not for analysis or reagent preparation. SRW is used for specific techniques like the HPLC, molecular diagnostics, or mass spectrophotometry, which may require specific parameters for the analysis. All SRW should meet CLRW standards and, depending on the application, CLRW should be stored in a manner that reduces any chemical or bacterial contamination and for short periods.

Testing procedures to determine the quality of reagent-grade water include measurements of resistance, pH, colony counts on selective and nonselective media for the detection of bacterial contamination, chlorine, ammonia, nitrate or nitrite, iron, hardness, phosphate, sodium, silica, carbon dioxide, chemical oxygen demand, and metal detection. Some accreditation agencies[10] recommend that laboratories document culture growth, pH, and specific resistance on water used in reagent preparation. Resistance is measured because pure water, devoid of ions, is a poor conductor of electricity and has increased resistance. The relationship of water purity to resistance is linear; generally, as purity increases, so does resistance. This one measurement does not suffice for determination of true water purity because a nonionic contaminant may be present that will have little effect on resistance. Reagent water meeting specifications from other organizations, such as the American Society for Testing and Materials (ASTM), may not be equivalent to those established by the CLSI, so care should be taken to meet the assay procedural requirements for water type.

Solution Properties

In clinical chemistry, substances found in biologic fluids, including serum, plasma, urine, and spinal fluid, are quantified. A substance that is dissolved in a liquid is called a **solute**; a biologic solute is also known as an **analyte**. The liquid in which the solute is dissolved—for example, a biologic fluid—is the **solvent**. Together, solute and solvent represent a **solution**. Any chemical or biologic solution can be described by its basic properties, including concentration, saturation, colligative properties, redox potential, conductivity, density, pH, and ionic strength.

Concentration

The analyte concentration in solution can be expressed in many ways. Concentration is commonly expressed as *percent solution*, *molarity*, *molality*, or *normality*. These are non-SI units, however; the SI unit for the amount of a substance is the *mole*. Examples of concentration calculations are provided later in this chapter.

Percent solution is expressed as the amount of solute per 100 total units of solution. Three expressions of percent solutions are weight per weight (w/w), volume per volume (v/v), and weight per volume (w/v). Weight per weight (% w/w) refers to the number of grams of solute per 100 g of solution. Volume per volume (% v/v) is used for liquid solutes and gives the milliliters of solute in 100 mL of solution. For v/v solutions, it is recommended that grams per deciliter (g/dL) be used instead of % v/v. Weight per volume (% w/v) is the most commonly used percent solution in the clinical laboratory and is defined as the number of grams of solute in 100 mL of solution. Weight per volume is not the same as molarity, and care must be taken to not confuse the two. Examples of percent solution calculations can be found later in this chapter.

Molarity (M) is expressed as the number of moles per 1 L of solution. One mole of a substance equals its gram molecular weight (gmw), so the customary units of molarity (M) are moles/liter. The SI representation for the traditional molar concentration is moles

of solute per volume of solution, with the volume of the solution given in liters. The SI expression for concentration should be represented as moles per liter (mol/L), millimoles per liter (mmol/L), micromoles per liter (µmol/L), or nanomoles per liter (nmol/L). The common concentration term *molarity* is not an SI unit for concentration. Molarity depends on volume, and any significant physical changes that influence volume, such as changes in temperature and pressure, will also influence molarity. Calculations can be found in the Laboratory Mathematics and Calculations section of this chapter.

Molality (m) represents the amount of solute per 1 kg of solvent. Molality is sometimes confused with molarity; however, it can be easily distinguished because molality is always expressed in terms of moles per kilogram (weight per weight) and describes moles per 1000 g (1 kg) of solvent. Note that the common abbreviation (m) for molality is a lowercase "m," while the uppercase "M" refers to molarity. Molality is not influenced by temperature or pressure because it is based on mass rather than volume.

Normality is the least likely of the four concentration expressions to be encountered in clinical laboratories, but it is often used in chemical titrations and chemical reagent classification. It is defined as the number of gram equivalent weights per 1 L of solution. An **equivalent weight** is equal to the gmw of a substance divided by its valence. The **valence** is the number of units that can combine with or replace 1 mole of hydrogen ions for acids and hydroxyl ions for bases and the number of electrons exchanged in oxidation–reduction reactions. Normality is always equal to or greater than the molarity of the compound. Calculations can be found later in this chapter. Normality was previously used for reporting electrolyte values, expressed as milliequivalents per liter (mEq/L); however, this convention has been replaced with millimoles per liter (mmol/L). The College of American Pathologists (CAP) currently requires chloride to be reported in mmol/L. Because the four main electrolytes, Na^+, K^+, CO_2^- (HCO_3^-), and Cl^-, all have a valence of 1, the concentration reported will remain the same whether the unit is mEq/L or mmol/L.

Solution saturation gives little specific information about the concentration of solutes in a solution. A solution is considered *saturated* when no more solvent can be dissolved in the solution. Temperature, as well as the presence of other ions, can influence the solubility constant for a solute in a given solution and thus affect the saturation. Routine terms in the clinical laboratory that describe the extent of saturation are *dilute*, *concentrated*, *saturated*, and *supersaturated*. A *dilute solution* is one in which there is relatively little solute or one that has a lower solute concentration per volume of solvent than the original, such as when making a dilution. In contrast, a *concentrated solution* has a large quantity of solute in solution. A solution in which there is an excess of undissolved solute particles can be referred to as a *saturated solution*. As the name implies, a *supersaturated solution* has an even greater concentration of undissolved solute particles than a saturated solution of the same substance. Because of the greater concentration of solute particles, a supersaturated solution is thermodynamically unstable. The addition of a crystal of solute or mechanical agitation disturbs the supersaturated solution, resulting in crystallization of any excess material out of solution. An example is when measuring serum osmolality by freezing point depression.

Colligative Properties

Colligative properties are those properties related to the number of solute particles per solvent molecules, not on the type of particles present. The behavior of particles or solutes in solution demonstrates four properties: **osmotic pressure**, vapor pressure, freezing point, and boiling point. These are called **colligative properties**. *Osmotic pressure* is the pressure that opposes osmosis when a solvent flows through a semipermeable membrane to establish equilibrium between compartments of differing concentration. *Vapor pressure* is the pressure exerted by the vapor when the liquid solvent is in equilibrium with the vapor. *Freezing point* is the temperature at which the first crystal (solid) of solvent forms in equilibrium with the solution. *Boiling point* is the temperature at which the vapor pressure of the solvent reaches atmospheric pressure (usually 1 atmosphere).

The osmotic pressure of a dilute solution is directly proportional to the concentration of the molecules in solution. The expression for concentration is the osmole. One osmole of a substance equals the molarity or molality multiplied by the number of particles, not the kind of particle, at dissociation. If molarity is used, the resulting expression would be termed osmolarity; if molality is used, the expression changes to osmolality. Osmolality is preferred since it depends on the weight rather than volume and is not readily influenced by temperature and pressure changes. When a solute is dissolved in a solvent, the colligative properties change in a predictable manner for each osmole of substance present. In the clinical setting, freezing point and vapor pressure depression can be measured as a function

of osmolality. Freezing point is preferred since vapor pressure measurements can give inaccurate readings when some substances, such as alcohols, are present in the samples.

Redox Potential

Redox potential, or *oxidation–reduction potential*, is a measure of the ability of a solution to accept or donate electrons. Substances that donate electrons are called **reducing agents**; those that accept electrons are considered **oxidizing agents**. The mnemonic—LEO (lose electrons **oxidized**) the lion says GER (gain electrons **reduced**)—may prove useful when trying to recall the relationship between reducing/oxidizing agents.

Conductivity

Conductivity is a measure of how well electricity passes through a solution. A solution's conductivity quality depends principally on the number of respective charges of the ions present. *Resistivity*, the reciprocal of conductivity, is a measure of a substance's resistance to the passage of electrical current. The primary application of resistivity in the clinical laboratory is for assessing the purity of water. Resistivity (resistance) is expressed as ohms and conductivity is expressed as $ohms^{-1}$.

pH and Buffers

Buffers are weak acids or bases and their related salts that minimize changes in the hydrogen ion concentration. Hydrogen ion concentration is often expressed as pH. A lowercase *p* in front of certain letters or abbreviations operationally means the "negative logarithm of" or "inverse log of" that substance. In keeping with this convention, the term **pH** represents the negative or inverse log of the hydrogen ion concentration. Mathematically, pH is expressed as

$$\mathrm{pH} = \log\left(\frac{1}{[\mathrm{H^+}]}\right) \qquad \text{(Eq. 1.1)}$$

$$\mathrm{pH} = -\log[\mathrm{H^+}]$$

where $[H^+]$ equals the concentration of hydrogen ions in moles per liter (M). The pH scale ranges from 0 to 14 and is a convenient way to express hydrogen ion concentration.

Unlike a strong acid or base, which dissociates almost completely, the dissociation constant for a weak acid or base solution (like a buffer) tends to be very small, meaning little dissociation occurs.

The dissociation of acetic acid (CH_3COOH), a weak acid, can be illustrated as follows:

$$[\mathrm{HA}] \leftrightarrow [\mathrm{A^-}] + [\mathrm{H^+}] \qquad \text{(Eq. 1.2)}$$

$$[\mathrm{CH_3COOH}] \leftrightarrow [\mathrm{CH_3COO^-}] + [\mathrm{H^+}]$$

HA = weak acid, A^- = conjugate base, H^+ = hydrogen ions, [] = concentration of item in the bracket.

Sometimes, the conjugate base (A^-) will be referred to as a "salt" since, physiologically, it will be associated with some type of cation, such as sodium (Na^+).

The dissociation constant, K_a, for a weak acid may be calculated using the following equation:

$$K_a = \frac{[\mathrm{A^-}][\mathrm{H^+}]}{[\mathrm{HA}]} \qquad \text{(Eq. 1.3)}$$

Rearrangement of this equation reveals

$$[\mathrm{H^+}] = K_a \times \frac{[\mathrm{HA}]}{[\mathrm{A^+}]} \qquad \text{(Eq. 1.4)}$$

Taking the log of each quantity and then multiplying by minus 1 (–1), the equation can be rewritten as

$$-\log[\mathrm{H^+}] = -\log K_a \times -\log\frac{[\mathrm{HA}]}{[\mathrm{A^-}]} \qquad \text{(Eq. 1.5)}$$

By convention, lowercase *p* means "negative log of"; therefore, $-\log[H^+]$ may be written as pH, and $-K_a$ may be written as pK_a. The equation now becomes

$$\mathrm{pH} = \mathrm{p}K_a - \log\frac{[\mathrm{HA}]}{[\mathrm{A^-}]} \qquad \text{(Eq. 1.6)}$$

Eliminating the minus sign in front of the log of the quantity $\frac{[\mathrm{HA}]}{[\mathrm{A^-}]}$ results in an equation known as the **Henderson-Hasselbalch equation**, which mathematically describes the dissociation characteristics of weak acids (pK_a) and bases (pK_b) and the effect on pH:

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{A^-}]}{[\mathrm{HA}]} \qquad \text{(Eq. 1.7)}$$

When the ratio of $[A^-]$ to [HA] is 1, the pH equals the p*K* and the buffer has its greatest buffering capacity. The dissociation constant K_a, and therefore the pK_a, remains the same for a given substance. Any changes in pH are solely due to the ratio of conjugate base $[A^-]$

concentration to weak acid [HA] concentration. Refer to Chapter 12, *Blood Gases, pH, and Buffer Systems,* for more information.

Ionic strength is another important aspect of buffers, particularly in separation techniques. **Ionic strength** is the concentration or activity of ions in a solution or buffer. Increasing ionic strength increases the ionic cloud surrounding a compound and decreases the rate of particle migration. It can also promote compound dissociation into ions effectively increasing the solubility of some salts, along with changes in current, which can also affect electrophoretic separation.

Laboratory Equipment

In today's clinical chemistry laboratory, there are many different types of equipment in use. Most manual techniques have been replaced by automation, but it is still necessary for the laboratorian to be knowledgeable in the operation and use of common laboratory equipment. The following is a brief discussion of the composition and general use of common equipment found in a clinical chemistry laboratory, including heating units, thermometers, pipettes, flasks, beakers, balances, and centrifuges.

Heating Units

Heat blocks and water baths are common heating units within the laboratory. The temperature of these heating units must be monitored daily when in use. The predominant practice for temperature measurement uses the Celsius (°C) scale; however, Fahrenheit (°F) and Kelvin (°K) scales are also used.[11] The SI designation for temperature is the Kelvin scale. **Table 1.3** gives the conversion formulas between Fahrenheit and Celsius scales, and Appendix C (found in the Navigate 2 digital component) lists the various conversion formulas.

All analytic reactions occur at an optimal temperature. Some laboratory procedures, such as enzyme determinations, require precise temperature control, whereas others work well over a wide range of temperatures. Reactions that are temperature dependent use some type of heating/cooling cell, heating/cooling block, or water/ice bath to provide the correct temperature environment. Laboratory refrigerator temperatures are often critical and need periodic verification. Thermometers can be an integral part of an instrument or need to be placed in the device for temperature maintenance and monitoring. Several types of temperature devices are currently used in the clinical laboratory, including liquid-in-glass and electronic (**thermistor**) devices. Regardless of which type is being used, all temperature-reading devices must be calibrated for accuracy. Liquid-in-glass thermometers use a colored liquid (red or other colored material), encased in plastic or glass, measuring temperatures between 20°C and 400°C. Visual inspection of the liquid-in-glass thermometer should reveal a continuous line of liquid, free from separation or bubbles. If separation or bubbles are present, then replace the thermometer.

Liquid-in-glass thermometers should be calibrated against a NIST-certified or NIST-traceable thermometer for critical laboratory applications.[11] NIST has an SRM thermometer with various calibration points (0°C, 25°C, 30°C, and 37°C) for use with liquid-in-glass thermometers. Gallium, another SRM, has a known melting point and can also be used for thermometer verification.

As automation advances and miniaturizes, the need for an accurate, fast-reading electronic thermometer (thermistor) has increased and is now routinely incorporated in many devices. The advantages of a thermistor over the more traditional liquid-in-glass thermometers are size and millisecond response time. Similar to the liquid-in-glass thermometers, the thermistor can be calibrated against an SRM thermometer.

Table 1.3 Common Temperature Conversions

Celsius (Centigrade) to Fahrenheit	°C (9/5) + 32 (multiply Celsius temperature by 9; divide the answer by 5, then add 32)
Fahrenheit to Celsius (Centigrade)	(°F – 32)5/9 (subtract 32 and divide the answer by 9; then multiply that answer by 5)

Glassware and Plasticware

Until recently, laboratory supplies (e.g., pipettes, flasks, beakers) consisted of some type of glass and could be correctly termed *glassware*. As plastic material was refined and made available to manufacturers, plastic has been increasingly used to make laboratory supplies. A brief summary of the types and uses of glass and plastic commonly seen today in laboratories can be found in the Navigate 2 digital component. Regardless of design, most laboratory supplies must satisfy certain tolerances of accuracy

and fall into two classes of precision tolerance, either Class A or Class B as given by ASTM.[12,13] Those that satisfy Class A ASTM precision criteria are stamped with the letter "A" on the glassware and are preferred for laboratory applications. Class B glassware generally have twice the tolerance limits of Class A, even if they appear identical, and are often found in student laboratories where durability is needed. Vessels holding or transferring liquid are designed either *to contain* (TC) or *to deliver* (TD) a specified volume. The major difference is that TC devices do not deliver the volume measured when the liquid is transferred into a container, whereas the TD designation means that the labware will deliver the amount measured.

Glassware used in the clinical laboratory usually fall into one of the following categories: Kimax/Pyrex (borosilicate), Corex (aluminosilicate), high silica, Vycor (acid and alkali resistant), low actinic (amber colored), or flint (soda lime) glass used for disposable material.[14] Glassware routinely used in clinical chemistry should consist of high thermal borosilicate or aluminosilicate glass. The manufacturer is the best source of information about specific uses, limitations, and accuracy specifications for glassware.

Plasticware is beginning to replace glassware in the laboratory setting; high resistance to corrosion and breakage, as well as varying flexibility, has made plasticware appealing. Relatively inexpensive, it allows most items to be completely disposable after each use. The major types of resins frequently used in the clinical chemistry laboratory are polystyrene, polyethylene, polypropylene, Tygon, Teflon, polycarbonate, and polyvinyl chloride. Again, the individual manufacturer is the best source of information concerning the proper use and limitations of any plastic material.

In most laboratories, glass or plastic that is in direct contact with biohazardous material is usually disposable. If not, it must be decontaminated according to appropriate protocols. Should the need arise, cleaning of glass or plastic may require special techniques. Immediately rinsing glass or plastic supplies after use, followed by washing with a detergent designed for cleaning laboratory supplies and several distilled water rinses, may be sufficient. Presoaking glassware in soapy water is highly recommended whenever immediate cleaning is impractical. Many laboratories use automatic dishwashers and dryers for cleaning. Detergents and temperature levels should be compatible with the material and the manufacturer's recommendations. To ensure that all detergent has been removed from the labware, multiple rinses with appropriate grade water is recommended. Check the pH of the final rinse water and compare it with the initial pH of the prerinse water. Detergent-contaminated water will have a more alkaline pH as compared with the pH of the prerinse water. Visual inspection should reveal spotless vessel walls. Any biologically contaminated labware should be disposed of according to the precautions followed by the laboratory.

Some determinations, such as those used in assessing heavy metals or assays associated with molecular testing, require scrupulously clean or disposable glassware. Other applications may require plastic rather than glass because glass can absorb metal ions. It is suggested that disposable glass and plastic be used whenever possible.

Dirty reusable pipettes should be placed, with the pipette tips up, immediately in a specific pipette soaking/washing/drying container. This container should have soapy water high enough to cover the entire pipette. For each final water rinse, fresh reagent-grade water should be used; if possible, designate a pipette container for final rinses only. Cleaning brushes are available to fit almost any size glassware and are recommended for any articles that are washed routinely.

Although plastic material is often easier to clean because of its nonwettable surface, it may not be appropriate for some applications involving organic solvents or autoclaving. Brushes or harsh abrasive cleaners should not be used on plasticware. Many initial cleaning procedures, described in Appendix J (found in the Navigate 2 digital component), can be adapted for plasticware. Ultrasonic cleaners can help remove debris coating the surfaces of glass or plasticware. Properly cleaned laboratory glass and plasticware should be completely dried before using.

Laboratory Glassware

Flasks, beakers, and graduated cylinders are used to hold solutions. Volumetric and Erlenmeyer flasks are two types of containers in general use in the clinical laboratory.

A **volumetric** flask is calibrated to hold one exact volume of liquid (TC). The flask has a round, lower portion with a flat bottom and a long, thin neck with an etched calibration line. Volumetric flasks are used to bring a given reagent to its final volume with the recommended diluent. When bringing the bottom of the meniscus to the calibration mark, a pipette should be used for adding the final drops of diluent to ensure

maximum control is maintained and the calibration line is not missed.

Erlenmeyer flasks and **Griffin beakers** are designed to hold different volumes rather than one exact amount. Because Erlenmeyer flasks and Griffin beakers are often used in reagent preparation, flask size, chemical inertness, and thermal stability should be considered. The Erlenmeyer flask has a wide bottom that gradually evolves into a smaller, short neck. The Griffin beaker has a flat bottom, straight sides, and an opening as wide as the flat base, with a small spout in the lip.

Graduated cylinders are long, cylindrical tubes usually held upright by an octagonal or circular base. The cylinder has horizontal calibration marks and is used to measure volumes of liquids. Graduated cylinders do not have the accuracy of volumetric labware. The sizes routinely used are 10, 25, 50, 100, 500, 1000, and 2000 mL.

All laboratory glassware used for critical measurements should be Class A whenever possible to maximize accuracy and precision and thus decrease calibration time (**Figure 1.1** illustrates representative laboratory glassware).

Pipettes

Pipettes are a type of laboratory equipment used to transfer liquids; they may be reusable or disposable. Although pipettes may transfer any volume, they are usually used for volumes of 20 mL or less; larger volumes are usually transferred or dispensed using automated pipetting devices. **Table 1.4** outlines the pipette classification.

Similar to other laboratory equipment, pipettes are designed to contain (TC) or to deliver (TD) a particular volume of liquid. The major difference is the amount of liquid needed to wet the interior surface of the pipette and the amount of any residual liquid left in the pipette tip. Most manufacturers stamp *TC* or *TD* near the top of the pipette to alert the user as to the type of pipette. Like other TC-designated labware, a TC pipette holds or contains a particular volume but does not dispense that exact volume, whereas a TD pipette will dispense the volume indicated.

When using either pipette, the tip must be immersed in the intended transfer liquid to a level that will allow the tip to remain in solution after the volume of liquid has entered the pipette—without touching the vessel walls. The pipette is held upright, not at an angle (**Figure 1.2**). Using a pipette bulb or similar device, a slight suction is applied to the opposite end until the liquid enters the pipette and the meniscus is brought above the desired graduation line (**Figure 1.3A**), and suction is then stopped. While the meniscus level is held in place, the pipette tip is raised slightly out of the solution and wiped with a laboratory tissue to remove any adhering liquid. The liquid is allowed to drain until the bottom of the meniscus touches the desired calibration mark (**Figure 1.3B**). With the pipette held in a vertical position and the tip against the side of the receiving vessel, the pipette contents are allowed to drain into the vessel (e.g., test tube, cuvette, or flask). A *blowout pipette* has a continuous etched ring or two small,

Table 1.4 Pipette Classification

I.	Design
	A. To contain (TC)
	B. To deliver (TD)
II.	Drainage characteristics
	A. Blowout
	B. Self-draining
III.	Type
	A. Measuring or graduated
	1. Serologic
	2. Mohr
	3. Bacteriologic
	4. Ball, Kolmer, or Kahn
	5. Micropipette
	B. Transfer
	1. Volumetric
	2. Ostwald-Folin
	3. Pasteur pipettes
	4. Automatic macropipettes or micropipettes

Figure 1.1 Laboratory glassware.

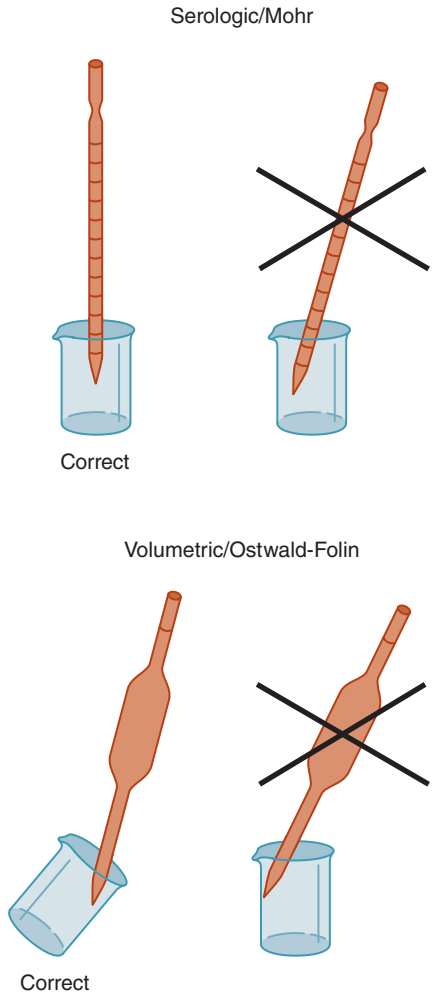

Figure 1.2 Correct and incorrect pipette positions.

close, continuous rings located near the top of the pipette. This means that the last drop of liquid should be expelled into the receiving vessel. Without these markings, a pipette is *self-draining*, and the user allows the contents of the pipette to drain by gravity. The tip of the pipette should not be in contact with the accumulating fluid in the receiving vessel during drainage. With the exception of the Mohr pipette, the tip should remain in contact with the side of the vessel for several seconds after the liquid has drained. The pipette is then removed (Figure 1.2).

Measuring or graduated pipettes are capable of dispensing several different volumes. Measuring pipettes are used to transfer reagents or make dilutions and can be used to repeatedly transfer a particular solution. The markings at the top of a measuring or graduated pipette indicate the volume(s) it is designed to dispense. Because the graduation lines located on the pipette may vary, the increments will be indicated on the top of each pipette. For example, a 5-mL pipette can be used to

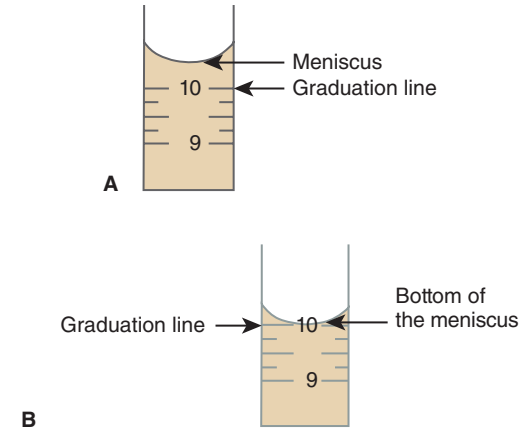

Figure 1.3 Pipetting technique. **(A)** Meniscus is brought above the desired graduation line. **(B)** Liquid is allowed to drain until the bottom of the meniscus touches the desired calibration mark.

measure 5, 4, 3, 2, or 1 mL of liquid, with further graduations between each milliliter. The pipette is designated as 5 in 1/10 increments (**Figure 1.4**) and could deliver any volume in tenths of a milliliter, up to 5 mL. Another pipette, such as a 1-mL pipette, may be designed to dispense 1 mL and have subdivisions of hundredths of a milliliter. The subgroups of measuring or graduated pipettes are Mohr, serologic, and micropipettes. A *Mohr pipette* does not have graduations to the tip. It is a self-draining pipette, but the tip should not be allowed to touch the vessel while the pipette is draining. A *serologic pipette* has graduation marks to the tip and is generally a blowout pipette. A *micropipette* is a pipette with a total holding volume of less than 1 mL; it may be designed as either a Mohr or a serologic pipette.

Transfer pipettes are designed to dispense one volume without further subdivisions. Ostwald-Folin pipettes are used with biologic fluids having a viscosity greater than that of water. They are blowout pipettes, indicated by two etched, continuous rings at the top. The volumetric pipette is designed to dispense or transfer aqueous solutions and is always self-draining. The bulb-like enlargement in the pipette stem easily

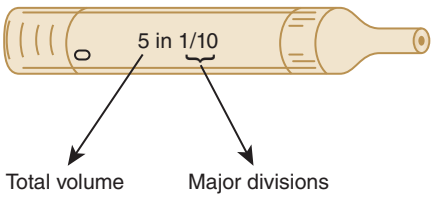

Figure 1.4 Volume indication of a pipette.

identifies the volumetric pipette. This type of pipette usually has the greatest degree of accuracy and precision and should be used when diluting standards, calibrators, or quality control material. They should only be used once prior to cleaning. Disposable transfer pipettes may or may not have calibration marks and are used to transfer solutions or biologic fluids without consideration of a specific volume. These pipettes should not be used in any quantitative analytic techniques (**Figure 1.5**).

The *automatic pipette* is the most routinely used pipette in today's clinical chemistry laboratory. Automatic pipettes come in a variety of types including fixed volume, variable volume, and multichannel. The term *automatic*, as used here, implies that the mechanism that draws up and dispenses the liquid is an integral part of the pipette. It may be a fully automated/self-operating, semiautomatic, or completely manually operated device. Automatic and semiautomatic pipettes have many advantages, including safety, stability, ease of use, increased precision, the ability to save time, and less cleaning required because the pipette tips are

Figure 1.5 Disposable transfer pipettes.

disposable. **Figure 1.6** illustrates many common automatic pipettes. A pipette associated with only one volume is termed a *fixed* volume, and models able to select different volumes are termed *variable*; however, only one volume may be used at a time. The available range of pipette volumes is 1 µL to 5000 mL. A pipette with a capability of

Figure 1.6 **(A)** Adjustable volume pipette. **(B)** Fixed volume pipette with disposable tips. **(C)** Multichannel pipette. **(D)** Multichannel pipette in use.

less than 1 mL is considered a *micropipette*, and a pipette that dispenses greater than 1 mL is called an *automatic macropipette*. Multichannel pipettes are able to attach multiple pipette tips to a single handle and can then be used to dispense a fixed volume of fluid to multiple wells, such as to a multiwell microtiter plate. In addition to classification by volume delivery amounts, automatic pipettes can also be categorized according to their mechanism: air-displacement, positive displacement, and dispenser pipettes. An *air-displacement pipette* relies on a piston for creating suction to draw the sample into a disposable tip that must be changed after each use. The piston does not come in contact with the liquid. A *positive-displacement pipette* operates by moving the piston in the pipette tip or barrel, much like a hypodermic syringe. It does not require a different tip for each use. Because of carryover concerns, rinsing and blotting between samples may be required. *Dispensers* and *dilutor/dispensers* are automatic pipettes that obtain the liquid from a common reservoir and dispense it repeatedly. The dispensing pipettes may be bottle-top, motorized, handheld, or attached to a dilutor. The dilutor often combines sampling and dispensing functions. Many automated pipettes use a wash between samples to eliminate carryover problems. However, to minimize carryover contamination with manual or semiautomatic pipettes, careful wiping of the tip may remove any liquid that adhered to the outside of the tip before dispensing any liquid. Care should be taken to ensure that the orifice of the pipette tip is not blotted, drawing sample from the tip. Another precaution in using manually operated semiautomatic pipettes is to move the plunger in a continuous and steady manner. Pipettes should be operated according to the manufacturer's directions.

Disposable, one-use pipette tips are designed for use with air-displacement pipettes. The laboratorian should ensure that the pipette tip is seated snugly onto the end of the pipette and free from any deformity. Plastic tips used on air-displacement pipettes can vary. Different brands can be used for one particular pipette, but they do not necessarily perform in an identical manner. Tips for positive-displacement pipettes are made of straight columns of glass or plastic. These tips must fit snugly to avoid carryover and can be used repeatedly without being changed after each use. As previously mentioned, these devices may need to be rinsed and dried between samples to minimize carryover.

Class A pipettes do not need to be recalibrated by the laboratory. Automatic pipetting devices, as well as non–Class A materials, do need recalibration.[15,16] Calibration of pipettes is done to verify accuracy and precision of the device and may be required by the laboratory's accrediting agency. A gravimetric method (see the Navigate 2 digital component resources for this procedure) can accomplish this task by delivering and weighing a solution of known specific gravity, such as water. A currently calibrated analytic balance and at least Class 2 weights should be used.[17] Deviation from the chosen volume is calculated based on the type of pipette tested. Pipettes that fall outside of the maximum allowable error will need to be adjusted following the manufacturer's instructions. Although gravimetric validation is the most desirable method,[18,19] pipette calibration may also be accomplished by using photometric methods, particularly for automatic pipetting devices. When a spectrophotometer is used, the molar absorptivity of a compound, such as potassium dichromate, is obtained. After an aliquot of diluent is pipetted, the change in concentration will reflect the volume of the pipette. Another photometric technique used to assess pipette accuracy compares the absorbances of dilutions of potassium dichromate, or another colored liquid with appropriate absorbance spectra, using Class A volumetric labware versus equivalent dilutions made with the pipetting device.

These calibration techniques are time consuming and impractical for use in daily checks. It is recommended that pipettes be checked initially and subsequently three or four times per year, or as dictated by the laboratory's accrediting agency. Many companies offer calibration services; the one chosen should also satisfy any accreditation requirements. A quick, daily check for many larger volume automatic pipetting devices involves the use of volumetric flasks. For example, a bottle-top dispenser that routinely delivers 2.5 mL of reagent may be checked by dispensing four aliquots of the reagent into a 10-mL Class A volumetric flask. The bottom of the meniscus should meet with the calibration line on the volumetric flask.

Syringes

Syringes are sometimes used for transfer of small volumes (< 500 µL) in blood gas analysis or in separation techniques such as chromatography or electrophoresis (**Figure 1.7**). The syringes are glass and have fine barrels. The plunger is often made of a fine piece of wire. Tips are not used when syringes are used for injection of sample into a gas chromatographic or

Figure 1.7 Microliter glass syringe.

high-pressure liquid chromatographic system. In electrophoresis work, however, disposable Teflon tips may be used.

Desiccators and Desiccants

Many compounds combine with water molecules to form loose chemical crystals. The compound and the associated water are called a **hydrate**. When the water of crystallization is removed from the compound, it is said to be **anhydrous**. Substances that take up water on exposure to atmospheric conditions are called **hygroscopic**. Materials that are very hygroscopic can remove moisture from the air as well as from other materials. These materials make excellent drying substances and are sometimes used as **desiccants** (drying agents) to keep other chemicals from becoming hydrated. Closed and sealed containers that include desiccant material are referred to as desiccators and may be used to store more hygroscopic substances. Many sealed packets or shipping containers, often those that require refrigeration, include some type of small packet of desiccant material to prolong storage.

Balances

A properly operating balance is essential in producing high-quality reagents and standards. However, because many laboratories discontinued in-house reagent preparation, balances may no longer be as widely used. Balances are classified according to their design, number of pans (single or double), and whether they are mechanical or electronic or classified by operating ranges.

Analytic and electronic balances are currently the most popular in the clinical laboratory. Analytic

Figure 1.8 Analytic balance.

balances (**Figure 1.8**) are required for the preparation of any primary standard. It has a single pan enclosed by sliding transparent doors, which minimize environmental influences on pan movement, tared weighing vessel, and sample. An optical scale allows the operator to visualize the mass of the substance. The weight range for many analytic balances is from 0.01 mg to 160 g.

Electronic balances (**Figure 1.9**) are single-pan balances that use an electromagnetic force to counterbalance the weighed sample's mass. Their measurements equal the accuracy and precision of any available mechanical balance, with the advantage of a fast response time (< 10 seconds).

Test weights used for calibrating balances should be selected from the appropriate ANSI/ASTM Classes 1 through 4.[19] Weighing instruments will need to be calibrated and adjusted periodically due to wear and tear from frequent use. Mechanisms for automatic adjustments are built into many newer instruments. These instruments will test and adjust the sensitivity of the device. Periodic verification is still necessary to assure the performance of that device. The frequency of calibration is dictated by the

Figure 1.9 Electronic top-loading balance.

accreditation/licensing guidelines for a specific laboratory. Balances should be kept clean and be located in an area away from heavy traffic, large pieces of electrical equipment, and open windows to prevent inaccurate readings. The level checkpoint should always be corrected before weighing occurs.

Centrifuges

Centrifugation is a process in which centrifugal force is used to separate serum or plasma from the blood cells as the blood samples are being processed; to separate a supernatant from a precipitate during an analytic reaction; to separate two immiscible liquids, such as a lipid-laden sample; or to expel air. When samples are not properly centrifuged, small fibrin clots and cells can cause erroneous results during analysis. The centrifuge separates the mixture based on mass and density of the component parts. It consists of a head or rotor, carriers, or shields that are attached to the vertical shaft of a motor or air compressor and enclosed in a metal covering. The centrifuge always has a lid, with new models having a locking lid for safety. Many models include a brake or a built-in tachometer, which indicates speed, and some centrifuges are refrigerated.

Centrifugal force depends on three variables: mass, speed, and radius. The speed is expressed in revolutions per minute (rpm), and the centrifugal force generated is expressed in terms of relative centrifugal force (RCF) or gravities (*g*). The speed of the centrifuge is related to the RCF by the following equation:

$$RCF = 1.118 \times 10^{-5} \times r \times (rpm)^2$$

where 1.118×10^{-5} is a constant, determined from the angular velocity, and r is the radius in centimeters, measured from the center of the centrifuge axis to the bottom of the test tube shield or bucket. Centrifuge classification is based on several criteria, including benchtop (**Figure 1.10A**) or floor model; refrigeration; rotor head (e.g., fixed angle, hematocrit, cytocentrifuge, swinging bucket [**Figure 1.10B**], or angled); or maximum speed attainable (i.e., ultracentrifuge).

Centrifuge maintenance includes daily cleaning of any spills or debris, such as blood or glass, and

Figure 1.10 **(A)** Benchtop centrifuge. **(B)** Swinging-bucket rotor.

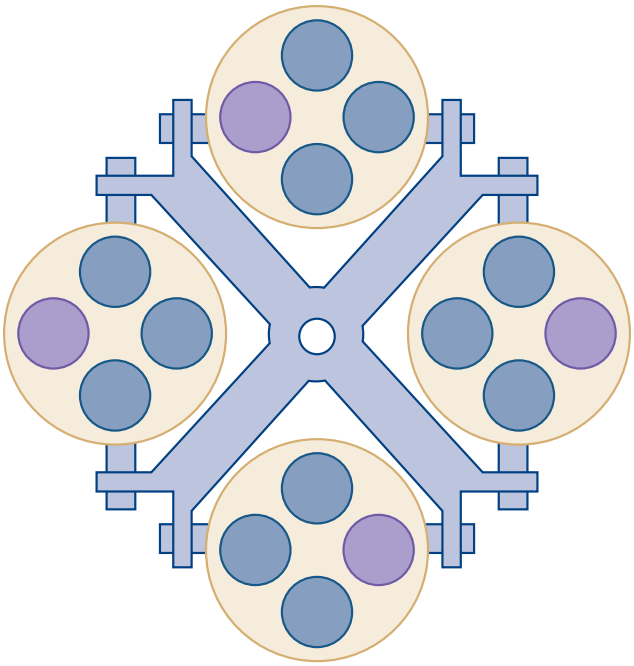

Figure 1.11 Properly balanced centrifuge. *Colored circles* represent counterbalanced positions for sample tubes.

ensuring that the centrifuge is properly balanced and free from any excessive vibrations. Balancing the centrifuge load is critical (**Figure 1.11**). Many newer centrifuges will automatically decrease their speed if the load is not evenly distributed, but more often, the centrifuge will shake and vibrate or make more noise than expected. A centrifuge needs to be balanced by equalizing both the volume and weight distribution across the centrifuge head. Many laboratories will have "balance" tubes of routinely used volumes and tube sizes, which can be used to match those from patient samples. A good rule of thumb is one

Figure 1.12 Properly loaded centrifuge.

of even placement and "opposition" (**Figure 1.12**). Exact positioning of tubes depends on the design of the centrifuge holders.

The centrifuge cover should remain closed until the centrifuge has come to a complete stop to avoid any aerosol production. It is recommended that the timer, brushes (if present), and speed be periodically checked. The brushes, which are graphite bars attached to a retainer spring, create an electrical contact in the motor. The specific manufacturer's service manual should be consulted for details on how to change brushes and on lubrication requirements. The speed of a centrifuge is easily checked using a tachometer or strobe light. The hole located in the lid of many centrifuges is designed for speed verification using these devices but may also represent an aerosol biohazard if the hole is uncovered. Accreditation agencies require periodic verification of centrifuge speeds.

CASE STUDY 1.2, PART 2

Recall Mía, the new graduate.

1. How should Mía place the chemistry tubes in the centrifuge?
2. If the centrifuge starts vibrating, what is the first troubleshooting step Mía should take?
3. If the rubber cap came off the tube during centrifugation, how should Mía decontaminate the centrifuge?

Laboratory Mathematics and Calculations

Significant Figures

Significant figures are the minimum number of digits needed to express a particular value in scientific notation without loss of accuracy. There are several rules in regard to identifying significant figures:

1. All nonzero numbers are significant (1, 2, 3, 4, 5, 6, 7, 8, 9).
2. All zeros between nonzero numbers are significant.
3. All zeros to the right of the decimal are not significant when followed by a nonzero number.
4. All zeros to the left of the decimal are not significant.

The number 814.2 has four significant figures, because in scientific notation, it is written as 8.142×10^2. The number 0.000641 has three significant figures, because the scientific notation expression for this value is 6.41×10^{-4}. The zeros to the right of the decimal preceding the nonzero digits are merely holding decimal places and are not needed to properly express the number in scientific notation. However, by convention, zeros following a decimal point are considered significant. For example, 10.00 has four significant figures. The zeros to the right of the decimal indicate the precision of this value.

Logarithms

Logarithms are the inverse of exponential functions and can be related as such:

$$x = A^B \text{ or } B = \log_A (x)$$

This is then read as B is the log base A of X, where B must be a positive number, A is a positive number, and A cannot be equal to 1. Calculators with a log function do not require conversion to scientific notation.

To determine the original number from a log value, the process is performed in reverse. This process is termed the *antilogarithm* or *antilog* as it is the inverse of the logarithm. Most calculators require using an inverse or secondary/shift function when entering this value. If given a log of 3.1525, the resulting value is 1.424×10^3 on the base 10 system. Consult the specific manufacturer's directions of the calculator to become acquainted with the proper use of these functions.

pH (Negative Logarithms)

In certain circumstances, the laboratorian may work with negative logs. Such is the case with pH or pK_a. As previously stated, the pH of a solution is defined as the negative log of the hydrogen ion concentration. The following is a convenient formula to determine the negative logarithm when working with pH or pK_a:

$$\frac{\text{pH}}{pK_a} = x - \log N \qquad \textbf{(Eq. 1.11)}$$

where x is the negative exponent base 10 expressed and N is the decimal portion of the scientific notation expression.

For example, if the hydrogen ion concentration of a solution is 5.4×10^{-6}, then $x = 6$ and $N = 5.4$. Substitute this information into **Equation 1.11**, and it becomes

$$\text{pH} = 6 - \log 5.4 \qquad \textbf{(Eq. 1.12)}$$

The logarithm of N (5.4) is equal to 0.7324, or 0.73. The pH becomes

$$\text{pH} = 6 - 0.73 = 5.27 \qquad \textbf{(Eq. 1.13)}$$

The same formula can be applied to obtain the hydrogen ion concentration of a solution when only the pH is given. Using a pH of 5.27, the equation becomes

$$5.27 = x - \log N \qquad \textbf{(Eq. 1.14)}$$

In this instance, the x term is always the next largest whole number. For this example, the next largest whole number is 6. Substituting for x, the equation becomes

$$5.27 = 6 - \log N \qquad \textbf{(Eq. 1.15)}$$

A shortcut is to simply subtract the pH from x (6 – 5.27 = 0.73) and take the antilog of that answer 5.73. The final answer is 5.73×10^{-6}. Note that rounding, while allowed, can alter the answer. A more algebraically correct approach follows in **Equations 1.16** through **1.18**. Multiply all the variables by –1:

$$\begin{aligned}(-1)(5.27) &= (-1)(6) - (-1)(\log N) \\ -5.27 &= -6 + \log N\end{aligned} \qquad \textbf{(Eq. 1.16)}$$