PART 1

Basic Principles and Practice in Clinical Chemistry

Basic Principles and Practices of Clinical Chemistry

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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

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

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

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:

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

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

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:

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

Rearrangement of this equation reveals

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

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

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

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

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

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:

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

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.

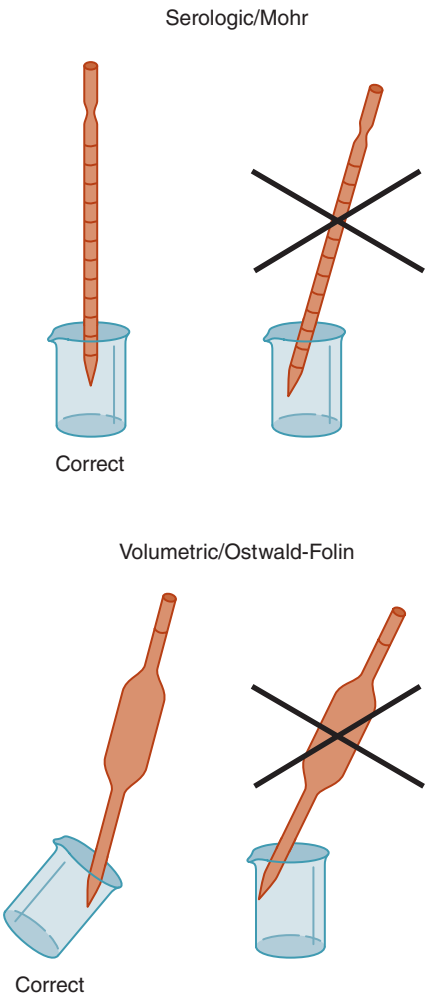


Figure 1.2 Correct and incorrect pipette positions.

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

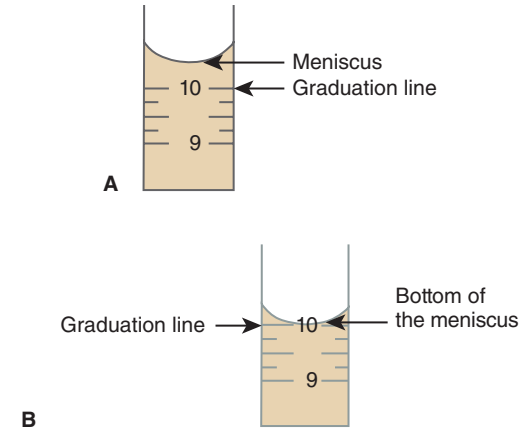


Figure 1.3 Pipetting technique. **(A)** Meniscus is brought above the desired graduation line. **(B)** Liquid is allowed to drain until the bottom of the meniscus touches the desired calibration mark.

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

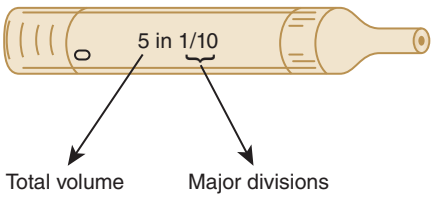


Figure 1.4 Volume indication of a pipette.

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

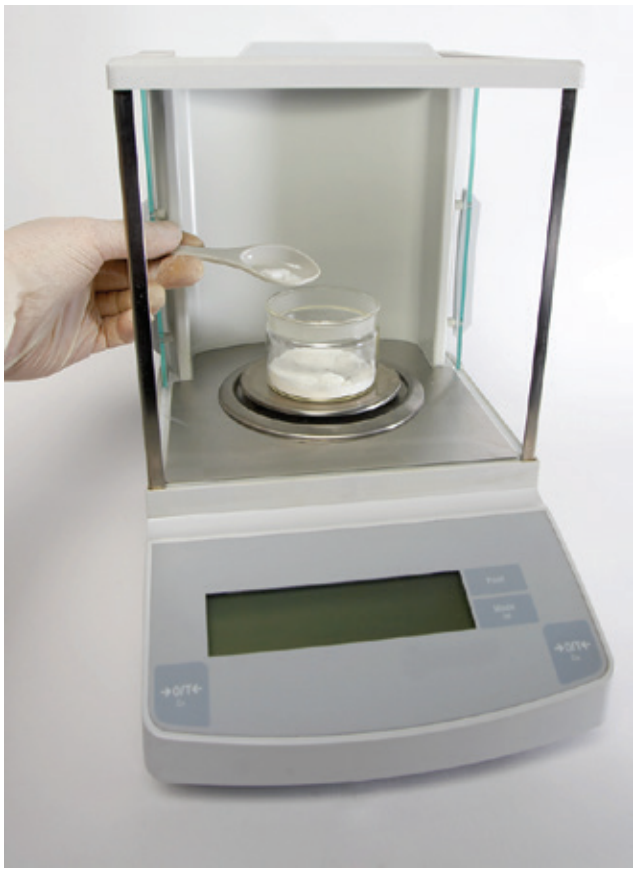


Figure 1.8 Analytic balance.

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.

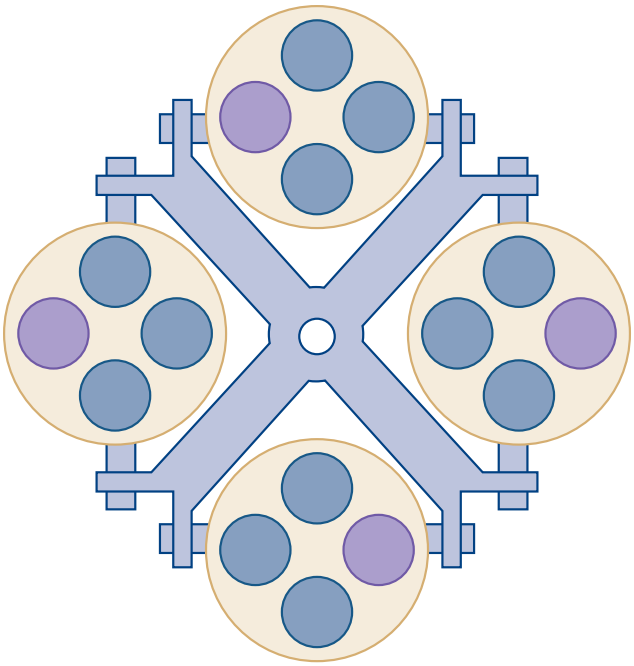


Figure 1.11 Properly balanced centrifuge. *Colored circles* represent counterbalanced positions for sample tubes.

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:

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

Solve the equation for the unknown quantity by adding a positive 6 to both sides of the equal sign, and the equation becomes

$$6 - 5.27 = \log N$$
$$0.73 = \log N \qquad \text{(Eq. 1.17)}$$

The result is 0.73, which is the antilogarithm value of N, which is 5.37, or 5.4:

$$\text{Antilog } 0.73 = N;\ N = 5.37 = 5.4 \qquad \text{(Eq. 1.18)}$$

The hydrogen ion concentration for a solution with a pH of 5.27 is 5.4×10^{-6}. Many scientific calculators have an inverse function that allows for more direct calculation of negative logarithms.

Concentration

A description of each concentration term is provided at the beginning of this chapter. The following discussion focuses on the basic mathematical expressions needed to prepare reagents of a stated concentration.

Percent Solution

A percent solution is determined in the same manner regardless of whether weight/weight, volume/volume, or weight/volume units are used. *Percent* implies "parts per 100," which is represented as percent (%) and is independent of the molecular weight of a substance.

Example 1.1: Weight/Weight (w/w)

To make up 250 g of a 5% aqueous solution of hydrochloric acid (using 12 M HCl), multiply the total amount by the percent expressed as a decimal. The 5% aqueous solution can be expressed as

$$5\% = \frac{5}{100} = 0.050 \qquad \text{(Eq. 1.19)}$$

Therefore, the calculation becomes

$$0.050 \times 250 \text{ g} = 12.5 \text{ g of 12M HCl} \qquad \text{(Eq. 1.20)}$$

Another way of arriving at the answer is to set up a ratio so that

$$\text{Desired solution concentration} = \text{Final product of 12 M HCl}$$
$$\frac{5}{100} = \frac{x}{250}$$
$$x = 12.5 \qquad \text{(Eq. 1.21)}$$

Example 1.2: Weight/Volume (w/v)

The most frequently used term for a percent solution is weight per volume, which is often expressed as grams per 100 mL of the diluent. To make up 1000 mL of a 10% (w/v) solution of NaOH, use the preceding approach. Restate the w/v as a fraction:

$$10\% = \frac{10 \text{ g}}{100 \text{ mL}} = 0.10$$

Next, set up a ratio and solve for x

$$\frac{10\text{g}}{100 \text{ mL}} = \frac{x}{1000 \text{ mL}}$$
$$x = 100\text{g} \qquad \text{(Eq. 1.22)}$$

Lastly, add 100 g of 10% NaOH to a 1000-mL volumetric Class A flask and add sufficient volume of reagent-grade water to the calibration mark.

Example 1.3: Volume/Volume (v/v)

If both chemicals in a solution are in the liquid form, the volume per unit volume is used to give the volume of solute present in 100 mL of solution. To make up 50 mL of a 2% (v/v) concentrated hydrochloric acid solution, a similar approach is used. The (v/v) is restated as a fraction:

$$2\% = \frac{2 \text{ mL}}{100 \text{ mL}} = 0.02$$

Then, the calculation becomes

$$0.02 \times 50 \text{ mL} = 1 \text{ mL}$$

or using a ratio

$$\frac{2 \text{ mL}}{100 \text{ mL}} = \frac{x}{50 \text{ mL}}$$
$$x = 1 \text{ mL} \qquad \text{(Eq. 1.23)}$$

Therefore, add 40 mL of reagent-grade water to a 50-mL Class A volumetric flask, add 1 mL of concentrated HCl, mix, and dilute up to the calibration mark with reagent-grade water. Remember, always add acid to water!

Molarity

Molarity (M) is routinely expressed in units of moles per liter (mol/L) or sometimes millimoles per milliliter (mmol/mL). Remember that 1 mol of a substance is equal to the gram molecular weight (gmw) of that

substance. When trying to determine the amount of substance needed to yield a particular concentration, initially decide what final concentration units are needed. For molarity, the final units will be moles per liter (mol/L) or millimoles per milliliter (mmol/mL). The second step is to consider the existing units and the relationship they have to the final desired units. Essentially, try to put as many units as possible into like terms and arrange so that the same units cancel each other out, leaving only those needed in the final answer. To accomplish this, it is important to remember what units are used to define each concentration term. It is key to understand the relationship between molarity (moles/liter), moles, and gmw. While molarity is given in these examples, the approach for molality is the same except that one molal is expressed as one mole of solute per kilogram of solvent. For water, one kilogram is proportional to one liter, so molarity and molality are equivalent.

Example 1.4

How many grams are needed to make 1 L of a 2 M solution of HCl?

Step 1: What *units* are needed in the final answer? *Answer:* Grams per liter (g/L).

Step 2: Assess other mass/volume terms used in the problem. In this case, moles are also needed for the calculation: How many grams are equal to 1 mole? The gmw of HCl, which can be determined from the periodic table, will be equal to 1 mole. For HCl, the gmw is 36.5g/mol, so the equation may be written as

$$\frac{36.5\ \text{g HCl}}{\cancel{\text{mol}}} \times \frac{2\ \cancel{\text{mol}}}{\text{L}} = \frac{73\ \text{g HCl}}{\text{L}} \qquad \textbf{(Eq. 1.24)}$$

Cancel out like units, and the final units should be grams per liter. In this example, 73 g HCl per liter is needed to make a 2 M solution of HCl.

Example 1.5

A solution of NaOH is contained within a Class A 1-L volumetric flask filled to the calibration mark. The content label reads 24 g of NaOH. Determine the molarity.

Step 1: What *units* are needed? *Answer:* Moles per liter (mol/L).

Step 2: The units that exist are grams and L. NaOH may be expressed as moles and grams. The calculated gmw of NaOH is 40 g/mol. Rearrange the equation so that grams can be canceled, and the remaining units reflect those needed in the answer, which are mole/L.

Step 3: The equation becomes

$$\frac{24\ \cancel{\text{g}}\ \text{NaOH}}{\text{L}} \times \frac{1\text{mol}}{40\ \cancel{\text{g}}\ \text{NaOH}} = 0.6\frac{\text{mol}}{\text{L}} \qquad \textbf{(Eq. 1.25)}$$

By canceling out like units and performing the appropriate calculations, the final answer of 0.6 M or 0.6 mol/L.

Example 1.6

Prepare 250 mL of a 4.8 M solution of HCl.

Step 1: Start with what is given: 4.8 moles/L

Step 2: Determine the gmw of HCl ([H = 1] + [Cl = 35.5] = 36.5 g/mol)

Step 3: Set up the equation, cancel out like units, and perform the appropriate calculations:

$$\frac{4.8\ \text{mol}}{1\ \text{L}} \times \frac{36.5\ \text{g HCl}}{1\ \text{mol}} \times \frac{1\ \text{L}}{1000\ \text{mL}} \times 250\ \text{mL} = 43.8\ \text{g HCl} \qquad \textbf{(Eq. 1.26)}$$

In a 250-mL Class A volumetric flask, add 200 mL of reagent-grade water. Add 43.8 g of HCl and mix. Dilute up to the calibration mark with reagent-grade water.

Normality

Normality (N) is expressed as the number of equivalent weights per liter (Eq/L) or milliequivalents per milliliter (mmol/mL). Equivalent weight is equal to gmw divided by the valence (*V*). Normality has often been used in acid–base calculations because an equivalent weight of a substance is also equal to its combining weight (or the weight that will combine with or displace 1 mole of hydrogen). Another advantage in using equivalent weight is that an equivalent weight of one substance is equal to the equivalent weight of any other chemical.

Example 1.7

Give the equivalent weight, in grams, for each substance listed below.

1. NaCl (gmw = 58 g/mol, valence = 1)

$$\frac{58\ \text{g}}{\text{L}} = 58\ \text{g per equivalent weight} \qquad \textbf{(Eq. 1.27)}$$

2. H_2SO_4 (gmw = 98 g/mol, valence = 2)

$$\frac{98\text{ g}}{2} = 49\text{ g per equivalent weight} \qquad \textbf{(Eq. 1.28)}$$

Example 1.8

What is the normality of a 500 mL solution that contains 7 g of H_2SO_4?

Step 1: What units are needed? *Answer:* Normality expressed as equivalents per liter (Eq/L).

Step 2: Start with what is given: 7 g/500 mL

Step 3: Calculate the gmw of H_2SO_4 (98 g/mol) and determine the valence (2)

Step 4: Add a conversion factor to convert mL to L (1000 mL/1 L)

Step 5: Cancel out like terms and calculate the result in Eq/L.

This equation is:

$$\frac{7\text{ g } H_2SO_4}{500\text{ mL}} \times \frac{1\text{ Eq}}{49\text{ g } H_2SO_4} \times \frac{1000\text{ mL}}{1\text{ L}} \qquad \textbf{(Eq. 1.29)}$$
$$= 0.285\text{ Eq/L} = 0.285\text{ N}$$

Example 1.9

What is the normality of a 0.5 M solution of H_2SO_4? Continuing with the previous approach, the final equation is

$$\frac{0.5\text{ mol } H_2SO_4}{L} \times \frac{98\text{ g } H_2SO_4}{\text{mol } H_2SO_4} \times \frac{1\text{ Eq } H_2SO_4}{49\text{ g } H_2SO_4}$$
$$= 1\text{ Eq/L} = 1\text{N}$$

(Eq. 1.30)

When converting between molarity and normality, the following conversion formula may be applied:

$$M \times V = N \qquad \textbf{(Eq. 1.31)}$$

where V is the valence of the compound. Using this formula, **Example 1.9** becomes

$$0.5\text{ M} \times 2 = 1\text{N} \qquad \textbf{(Eq. 1.32)}$$

Example 1.10

What is the molarity of a 2.5 N solution of HCl? This problem may be solved in several ways. One way is to use the stepwise approach in which existing units are exchanged for units needed. The equation is

$$\frac{2.5\text{ Eq HCl}}{\text{L}} \times \frac{36.5\text{ g} \times \text{HCl}}{1\text{ Eq}} \times \frac{1\text{ mol HCl}}{36.5\text{ g HCl}} \qquad \textbf{(Eq. 1.33)}$$
$$= 2.5\text{ mol/L HCl}$$

The second approach is to use the normality-to-molarity conversion formula. The equation now becomes

$$M \times V = 2.5\text{ N}$$
$$V = 1$$
$$M = \frac{2.5\text{ N}}{1} = 2.5\text{ N} \qquad \textbf{(Eq. 1.34)}$$

When the valence of a substance is 1, the molarity will equal the normality. As previously mentioned, normality either equals or is greater than the molarity.

Although there are various methods to calculate laboratory mathematical problems, the technique of using conversion factors and canceling like units is used in most clinical chemistry calculation, regardless of whether the problem requests molarity and normality or exchanging one concentration term for another. However, it is necessary to recall the interrelationship between all the units in the expression.

Specific Gravity

Density is expressed as mass per unit volume of a substance. The **specific gravity** is the ratio of the density of a material when compared with the density of pure water at a given temperature and allows the laboratorian a means of expressing density in terms of volume. The units for density are grams per milliliter (g/mL). Specific gravity is often used with very concentrated materials, such as commercial acids (e.g., sulfuric and hydrochloric acids).

The density of a concentrated acid can also be expressed in terms of an assay or percent purity. The actual concentration is equal to the specific gravity multiplied by the assay or per*cent purity value* (expressed as a decimal) stated on the label of the container.

Example 1.11

What is the actual weight of a supply of concentrated HCl on which the label reads, specific gravity 1.19 with an assay value of 37%?

$$1.19\text{ g/mL} \times 0.37 = 0.44\text{g/mL of HCl} \qquad \textbf{(Eq. 1.35)}$$

Example 1.12

What is the molarity of this same stock solution? The final units desired are moles per liter (mol/L). The molarity of the solution is

$$\frac{0.44\ \text{g HCl}}{\text{mL}} \times \frac{1\ \text{mol HCl}}{36.5\ \text{g HCl}} \times \frac{1000\ \text{mL}}{\text{L}} \quad \text{(Eq. 1.36)}$$

$$= 12.05\ \text{mol/L or } 12\text{M}$$

Conversions

To convert one unit into another, the same approach of canceling out like units can be applied. In some instances, a chemistry laboratory may report a given analyte using two different concentration units—for example, calcium. The recommended SI unit for calcium is millimoles per liter. The more traditional units are milligrams per deciliter (mg/dL). Again, it is important to understand the relationship between the units given and those needed in the final answer.

Example 1.13

Convert 8.2 mg/dL calcium to millimoles per liter (mmol/L). The gmw of calcium is 40 g. If there are 40 g per mol, then it follows that there are 40 mg per mmol. The final units needed are mmol/L. The equation becomes

$$\frac{8.2\ \text{mg}}{\text{dL}} \times \frac{10\ \text{dL}}{1\ \text{L}} \times \frac{1\ \text{mmol}}{40\ \text{mg}} = \frac{2.05\ \text{mmol}}{\text{L}} \quad \text{(Eq. 1.37)}$$

Once again, the systematic stepwise approach of canceling similar units can be used for this conversion problem.

A frequently encountered conversion problem or, more precisely, a dilution problem occurs when a weaker concentration or different volume is needed than the stock substance available, but the *concentration terms* are the same. The following formula is used where V_1 is the volume of the first substance, C_1 is the concentration of the first substance, V_2 is the volume of the second substance, and C_2 is the concentration of the second substance:

$$V_1 \times C_1 = V_2 \times C_2 \quad \text{(Eq. 1.38)}$$

This formula is useful only if the concentration and volume units between the substances are the *same* and if three of four variables are known.

Example 1.14

What volume is needed to make 500 mL of a 0.1 M solution of Tris buffer from a solution of 2 M Tris buffer?

Identify the known values:

Concentration of initial substance (C_1) = 2 M

Volume of the product (V_2) = 500 mL

Concentration of the product (C_2) = 0.1 M

And the equation becomes:

$$V_1 \times 2\ \text{M} = 0.1\ \text{M} \times 500\ \text{mL}$$
$$(V_1)(2\ \text{M}) = (0.1\ \text{M})(500\ \text{mL});$$
$$(V_1)(2\ \text{M}) = 50\ \text{mL}$$

$$\text{Therefore, } V_1 \frac{50\ \text{mL}}{2} = 25\ \text{mL} \quad \text{(Eq. 1.39)}$$

It requires 25 mL of the 2 M solution to make up 500 mL of a 0.1 M solution.

This problem differs from the other conversions in that it is actually a dilution of a stock solution. While this approach will provide how much stock is needed when making the solution, the laboratorian must subtract the obtained volume value from the final desired volume to determine the amount of diluent needed, in this case 475 mL. A more involved discussion of dilution problems follows.

Dilutions

A **dilution** represents the part(s) of concentrated material to the total final volume of a solution. It consists of the parts of the substance being diluted in the total numbers of parts of the solution. In contrast, ratio refers to part substance to part substance. The most common dilution uses one part patient serum plus one part saline. This is a 1:1 **ratio** of serum to saline. It is a 1:2 *dilution* which can also be expressed as a fraction (½ dilution). After analysis, the laboratory result is multiplied by the reciprocal of the dilution (2/1) which is known as the *dilution factor*. Dilutions are required when the result is above the linearity of the assay. A dilution is an expression of concentration. Because a dilution is made by adding a more concentrated substance to a diluent, the dilution is always less concentrated than the original substance. There is an inverse relationship between the dilution factor and concentration. As the *dilution factor* increases, the *concentration decreases*. A dilution can be expressed as either a fraction or a ratio.[20]

Example 1.15

What dilution is needed to make a 100 mmol/L sodium solution from a 3000 mmol/L stock solution?

$$\frac{100\ \text{mmol}}{\cancel{L}} \times \frac{\cancel{L}}{3000\ \text{mmol}} = \frac{1}{30} \qquad \text{(Eq. 1.40)}$$

The dilution indicates 1 part stock solution is needed to make a *total volume* of 30 mL. To prepare this dilution, 1 mL of stock solution is added to 29 mL of diluent to achieve a total final volume of 30 mL. Note that the *dilution* indicates the *parts per total* amount. In making the dilution, the sum of the amount of the stock material plus the amount of the diluent must equal the *total volume*.

It is important to differentiate when a dilution or a ratio is stated within a procedure. For example, making a "1-**in**-4" dilution means adding one part stock to obtain a *total* of four parts. That is, one part of stock would be added to three parts of diluent. The dilution would be 1/4. Analyses performed on the diluted material would need to be multiplied by 4, the dilution factor, to get the final concentration. The *dilution factor* is the reciprocal of the dilution. This is very different from a procedure indicating preparation of a "1-**to**-4" ratio! In this instance, the dilution would be 1/5, where there is 1 part of serum and 4 parts of diluent. It is important that you fully understand the meaning of these expressions. Dilutions should be made using reagent-grade water, saline, or method-specific diluent. The diluted sample should be thoroughly mixed before analysis.

Example 1.16

Make 150 mL of the 100 mmol/L sodium solution using a 1:30 dilution.

Begin with the dilution and set up a ratio between the desired part (mL) to the total parts (150 mL) to determine the amount of sodium solution needed. The equation becomes

$$\frac{1}{30} = \frac{x\ \text{mL}}{150\ \text{mL}} \qquad \text{(Eq. 1.41)}$$

$$x = 5\ \text{mL}$$

Note that 5/150 reduces to the dilution of 1/30. To make up this solution, 5 mL of sodium solution is added to 145 mL of diluent, making the total volume equal to 150 mL.

Example 1.17

The formula $(V_1)(C_1) = (V_2)(C_2)$ may also be used for simple dilution calculations. This is acceptable, as long as stock volume is subtracted from the total final volume for the correct *diluent* volume.

$$(V_1)(C_1) = (V_2)(C_2)(x)(3000\ \text{mmol/L})$$

$$= (150\ \text{mL})(100\ \text{mmol/L})$$

$$x = 5$$

150 − 5 = 145 mL of diluent shouldbe added to 5 mL of stock (Eq. 1.42)

Simple Dilutions

When making a *simple dilution*, the laboratorian must decide on the total volume desired and the amount of stock to be used.

Example 1.18

A 1/10 dilution of serum can be achieved by using any of the following approaches. A ratio of 1:9—one part serum and nine parts diluent (saline) can be achieved using the following:

- 100 µL of serum added to 900 µL of saline
- 20 µL of serum added to 180 µL of saline
- 1 mL of serum added to 9 mL of saline
- 2 mL of serum added to 18 mL of saline

Note that the sum of the ratio of serum to diluent (1:9) needed to make up each dilution satisfies the dilution factor (1/10) of stock material to total volume. When thinking about the stock to diluent volume, subtract the parts of stock needed from the total volume or parts to get the number of diluent "parts" needed. Once the volume of each part, usually stock, is known, multiply the diluent parts needed to obtain the correct volume.

Example 1.19

You have a 10 g/dL stock of protein standard. You need a 2 g/dL standard. You only have 0.200 mL of 10 g/dL stock to use. The procedure requires 0.100 mL.

Solution:

$$\frac{2\ \text{g/dL}}{10\ \text{g/dL}} = \frac{1}{5} = \text{Dilution} \qquad \text{(Eq. 1.43)}$$

You will need 1 part or volume of stock in a total of 5 parts or volumes. Subtracting 1 from 5 yields that 4 parts or volumes of diluent is needed (**Figure 1.13**). In this instance, you need at least

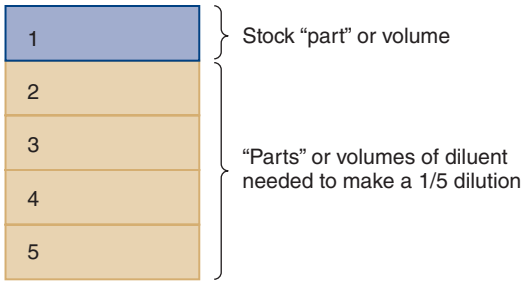


Figure 1.13 Simple dilution. Consider this diagram depicting a substance having a 1/5 dilution factor. The dilution factor represents that 1 part of stock is needed from a total of 5 parts. To prepare this dilution, you would determine the volume of 1 "part," usually the stock or patient sample. The remainder of the "parts" or total would constitute the amount of diluent needed, or four times the volume used for the stock.

0.100 mL for the procedure. You have 0.200 mL of stock. You can make the dilution in various ways, as seen in **Example 1.20**.

Example 1.20

There are several ways to prepare a 1/5 dilution having only 0.200 mL of stock and needing a total minimum volume of 0.100 mL.

- Add 0.050 mL stock (1 part) to 0.200 mL of diluent (4 parts × 0.050 mL).
- Add 0.100 mL of stock (1 part) to 0.400 mL of diluent (4 parts × 0.100 mL).
- Add 0.200 mL of stock (1 part) to 0.800 mL of diluent (4 parts × 0.200 mL).

The dilution factor is also used to determine the final concentration of a dilution by multiplying the original concentration by the inverse of the dilution factor or the dilution factor denominator when it is expressed as a fraction.

Example 1.21

Determine the concentration of a 200 µmol/mL human chorionic gonadotropin (hCG) standard that was diluted 1/50. This value is obtained by multiplying the original concentration, 200 µmol/mL hCG, by the dilution factor, 1/50. The result is 4 µmol/mL hCG. Quite often, the concentration of the original material is needed.

Example 1.22

A 1/2 dilution of serum with saline had a creatinine result of 8.6 mg/dL. Calculate the actual serum creatinine concentration.

Dilution factor: 1/2

Dilution result = 8.6 mg/dL

Because this result represents 1/2 of the concentration, the inverse (or reciprocal) of the dilution is used, and the serum creatinine value must take into consideration this dilution, so the actual value is

$$2 \times 8.6 \text{ mg/dL} = 17.2 \text{ mg/dL} \qquad \textbf{(Eq. 1.44)}$$

Serial Dilutions

A **serial dilution** may be defined as multiple, progressive dilutions that dilutes highly concentrated solutions to produce solutions with lower concentration. Serial dilutions are extremely useful when the volume of concentrate or diluent is in short supply and its use needs to be minimized, or when a number of dilutions are required, such as in determining a titer. For instance, the volume of patient sample available to the laboratory may be small (e.g., pediatric samples), and a serial dilution may be needed to ensure that sufficient sample is available for analysis.

The serial dilution is initially made in the same manner as a simple dilution. Subsequent dilutions will then be made from each preceding dilution. When a serial dilution is made, certain criteria may need to be satisfied. The criteria vary with each situation but usually include such considerations as the total volume desired, the amount of diluent or concentrate available, the dilution factor, the final concentration needed, and the support materials required.

Example 1.23

A three-tube, twofold serial dilution is to be made on a sample. To start, the three tubes must be labeled. It is arbitrarily decided that the total volume for each dilution is to be 1 mL. Into the first tube, 0.5 mL of diluent is added and then 0.5 mL of patient sample. This satisfies the "twofold" or 1/2 dilution for tube 1. In the next tube, 0.5 mL of diluent is again added, along with 0.5 mL of well-mixed liquid from tube 1. This satisfies the 1/2 dilution in tube 2, bringing the total tube dilution to 1/4. For the third tube, 0.5 mL of diluent is added, along with 0.5 mL of well-mixed liquid from tube 2. This satisfies the 1/2 dilution within the tube but brings the total tube dilution to 1/8. The calculation for these values is

$$\frac{1}{2}(\text{Tube 1 Dilution}) \times \frac{1}{2}(\text{Tube 2 Dilution}) = \frac{1}{4} \text{ total dilution for tube 2} \qquad \textbf{(Eq. 1.45)}$$

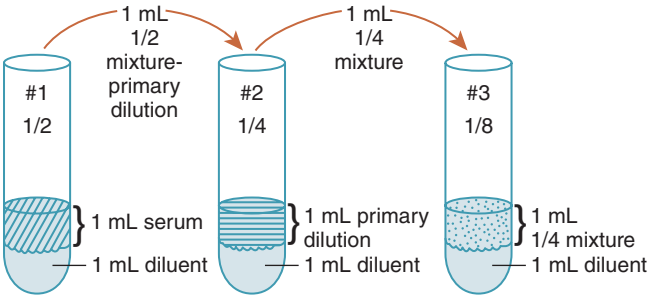


Figure 1.14 Serial dilution.

Making a 1/2 dilution of the 1/4 dilution will result in the next dilution (1/8) in Tube 3. To establish the dilution factor needed for subsequent dilutions, it is helpful to solve the following equation for (*x*):

$$\text{Stock/preceding concentration} \times (x) = (\text{final dilution factor}) \quad \textbf{(Eq. 1.46)}$$

Refer to **Figure 1.14** for an illustration of this serial dilution.

Example 1.24

Another type of dilution combines several dilution factors that are not multiples of one another. In our previous example, 1/2, 1/4, and 1/8 dilutions are all related to one another by a factor of 2. Consider the situation when 1/10, 1/20, 1/100, and 1/200 dilution factors are required. There are several approaches to solving this type of dilution problem. One method is to treat the 1/10 and 1/20 dilutions as one serial dilution problem, the 1/20 and 1/100 dilutions as a second serial dilution, and the 1/100 and 1/200 dilutions as the last serial dilution. Another approach is to consider what dilution factor of the concentrate is needed to yield the final dilution. In this example, the initial dilution is 1/10, with subsequent dilutions of 1/20, 1/100, and 1/200. The first dilution may be accomplished by adding 1 mL of stock to 9 mL of diluent. The total volume of solution is 10 mL. Our initial dilution factor has been satisfied. In making the remaining dilutions, 2 mL of diluent is added to each test tube.

$$\text{Initial/preceding dilution} \times (x) = \text{dilution needed}$$

Solve for (*x*).

Using the dilution factors listed above and solving for (*x*), the equations become

$$1/10 \times (x) = 1/20$$

where $(x) = 2$ (or 1 part stock to 1 part diluent)

$$1/20 \times (x) = 1/100$$

where $(x) = 5$ (or 1 part stock to 4 parts diluent)

$$1/100 \times (x) = 1/200$$

where $(x) = 2$ (or 1 part stock to 1 part diluent) **(Eq. 1.47)**

In practice, the 1/10 dilution must be diluted by a factor of 2 to obtain a subsequent 1/20 dilution. Because the second tube already contains 2 mL of diluent, 2 mL of the 1/10 dilution should be added (1 part stock to 1 part diluent). In preparing the 1/100 dilution from this, a 1/5 dilution factor of the 1/20 mixture is required (1 part stock to 4 parts diluent). Because this tube already contains 2 mL, the volume of diluent in the tube is divided by its parts, which is 4; thus, 500 µL, or 0.500 mL, of stock should be added. The 1/200 dilution is prepared in the same manner using a 1/2 dilution factor (1 part stock to 1 part diluent) and adding 2 mL of the 1/100 to the 2 mL of diluent already in the tube.

Water of Hydration

Some compounds are available in a hydrated form. A reagent protocol often designates the use of an anhydrous form of a chemical; frequently, however, all that is available is a hydrated form. To obtain a correct gmw for these chemicals, the attached water molecule(s) must be included.

Example 1.25

How much $CuSO_4 \cdot 5H_2O$ must be weighed to prepare 1 L of 0.5 *M* $CuSO_4$? When calculating the gmw of this substance, the water weight of 90 g must be considered so that the gmw is 250 g rather than gmw of $CuSO_4$ alone (160 g). Therefore,

$$\frac{250\ \cancel{g}\ CuSO_4\ 5H_2O}{\cancel{mol}} \times \frac{0.5\ \cancel{mol}}{1\ \cancel{g}} = 125\ g/L \quad \textbf{(Eq. 1.48)}$$

Cancel out like terms to obtain the result of 125 g/L of the hydrated form $CuSO_4 \cdot 5H_2O$.

Example 1.26

A procedure requires 0.9 g of $CuSO_4$. All that is available is $CuSO_4 \cdot 5H_2O$. What weight of $CuSO_4 \cdot 5H_2O$ is needed? Calculate the percentage of $CuSO_4$ present in $CuSO_4 \cdot 5H_2O$. Again, using the gmw, the percentage is

$$\frac{160}{250} = 0.64, \text{ or } 64\% \quad \textbf{(Eq. 1.49)}$$

Therefore, 1 g of $CuSO_4 \cdot 5H_2O$ contains 0.64 g of $CuSO_4$, so the equation becomes

$$\frac{0.9 \text{ g } CuSO_4 \text{ needed}}{0.64 \ CuSO_4 \text{ in } CuSO_4 \cdot 5H_2O} = 1.41 \text{ g } CuSO_4 \cdot 5H_2O \text{ required} \quad \textbf{(Eq. 1.50)}$$

Graphing and Beer's Law

The Beer-Lambert law (**Beer's law)** mathematically establishes the relationship between analyte concentration and absorbance of light in many photometric determinations. Beer's law states that the concentration of a substance is directly proportional to the amount of light absorbed or inversely proportional to the logarithm of the light transmitted. Beer's law can be expressed as

$$A = abc \quad \textbf{(Eq. 1.51)}$$

where A is absorbance; a is the absorptivity constant for a particular compound at a given wavelength under specified conditions (such as temperature and pH); b is the length of the light path; and c is the concentration.

If a method follows Beer's law, then absorbance is proportional to concentration as long as the length of the light path and the absorptivity of the absorbing species remain unaltered during the analysis. In practice, however, there are limits to the predictability of a linear response. In automated systems, adherence to Beer's law is often determined by checking the linearity of the test method over a wide concentration range. The *limits of linearity* often represent the reportable range of an assay. This term should not be confused with the reference ranges associated with clinical significance of a test. Assays measuring absorbance generally obtain the concentration results by using a graph of Beer's law, known as a standard graph or curve. This graph is made by plotting absorbance versus the concentration of known standards (**Figure 1.15**). Because most photometric assays set the initial absorbance to zero (0) using a reagent blank, the initial data points are 0,0. Graphs should be labeled properly and the concentration units must be given. The horizontal axis is referred to as the *x*-axis, whereas the vertical line is the *y*-axis. By convention in the clinical laboratory, concentration is usually plotted on the *x*-axis. On a standard graph, only the standard and the associated absorbances are plotted.

In terms of transmitted light, Beer's law is expressed as percent transmission (%T), where T is

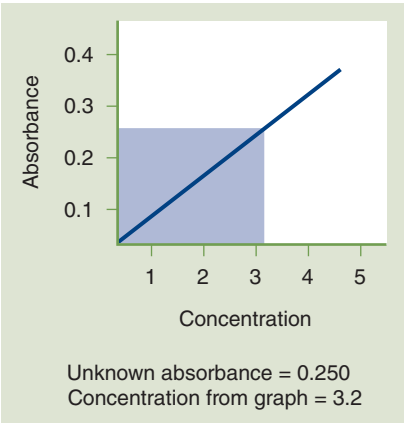


Figure 1.15 Standard curve.

defined as the ratio of the radiant energy transmitted divided by the radiant incident energy of the sample (I). The mathematical derivation of %T can be expressed as

$$\%T = \frac{I}{I_0} \times 100 \quad \textbf{(Eq. 1.52)}$$

Where I_0 is the incident light, and I is the transmitted light. The relationship between transmission and absorbance yields a nonlinear curve, and can be calculated using the following formula:

$$A = -\log\left(I/I_0\right) = \log(100\,\%) - \log\%T = 2 - \log\%T \quad \textbf{(Eq. 1.53)}$$

Once a standard curve has been established, it is permissible to run just one standard, or *calibrator*, as long as the system remains unchanged. A **One-point calibration** or calculation refers to the calculation of the comparison of the known standard or calibrator concentration and its corresponding absorbance to the absorbance of an unknown value according to the following ratio, where C_u and A_u indicate the concentration and absorbance, respectively, for the unknown value:

$$\frac{\text{Concentration of standard } (C_s)}{\text{Absorbance of standard } (A_s)} = \frac{\text{Concentration of standard } (C_u)}{\text{Absorbance of standard } (A_u)} \quad \textbf{(Eq. 1.54)}$$

Solving for the concentration of the unknown, the equation becomes

$$C_u = \frac{(A_u)(C_s)}{A_s} \quad \textbf{(Eq. 1.55)}$$

Example 1.27

The biuret assay method for protein is very stable and follows Beer's law. Rather than make up a standard graph, one protein standard of 6 g/dL concentration was assayed. The measured absorbance of the standard was 0.400, and the measured absorbance of the unknown was 0.350. Determine the value of the unknown in g/dL.

$$C_u = \frac{(0.350)(6\ \text{g/dL})}{(0.400)} = 5.25\ \text{g/dL} \qquad \textbf{(Eq. 1.56)}$$

This method of calculation is acceptable as long as everything in the system, including the instrument and lot number of reagents, remains the same. If anything in the system changes, a new standard graph should be generated. Verification of linearity and/or calibration is required whenever a system changes or becomes unstable. Regulatory agencies often prescribe the condition of verification as well as how frequently the linearity needs to be performed.

Enzyme Calculations

Another application of Beer's law is the calculation of enzyme assay results. When calculating enzyme results, the rate of change in absorbance over time is often monitored continuously during the reaction to give the difference in absorbance, known as the **delta absorbance**, or ΔA. Instead of using a standard graph or a one-point calculation, the molar absorptivity of the product is used. If the absorptivity constant and absorbance, in this case ΔA, are known, Beer's law can be used to calculate the enzyme concentration directly without the need of a standard graph, as follows:

$$A = abC$$
$$C = \frac{A}{ab} \qquad \textbf{(Eq. 1.57)}$$

When the absorptivity constant (a) is given in units of grams per liter (moles) through a 1-centimeter (cm) light path, the term *molar absorptivity* (ε) is used. Substitution of ε for a and ΔA for A produces the following Beer's law formula:

$$C = \frac{\Delta A}{\varepsilon} \qquad \textbf{(Eq. 1.58)}$$

For reporting enzyme activity, the IU, or **international unit**, is defined as the amount of enzyme that will catalyze 1 µmol of substrate per minute per liter. These units were often expressed as units per liter (U/L). The designations IU, U, and IU/L were adopted by many clinical laboratories to represent the IU. Although the reporting unit is the same, unless the analysis conditions are identical, use of the IU does not standardize the actual enzyme activity, and therefore, results between different methods of the same enzyme do not result in equivalent activity of the enzyme. For example, an alkaline phosphatase performed at 37°C will catalyze more substrate than if it is run at lower temperature, such as 25°C, even though the unit of expression, U/L, will be the same. The SI recommended unit is the *katal*, which is expressed as moles per liter per second. Whichever unit is used, calculation of the activity using Beer's law requires inclusion of the dilution and, depending on the reporting unit, possible conversion to the appropriate term (e.g., µmol to mol, mL to L, minute to second, and temperature factors). Beer's law for the IU now becomes

$$C = \frac{(\Delta A)10^{-6}(\text{TV})}{(\varepsilon)(b)(\text{SV})} \qquad \textbf{(Eq. 1.59)}$$

where TV is the total volume of sample plus reagents in mL and SV is the sample volume used in mL. The 10^{-6} converts moles to µmol for the IU. If another unit of activity is used, such as the katal, conversion into liters and seconds would be needed, but the conversions to and from micromoles are excluded.

Example 1.28

The ΔA per minute for an enzyme reaction is 0.250. The product measured has a molar absorptivity of 12.2×10^3 at 425 nm at 30°C. The incubation and reaction temperature are also kept at 30°C. The assay calls for 1 mL of reagent and 0.050 mL of sample. Give the enzyme activity results in international units.

Applying Beer's law and the necessary conversion information, the equation becomes

$$C = \frac{(0.250)(10^{-6})(1.050\ \text{mL})}{(12.2\times10^3)(1)(0.050\ \text{mL})} = 430\ \text{U} \qquad \textbf{(Eq. 1.60)}$$

Note: b is usually given as 1 cm; because it is a constant, it may not be considered in the calculation.

Specimen Collection and Handling

The process of specimen collection, handling, and processing remains one of the primary areas of *preanalytic* errors. Careful attention to each phase of the testing process is necessary to ensure proper subsequent testing and reporting of accurate and reliable results. All accreditation agencies require laboratories to clearly define and delineate the procedures used for proper collection, transport, and processing of patient samples and the steps used to minimize and detect any errors, along with the documentation of the resolution of any errors. The Clinical Laboratory Improvement Amendments Act of 1988 (CLIA 88)[21] specifies that procedures for specimen submission and proper handling be documented, including the disposition of any specimen that does not meet the laboratories' criteria of acceptability.

Types of Samples

Phlebotomy, or *venipuncture*, is the act of obtaining a blood sample from a vein using a needle attached to a collection device or a stoppered *evacuated tube*. The collection tubes are available in different volume sizes: from pediatric sizes (≈150 µL) to larger 10 mL tubes. The most frequent site for venipuncture is the medial antecubital vein of the arm. A tourniquet made of pliable nonlatex rubber flat band or tubing is wrapped around the arm, causing cessation of blood flow and dilation of the veins, making for easier detection. The gauge of the needle is inversely related to the size of the needle; the larger the number, the smaller the needle bore and length. Routine venipuncture uses a 23- or 21-gauge needle. A winged infusion set, sometimes referred to as a butterfly because of the appearance of the setup, may be used whenever the veins are fragile, small, or difficult to detect. The butterfly needle is attached to a piece of tubing, which is then attached to a hub or barrel. Because of potential for needlesticks and cost of the product, this practice may be discouraged. However, newer push-button safety devices are now available.

When selecting an appropriate vein, sites adjacent to IV (intravenous) therapy should be avoided. If both arms are involved in IV therapy and the IV cannot be discontinued for a short time, a site *below* the IV site may be considered. In such cases, the initial sample drawn (5 mL) should be discarded because it is most likely contaminated with IV fluid and only subsequent sample tubes should be used for analytic purposes.

In addition to venipuncture, blood samples can be collected using a capillary puncture technique that involves using either the outer area of the bottom of the foot (a heel stick) for infants or the lateral side of the middle or ring finger for individuals 1 year and older (finger stick). A sharp lancet device is used to pierce the skin, and an appropriate capillary or microtainer tube is used for sample collection.[22]

Analytic testing of blood involves the use of whole blood, serum, or plasma. **Whole blood**, as the name implies, contains the liquid portion of the blood, called *plasma*, and its cellular components (red blood cells, white blood cells, and platelets). The collection of whole blood requires the vacuum tube to contain an *anticoagulant*. Complete mixing of the blood immediately following venipuncture is necessary to ensure the anticoagulant adequately inhibits the specimen from clotting. As the tube of whole blood settles, the cells fall toward the bottom, leaving a clear yellow supernatant on top, which is the plasma.

If a tube does *not* contain an anticoagulant, the blood forms a fibrin clot incorporating the cells; this clot consumes fibrinogen. The remaining liquid is called **serum** rather than plasma (**Figure 1.16**). Most testing in the clinical chemistry laboratory is performed on either plasma or serum. The major difference between plasma and serum is that serum does not contain fibrinogen and some potassium is released from platelets (i.e., potassium levels are slightly higher in serum than in plasma). It is important that serum samples be allowed to completely clot (≈30 minutes) in an upright position at room temperature before being centrifuged. Plasma samples also require centrifugation but do not need time to clot, decreasing the turnaround time for testing and reporting results.

Centrifugation of the sample accelerates the process of separating the liquid portion and cellular portion. Specimens should be centrifuged according to recommendations by the tube manufacturer or test protocol, usually approximately 10 minutes at an RCF of 1000 to 2000 *g*, but should avoid the mechanical destruction of red blood cells that can result in hemoglobin release, which is called **hemolysis**.

Arterial blood samples measure blood gases (partial pressures of oxygen and carbon dioxide) and pH. Syringes containing heparin anticoagulant are used instead of evacuated tubes because of the pressure in an arterial blood vessel. The radial artery is the primary arterial site, while there may be times when the brachial or femoral artery may be considered. Arterial punctures are more difficult to perform

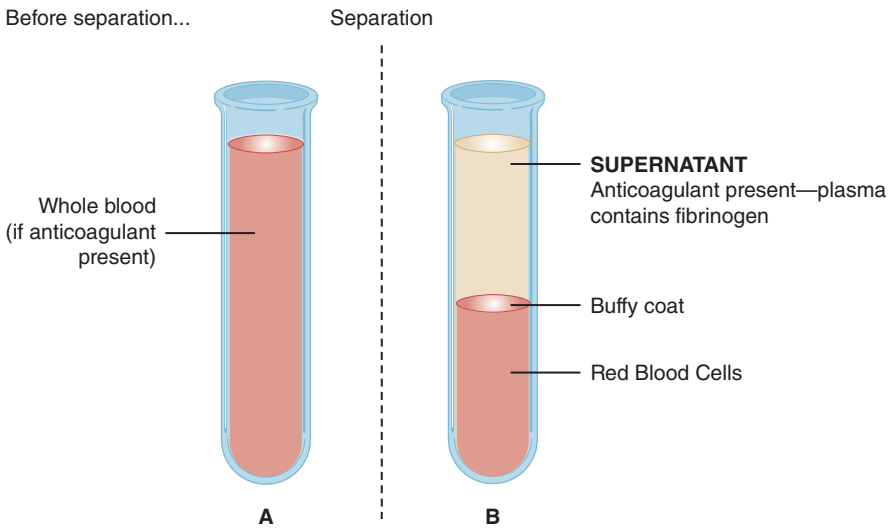


Figure 1.16 Blood sample. **(A)** Whole blood. **(B)** Whole blood after separation.

because of inherent arterial pressure, difficulty in stopping bleeding afterward, and the undesirable development of a hematoma, which cuts off the blood supply to the surrounding tissue.[23]

Continued metabolism may occur if the serum or plasma remains in contact with the cells for any period. Evacuated tubes may incorporate gel-like material that serves as a barrier between the cells and the plasma or serum and seals these compartments from one another during centrifugation. Some gels can interfere with certain analytes, and manufacturer recommendations should be followed.

Proper patient identification is the first step in sample collection. The importance of using the proper collection tube, avoiding prolonged tourniquet application, drawing tubes in the proper order, and proper labeling of tubes cannot be stressed strongly enough. Prolonged tourniquet application causes a stasis of blood flow and an increase in hemoconcentration and anything bound to proteins or the cells. Having patients open and close their hand during phlebotomy is of little value and may cause an increase in potassium or lactic acid and, therefore, should be avoided. IV contamination should be considered if a large increase occurs in the substances being infused, such as glucose, potassium, sodium, and chloride, with a decrease of other analytes such as urea and creatinine. In addition, the proper antiseptic must be used. Isopropyl alcohol wipes, for example, are used for cleaning and disinfecting the collection site; however, isopropyl alcohol is not recommended for disinfecting the site when drawing blood alcohol levels (in such cases, chlorhexidine is used as the disinfectant).

Blood is not the only sample analyzed in the clinical chemistry laboratory. Urine is the next most

CASE STUDY 1.2, PART 3

Recall Mía, the new graduate.

4. Which of the chemistry specimens provides the fastest turn-around time?
5. The whole blood analysis was performed and resulted. When Mía opened the centrifuge, she noticed that the specimens were grossly hemolyzed. What should Mía do?

common fluid for determination. Most quantitative analyses of urine require a timed sample (usually 24 hours); a complete sample (all urine collected within the specified time) can be difficult because many timed samples are collected by the patient in an outpatient situation. Creatinine analysis is often used to assess the completeness of a 24-hour urine sample because creatinine output is relatively free from interference and is stable, with little change in output within individuals. The average adult excretes 1 to 2 g of creatinine per 24 hours. Urine volume differs widely among individuals; however, a 4-L container is adequate (average output is ≈2 L). It should be noted that this analysis differs from the creatinine clearance test used to assess glomerular filtration rate, which compares urine creatinine output with that in the serum or plasma in a specified time interval and urine volume (often correcting for the surface area).

Other body fluids analyzed by the clinical chemistry laboratory include **cerebrospinal fluid (CSF)**, *paracentesis* fluids (pleural, pericardial, and peritoneal), and amniotic fluids. The color and characteristics of the fluid *before* centrifugation should be noted for these samples. Before centrifugation, a laboratorian should also verify that the sample is designated for clinical chemistry analysis *only* because a single fluid sample may be shared among several departments (i.e., hematology or microbiology), and centrifugation could invalidate other laboratory testing in those areas.

CSF is an ultrafiltrate of the plasma and is approximately two-thirds of the plasma glucose value. For glucose and total protein analysis, it is recommended that a blood sample be analyzed concurrently with the analysis of those analytes in the CSF. This will assist in determining the clinical utility of the values obtained on the CSF sample. This is also true for lactate dehydrogenase and protein assays requested on paracentesis fluids. All fluid samples should be handled immediately, without delay between sample procurement, transport, and analysis.

Amniotic fluid may be used to assess fetal lung maturity, congenital diseases, hemolytic diseases, genetic defects, and gestational age. The laboratorian should verify the specific handling of this fluid with the manufacturer of the testing procedure(s).

Sample Processing

When samples arrive in the laboratory, they are first processed. In the clinical chemistry laboratory, this means correctly matching the blood collection tube(s) with the appropriate test requisition and patient identification labels. This is a particularly sensitive area of preanalytic error. Bar code labels (either as 1D linear barcodes, or 2D QR barcodes) or radiofrequency ID chip labeling on primary sample tubes are vital in detecting errors and to minimizing clerical errors. The laboratorian must also ascertain if the sample is acceptable for further processing. The criteria used depends on the test involved but usually include volume considerations (i.e., is there sufficient volume for testing needs?), use of proper anticoagulants or preservatives (i.e., was it collected in the correct evacuated tube?), whether timing is clearly indicated and appropriate for timed testing, and whether the specimen is intact and has been properly transported (e.g., on ice, within a reasonable period, protected from light). Unless a whole blood analysis is being performed, the sample is then centrifuged as previously described and the serum or plasma should be separated from the cells if not analyzed immediately. Today, the use of serum separator tubes and plasma separator tubes is common practice.

Once the sample is processed, the laboratorian should note the presence of any serum or plasma characteristics such as *hemolysis* and **icterus** (increased bilirubin pigment) or the presence of turbidity often associated with **lipemia** (increased lipids). (**See Table 1.5**.) Many analytes are stable at room temperature between 24 to 72 hours. However, if testing is not to be performed within 8 hours, it is recommended that serum and/or plasma be

Table 1.5 Examples of Hemolyzed, Icteric, and Lipemic/Turbid Samples

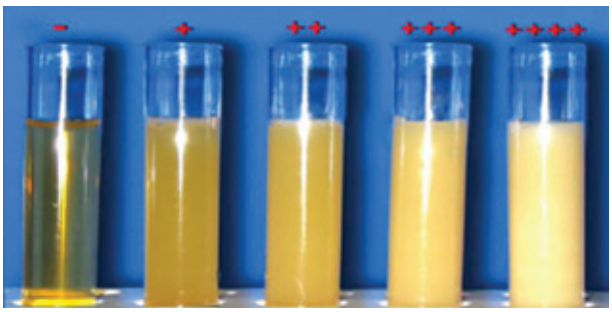
Hemolyzed (red)

Hemoglobin					
mg/dL	0	50	150	250	525
g/L	0	0.50	1.50	2.50	5.25

Icteric (yellow)

Total Bilirubin					
mg/dL	0	1.7	6.6	16	30
µmol/L	0	29.1	112.9	273.6	513

Lipemic (turbid)



Intralipid®					
mg/dL	0	125	250	500	1000
mmol/L*	0	1.41	2.83	5.65	11.83

*Concentrations based on dilutions of 20% Intralipid® (or the equivalent).

CLSI C56A_2012 Hemolysis, Icterus, and Lipemia_Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis, 1st Edition.

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refrigerated between 2°C and 8°C.[24] It is important to avoid exposing samples that are light sensitive, such as bilirubin, to artificial or ultraviolet light for extended periods of time.[24] Separated serum and/or plasma may be frozen at –20°C and stored for longer periods without deleterious effects on the results. Repeated cycles of freezing and thawing, like those that occur in so-called frost-free freezers, should be avoided.

Hemolysis can be visually observed in a centrifuged patient sample as a red color due to the release of hemoglobin. There are patient conditions where this may occur in vitro, such as hemolytic anemia, but hemolysis can also be present due to preanalytic collection variables such as inappropriate needle gauge, venipuncture site selection (small veins), and venous trauma or difficulty in specimen collection. Along with the release of hemoglobin, other intracellular components may be released, such as potassium, phosphate, and lactate dehydrogenase, which may impact patient values for these analytes. For analyzers utilizing spectrophotometric or enzymatic detection methods, hemolysis may also cause errors during assay.

Lipemia results when the lipid levels of the patient are elevated and, in turn, is visualized as a creamy or milky appearance to the serum or plasma upon centrifugation. Lipemia can cause a volume displacement for some analytes, such as electrolytes, as well as interference in light-scattering methodologies due to the turbidity present. Icterus is a deep yellow or golden appearance of the serum or plasma due to increased bilirubin levels, and may cause spectral interference on certain analyzers in the chemistry lab. To help determine if interference has occurred, many analyzers are capable of detecting, then estimating, the interferent and the effect on sample values. This assessment of hemolysis, icterus, and lipemia is known as the HIL index. Laboratories have the ability to determine values to establish the alert values of the HIL indices as a means of assessing specimen integrity and acceptability. Table 1.5 illustrates examples of HIL indices in serum or plasma.[25]

Sample Variables

Sample variables (**Table 1.6**) include physiologic considerations, proper patient preparation, and problems in collection, transportation, processing, and storage. Although laboratorians must include mechanisms to minimize the effect of these variables on testing and must document each preanalytic incident, it is often difficult to control the variables that involve individuals outside of the laboratory. The best course of action is to critically assess or anticipate variances, identify potential problems, and implement an action plan that contains policies, procedures, and checkpoints throughout the testing process. Good communication with all personnel involved helps ensure that the right specimen at the right time for the right patient is collected each and every time to meet the needs of the healthcare team including the patient, laboratory, and ordering physician. Most accreditation agencies require that

Table 1.6 Variables Affecting Select Chemistry Analytes

Physiological factors	Diet	Samples requiring fasting (glucose)	
	Medication or herbal supplements	Possible interference with analytical methods (biotin)	
	Circadian rhythm	Analyte changes based on diurnal variation (cortisol)	
		Timing of collection	Serial testing of an analyte (cardiac troponin) or timing based on medication (therapeutic drug monitoring)
	Patient posture	Shift of hemodynamic fluid volume (proteins)	
Patient preparation factors	Fasting	Proper instructions for 8- to 12-hour fasting	
	24-hour urine collection	Proper instructions for collection	
Collection and sample processing factors	Venipuncture technique	Needle selection, site selection to decrease opportunity of hemolysis	
	Tube selection	Appropriate sample tube, inversion following collection, appropriate clotting time	
	Tourniquet use	Prolonged use affects analytes (K^+, lactic acid)	
	Specimen transport and storage	Protection from light (bilirubin) or collect and store on ice (arterial blood gas, ammonia)	

laboratories consider all aspects of preanalytic variation as part of their quality assurance plans, including effective problem solving and documentation.

Physiologic variation refers to changes that occur within the body, such as cyclic changes (diurnal or circadian variation) or those resulting from exercise, diet, stress, gender, age, underlying medical conditions (e.g., fever, asthma, and obesity), drugs, or posture. Samples may be drawn on patients who are fasting (usually overnight for at least 8 hours). When fasting, many patients may drink water to avoid becoming dehydrated, which can lead to falsely elevated electrolyte results. Patient preparation for timed samples or those requiring specific diets or other instructions must be well written and verbally explained to patients. Elderly patients often misunderstand or are overwhelmed by the directions given to them. Collection and processing variations are related to those factors discussed under specimen processing. Clerical errors are the most frequently encountered, followed by other pre-analytical variables including inadequate separation of cells from serum, improper storage, and collection.

Chain of Custody

When laboratory tests are likely linked to a crime or accident, they become forensic in nature. In these cases, documented specimen identification is required at each phase of the process. Each facility has its own forms and protocols; however, the patient, and usually a witness, must identify the sample. The sample should be collected and then sealed with a tamper-proof seal. Any individual in contact with the sample must document receipt of the sample, the condition of the sample at the time of receipt, and the date and time it was received. In some instances, one witness verifies the entire process and cosigns as the sample moves along. Any analytic test could be used as part of legal testimony; therefore, the laboratorian should give each sample—even without the documentation—the same attention given to a forensic sample.

Electronic and Paper Reporting of Results

Electronic transmission of laboratory data and the use of physician order entry, electronic medical record, coding, billing, and other data management systems maintain the integrity of data generated by providing reporting guidelines and safeguards to ensure privacy of the data and records. There are various data management systems in use by healthcare agencies related to accessing laboratory information. For example, the Logical Observation Identifiers Names and Codes (LOINC) system, International Federation of Clinical Chemistry/International Union of Pure and Applied Chemistry (IFCC/IUPAC), ASTM, Health Level Seven International (HL7), and Systematized Nomenclature of Medicine, Clinical Terms (SNOMED CT) are databases that use unique coding systems for laboratory observations. There are also additional proprietary systems in use. To standardize these processes and to protect the confidentiality of patient information as required by the Health Insurance Portability and Accountability Act (HIPAA), the Healthcare Common Procedure Coding System Level II (HCPCS) test and services coding system was developed by the Centers for Medicare and Medicaid Services (CMS) to be recognized by all insurers for reimbursement purposes. The International Classification of Diseases (ICD) developed by the World Health Organization (WHO) uses codes identifying patient diseases and conditions. In the United States, ICD-11 is currently in place. The clinical modifications are maintained by the National Center for Health Statistics. Incorporated into the HCPCS system is the Current Procedural Terminology (CPT) codes, developed by the American Medical Association, which identify almost all laboratory tests and procedures. The CPT codes are divided into different subcategories, with tests or services assigned five-digit numbers followed by the name of the test or service. Together, these standard coding systems help patient data and tracking of disease transmission between all stakeholders such as physicians, patients, epidemiologists, and insurers.

Clinical laboratory procedures are found in CPT Category I with coding numbers falling between 80,000 and 89,999. There can be several codes for a given test based on the reason and type of testing, and there are codes given for common profiles or array of tests that represent each test's separate codes. For example, blood glucose testing includes the codes 82947 (quantitative except for strip reading), 82948 (strip reading), and 82962 (self-monitoring by FDA-cleared device), and the comprehensive metabolic panel (80053) includes albumin, alkaline phosphatase, total bilirubin, blood urea nitrogen, total calcium, carbon dioxide, chloride, creatinine, glucose, potassium, total protein, sodium, and alanine and aspartate transaminases and their associated codes. At a minimum, any laboratory reporting system must include a unique patient identifier, test name, and code that relates back to the HCPCS and ICD databases. For reporting purposes, whether paper or electronic, the report should include the unique patient identifier and test name (including any appropriate abbreviations), the test value with the unit of measure, date and time of collection, sample information, reference ranges, plus any other pertinent information for proper test interpretation. Results that are subject to autoverification should be indicated in the report. **Table 1.7** lists the information that is often required by accreditation agencies.[26]

Table 1.7 Minimum Elements of Paper or Electronic Patient Reports

Name and address of laboratory performing the analysis including any reference laboratories used
Patient name and identification number or unique identifier
Name of physician or person ordering the test
Date and time of specimen collection
Date and time of release of results (or available if needed)
Specimen source or type
Test results and units of measure if applicable
Reference ranges, when available
Comments relating to any sample or testing interferences that may alter interpretation

WRAP-UP

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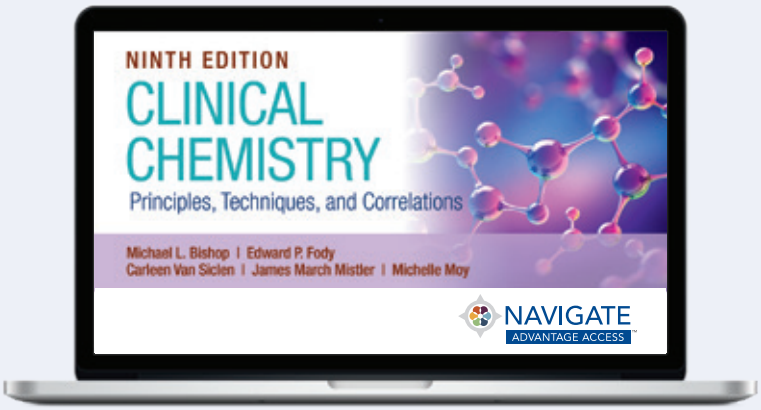

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