Burtis, et al: Tietz Fundamentals of Clinical Chemistry, 6th Edition

Instructor's Manual

Chapter 2: Introduction to Principles of Laboratory Analyses and Safety

Chapter Focus

The basics of clinical chemistry analyses are rooted in chemistry fundamentals that include laboratory mathematics; properties of solutions; basic techniques, such as pipetting and centrifugation; and the use of standards. This chapter focuses on the principles and performance of these laboratory essentials. Another requisite element of the laboratory is safety. Chapter 2 provides an overview of basic laboratory safety practices, OSHA standards, and universal precautions.

Although the use of radioactivity to examine analyte concentration is not widespread, its application provided an early approach to analytical assessment. Many laboratories no longer practice these procedures; however, a basic understanding of the principles and current applications of radioactivity completes the introduction to chemistry.

Teaching Strategies

Much of the instruction to be presented to students from this chapter relates to the basics of a chemistry laboratory, but applies to all laboratory situations. These basics include introduction to various reagent formulations, expressions of concentration, types of water, glassware and pipettes, and basic lab equipment. Visual presentation of different pipettes and glassware is most effective, so this would be best presented in a teaching lab setting (see Learning Activities). To introduce students to the terminology used in the chemistry lab, make lists of words with definitions (better yet, have students perform this task) such as standard, control, reagent, etc. Clarify the difference between standards and controls; show a standard curve constructed on graph paper and how control and unknown analyte concentration values are obtained from this curve. Explain how these simple standard curves are set up and used in complex chemistry analyzers.

To instruct (and reduce the fear in) students when calculating molarities, percent concentrations, normality, etc., the following formulae can be used as a simpler, kinder lesson in calculation.

Molarity = grams/liter divided by molecular weight

Example 1:

What is the molarity of a solution that contains 32 g of NaOH in 300 mL of buffer? MW of NaOH is 40

Equation: $\text{Mol/L} = \frac{32/0.3}{40} = \frac{106.7}{40} = 2.7$ mol/L

Therefore, 32 g of NaOH in 300 mL of buffer gives a 2.7 mol/L. Note that 300 mL must be converted to liters (300 mL = 0.3 L).

Example 2:

How many grams of sodium chloride (NaCl, MW = 58.5) are needed to make 1 L of a 2 mol/L solution?

Equation: $2 \text{ mol/L} = \frac{\text{grams per liter}}{58.5} = 2 = ?/58.5 \text{ or } 2 \times 58.5 = ?$

? = 117 g of NaCl in 1 L

Example 3:
How many grams of NaCl are required to prepare 250 mL of 6 mol/L NaCl?

Equation: $6 \text{ mol/L} = \frac{\text{g}/0.250}{58.5} = 6 \times 58.5 \times 0.250 = 87.75 \text{ g}$

Therefore, 87.75 g of NaCl are needed to prepare 250 mL of a 6 mol/L solution of NaCl.

Normality = N (or Eq/L) = grams/liter divided by equivalent weight

Example 1:
How many grams of HCl are required to make a 6N solution of HCl in 1 L of buffer?

Equation: gram molecular weight of HCl = 35 g
equivalent weight of HCl = 35 g

$$6N = \frac{\text{g}/1}{35} = 6 \times 35 = 210 \text{ g/L}$$

Therefore, 210 g of HCl must be placed in 1 L of buffer to make a 6N solution of HCl.

Example 2:
What is the normality of solution containing 50 g of NaOH in 300 mL of buffer?

Equation: gram molecular weight of NaOH = 40 g
equivalent weight of NaOH = 40 g

$$N = \frac{50 \text{ g}/0.300 \text{ L}}{40} \qquad N = \frac{166.7}{40} \qquad N = 4.2 \text{ Eq/L}$$

Example 3:
What is the normality of a solution containing 80 g of H_2SO_4 in 450 mL buffer?

Equation: gram molecular weight of H_2SO_4 = 98 g
equivalent weight of H_2SO_4 = 49 g

$$N = \frac{80\ g/0.450\ L}{49\ g} \qquad N = \frac{177}{49} \qquad N = 3.6\ Eq/L$$

Percent Concentration

In calculating percent solution problems, the first step is to determine the amount of solute per 100 parts of solvent. To determine how much solute to add to a solvent to obtain a given percentage solution of specified volume, a simple ratio equation is used.

Example 1:
Make 350 mL of an 8% solution of NaOH in buffer.

Equation: $\frac{8}{100} = \frac{X}{350}$ $2800 = 100X$ $X = 28$

Therefore, 28 g of NaOH is diluted up to 350 mL of buffer to obtain an 8% solution.

Example 2:
What is the percent concentration of a solution that contains 90 g of NaOH in 750 mL of buffer?

Equation: $\frac{90}{750} = \frac{X}{100}$ $750X = 9000$ $X = 12$

Therefore, 90 g of NaOH in 750 mL of buffer is a 12% solution.

Despite increased use of "kit" or prepackaged reagents for routine procedures performed in the clinical chemistry lab, special procedures require that the laboratory scientist be knowledgeable of chemicals, solutions, buffer, and water requirements. Practice with solution making is critical for students to understand how dilutions are made and how to use pipettes in making these solutions.

Be adamant that students understand the importance of laboratory safety. If you have institutional requirements for safety, prepare a separate presentation that introduces students to these and ensure that they comply with whatever training is needed. Clarify the use of the National Fire Protection Agency's hazard diamond. Enforce such directives as "no food or drink in the laboratory" and "no mouth pipetting." Review the meaning of and need for MSDS in the laboratory and show students examples of these safety sheets. It is imperative that students realize that compliance with OSHA and institutional safety plans is a critical issue, particularly in the case of chemical spills or needle sticks during routine phlebotomy. Understanding and observing safety requirements as a student will eventually become part of routine laboratory practice. If you have the space, set up a "mock" hazard laboratory and have students walk through it to locate all the hazards you've set up. Include biological hazards, chemical hazards, electrical hazards, and fire hazards in your scheme. Examples could include a cola can sitting on a lab bench, a frayed cord on a piece of equipment, open tubes of blood, blocked sprinklers, sinks hidden under papers, uninspected fire extinguishers, etc.

Atoms, atomic design, and different kinds of radioactivity are basic facets of chemistry. How these are currently applied to clinical chemistry, research, and genetics testing is an important, yet often overlooked, component of basic laboratory science education. If you need a review of these concepts, study the references provided. Review a brief history of radioactivity and its previous uses in the clinical laboratory with your students. Knowing how radioactivity is used in modern day laboratories and clinical practice will aid in presenting this information in an interesting way.

Chapter Outline

I. Concept of Solute and Solvent
- A. Definitions
- B. Expressing Concentrations of Solutions

II. Units of Measurement
- A. International System of Units
- B. Decimal Multiples and Submultiples of International Units
- C. Applications of SI in Laboratory Medicine
- D. Problem Areas in the Use of SI Units
- E. Standardized Reporting of Test Results
  - 1. LOINC System
  - 2. IFCC/IUPAC System

III. Chemicals and Reference Materials
- A. Reagent Grade Water
  - 1. Preparation of Reagent Grade Water
  - 2. Quality, Use, and Storage of Reagent Grade Water
  - 3. Testing for Water Purity
- B. Reagent Grade or Analytical Grade Chemicals
- C. Ultrapure Reagents
- D. Reference Materials

IV. Basic Techniques and Procedures
- A. Volumetric Sampling and Dispensing
  - 1. Pipettes
  - 2. Volumetric Flasks
- B. Centrifugation
  - 1. Types of Centrifuges
  - 2. Principles of Centrifugation
  - 3. Operation of the Centrifuge
  - 4. Operating Practice
- C. Measurements of Radioactivity—Basic Concepts
- D. Gravimetry
  - 1. Principles of Weighing
  - 2. Types of Balances
  - 3. Analytical Weights
- E. Thermometry
- F. Controlling Hydrogen Ion Concentration
- G. Procedures for Processing Solutions
  - 1. Dilutions

2. Evaporation
3. Lyophilization
4. Filtration

V. Safety
- A. Safety Program
- B. Safety Equipment
- C. Safety Inspections
- D. Mandated Plans
  1. Chemical Hygiene Plan
  2. Exposure Control Plan
  3. Tuberculosis Control Plan
- E. Ergonomics Program
- F. Hazards in the Laboratory
  1. Identification of Hazards
  2. Biological Hazards
  3. Chemical Hazards
  4. Electrical Hazards
  5. Fire Hazards

Chapter Objectives

1. State the properties of solutes, solvents, and solutions and express and calculate solution concentration using various methods.
2. Define units of measure and relate the differences among various units.
3. Distinguish between the different types of water used in the laboratory based on preparation and use.
4. List the different available pipettes, based on their use, type, and capability.
5. Understand centrifugation and balances and the terminology related to each and calculate RCF and rpm when given the appropriate information.
6. Describe an atom and define *atomic number, mass number, isotope, half-life,* and *nuclide.*
7. Define *radioactive decay.*
8. List four types of radioactive decay, the type of particle produced by each, and the manner in which each type of particle interacts with matter.
9. State the principles of autoradiography and scintillation counting.
10. List two types of scintillation counters and their uses in the laboratory.
11. Recognize and interpret various laboratory hazard signage and state the appropriate course of action when an accident occurs.
12. Describe Universal Precautions and the OSHA Hazard Exposure Plan.
13. State the purpose of an ergonomics program.

Key Words

Analyte: A substance or constituent for which the laboratory conducts testing.
Analysis: The procedural steps performed to determine the kind or amount of an analyte in a specimen.
Autoradiography: Use of a photographic emulsion (X-ray film) to visualize radioactively labeled molecules.

Balance: An instrument used for weighing.
Beta Particle: High-energy electron emitted as a result of radioactive decay.
Bloodborne Pathogens: Pathogenic microorganisms that are present in human blood. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV.)
Buffer: A solution or reagent that resists a change in pH upon addition of either an acid or a base.
Chemical Hygiene Plan: A set of written instructions describing the procedures required to protect employees from health hazards related to hazardous chemicals contained in the laboratory.
Centrifugation: The process of separating molecules by size or density using centrifugal forces generated by a spinning rotor. G-forces of several hundred thousand times gravity are generated in ultracentrifugation.
Certified Reference Material: A reference material that has one or more values certified by a technically valid procedure and is accompanied by, or is traceable to, a certificate or other document by a certifying body.
Desiccator: A container, filled with a desiccant, used to store substances in a water-free environment.
Dilution: The process (diluting) of reducing the concentration of a solute by adding additional solvent.
Ergonomics: The study of capabilities in relationship to work demands by defining postures that minimize unnecessary static work and reduce the forces working on the body.
Exposure Control Plan: A set of written instructions describing the procedures necessary to protect laboratory workers against potential exposure to bloodborne pathogens.
Gamma Ray: High-energy photons emitted as a result of radioactive decay.
Gravimetry: The process of measuring the mass (weight) of a substance.
Half-Life: The time period required for a radionuclide to decay to one-half the amount originally present.
Material Safety Data Sheet (MSDS): A technical bulletin that contains information about a hazardous chemical, such as chemical composition, chemical and physical hazard, and precautions for safe handling and use.
Metric System: A system of weights and measures based on the meter as a standard unit of length.
Primary Reference Material: A thoroughly characterized, stable, homogeneous material of which one or more physical or chemical properties have been experimentally determined within stated measurement uncertainties. Used for calibration of definitive methods; in the development, evaluation, and calibration of reference methods; and for assigning values to secondary reference material.
Radioactivity: Spontaneous decay of atoms (radionuclides) that produces detectable radiation.
Radiation Counter: Liquid or crystal scintillation counter or gas-filled (e.g., Geiger) counter used to detect and measure radiation.
Radiation Dose: The amount of radiation energy absorbed in matter, conventionally expressed in rads, defined as 100 ergs absorbed per gram of matter.
Radiation Safety: Regulations and practices to ensure that radiation is used safely.
Reagent Water: Water purified and classified for specific analytical uses.

Reference Material: A material or substance, one or more physical or chemical properties of which are sufficiently well established to be used for the calibration of an apparatus, the verification of a measurement method, or for assigning values to materials. Certified, primary, and secondary are types of reference materials.
Relative Centrifugal Force (RCF): The weight of a particle in a centrifuge relative to its normal weight.
Secondary Reference Material: A reference material that contains one or more analytes in a matrix that reproduces or stimulates the expected matrix. Used primarily for internal and external quality assurance purposes.
Système International d'Unites (SI): An internationally adopted system of measurement. The units of the system are called SI units.
Standard Reference Material (SRM): A certified reference material (CRM) that is certified and distributed by the National Institute of Standards and Technology (NIST), an Agency of the U.S. Government formerly known as the Bureau of Standards (NBS).
Test: In the clinical laboratory, a test is a qualitative, semiqualitative, quantitative, or semiquantitative procedure for detecting the presence, or measuring the quantity of an analyte in a specimen.
Total Effective Dose Equivalent (TEDE): Total radiation dose from both internal and external sources corrected for type of radiation. Limits for TEDE are stated in governmental regulations.
Universal Precautions: An approach to infection control. According to the concept of universal precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

Critical Thinking Questions

1. You are performing critical experimentation that involves expensive reagents and unique patient specimens. These items are stored in your laboratory refrigerators and freezers. How do you ascertain that this equipment is working properly? What would happen if your laboratory lost power? What protocols should be in place in the event of this occurrence? How does your specific institution handle this occurrence? What would you recommend?
2. Why would it be important for a research laboratory to self-impose maintenance of thermometers, refrigerator/freezer temperature checks, and pipette calibration?
3. You are practicing phlebotomy on a group of patients in an outpatient clinic and accidentally stick yourself with a needle. It is unclear if the patient you were drawing the blood sample from has any infectious disease. What procedures must you follow in reporting the incident, testing the blood, caring for yourself, following up, etc. What are the institutional requirements for these procedures at your facility?

Learning Activities

1. Include students in quality control procedures such as checking refrigerator/freezer and incubator temperatures in your teaching lab and keeping a record of these on a daily basis. If you don't have a teaching lab, ask permission to do these checks on a research lab's refrigerator/freezer or the ones in the school kitchen or break room. Other QC procedures that students can perform include centrifuge maintenance, pipette calibration, and equipment checks. These activities will prepare students for the time when they are in a

working laboratory. Emphasize that these procedures should be performed in research laboratories and clinical labs despite the lack of accrediting agency oversight.
2. Demonstrate the preparation of working solutions from stock standards. This will incorporate the lab math presented in this chapter. Have students make dilutions of a stock methyl orange solution (or use food coloring in water) using various types of pipettes and glassware if they are available. This activity will also provide students practice with using a pipette bulb or pipettor. Here are some examples of dilution practice:

1. Using a 10 mL graduated cylinder, place 5 mL of distilled water into a 25 mL Erlenmeyer flask. Next, using a 5 mL volumetric pipette, pipette 5 mL of stock methyl orange into the Erlenmeyer flask.
2. In a 16 × 100 mL test tube, using *serological* pipettes, dilute 1 mL of stock methyl orange with 9 mL of distilled water.
3. In a beaker, using a *repipettor and serological pipettes*, dilute 0.5 mL of stock methyl orange with distilled water (use whatever amount you like). Remember your dilution.
4. In a volumetric flask, using a volumetric pipette, make a 1:50 dilution of stock methyl orange using distilled water as the diluent. Use any size flask and pipette as long as a 1:50 is the final result.
5. Now obtain a micropipette and set it at 100 μL. Using a second pipette, make a 1:100 dilution.

Check on students as they work to make sure they are using the correct pipettes and glassware. Then ask the following questions:

1. Calculate the resulting dilutions made for steps 1, 2, and 3 above. Show all calculations.
2. For step 4 briefly outline the procedure you used to make the 1:50 dilution. State the pipette(s) used and the volumes used.
3. What would be the resulting dilution if 5 ml of serum is added to 20 mL of saline? If 5 mL of serum are added to 25 mL of saline?
4. What would be the resulting dilution if 5 mL of serum is diluted with 5 mL of saline?
5. Describe the dilution you made in step 5 and the pipette volumes you used.

3. Use the NFPA hazard diamond below to quiz students on various chemical substances, or find bottles of chemicals with the hazard diamond on them and ask students to interpret the colors, numbers, and other codes.