SUCCESS!
In Clinical Laboratory Science

FIFTH EDITION

Donald C. Lehman Janelle M. Chiasera

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WE DEDICATE THIS BOOK

To my wife, Terri, whose love, support, and encouragement
helped make this book possible.

Donald C. Lehman

and

To my parents, John and Arleen, for their unwavering support,
love, and encouragement; and to my husband, John,
who is my best friend, my greatest support,
my biggest comfort, and my strongest motivation.

Janelle M. Chiasera

and

To all the clinical laboratory professionals who contribute their expertise daily
as members of the healthcare team and to all current clinical
laboratory science students and those who will follow.

Contents

Preface

SUCCESS! in Clinical Laboratory Science: Complete Review, Fifth Edition, is designed as an all-in-one summary and review of the major clinical laboratory science content areas generally taught in an academic program. It is designed to help examination candidates prepare for national certification or state licensure examinations. It is also a resource for practicing clinical laboratory scientists wanting a "refresher." Students enrolled in clinical laboratory science programs can use the text to prepare for undergraduate examinations. The excellent reception received by the first four editions of the book spurred the writing of this fifth edition. Educators and students alike have commented that the strengths of the book are concise summaries of important information and the paragraph explanations that accompany each answer to the review questions. The explanations help readers of the book augment their knowledge or clear up misunderstandings.

In the fifth edition, we continued the concise outline of each content area that began in the fourth edition. The outlines are not intended to replace discipline-specific textbooks, but the outlines will provide a quick review of important material.

Color plates of 58 full-color pictures are included to provide the user with an experience in answering questions based on a color photograph. Additionally, a 200-question self-assessment test and a 100-question self-assessment test on the Student Resource Website are included as mechanisms for final evaluation of one's knowledge, thus allowing for the identification of one's strengths and weaknesses while there is still time to improve.

The book contains more than 2000 multiple choice questions that cover all the areas commonly tested on national certification and state licensure examinations. The questions are based on current clinical laboratory practice, and case study questions are incorporated to hone problem-solving and critical thinking skills. The paragraph rationales expand upon the correct answer, and matching puzzles on the Student Resource Website provide an alternate

means to assess recall knowledge. Overall, this book provides the essential components needed in an effective clinical laboratory science examination review book. We hope that you find this book and the accompanying Student Resource Website useful, and we wish you success in your academic studies, with the certification examination, and with your career as a clinical laboratory professional.

New to the fifth edition:

- We expanded the content on molecular biology and added more review questions.
- Information was added on matrix assisted-laser desorption/ionization time of flight for the identification of microorganisms.
- The section on antimicrobial testing was expanded to include detecting extended-spectrum beta-lactamases and carbapenemases.
- New medications used in hemostasis treatments were added.
- Celiac disease, pathology and diagnosis, was added to the immunology chapter.
- Revisions were made to many of the multiple-choice questions and answer explanations.
- Several color images were updated.

STUDENT RESOURCES

To access the material on student resources that accompany this book, visit www.pearsonhighered.com/healthprofessionsresources. Click on view all resources and select Clinical Lab Science from the choice of disciplines. Find this book and you will find the complimentary study materials.

Acknowledgments

This book is the end product of the labor and dedication of a number of outstanding professionals. The editors would like to acknowledge these individuals for their invaluable assistance in completing this project. The editors greatly appreciate the efforts of the contributing authors who worked so diligently to produce quality materials. A note of recognition and appreciation is extended to Karen A. Keller, Mary Ann McLane, and Linda Sykora, who allowed use of their color slides in the fourth edition, many of which were maintained in the fifth edition. We want to thank Linda Smith who contributed new images for the fifth edition. In addition, we extend a special acknowledgment to Elmer W. Koneman, MD, Professor Emeritus, University of Colorado School of Medicine and Medical Laboratory Director, Summit Medical Center, Frisco, CO for use of color slides from his private collection.

Certifying Agencies

Information pertaining to certification examinations, education and training requirements, and application forms may be obtained by contacting the certifying agency of your choice. The following is a list of the certification agencies that service clinical laboratory professionals.

American Society for Clinical Pathology Board of Certification (ASCP/BOC)
33 West Monroe Street, Suite 1600
Chicago, IL 60603
312-541-4999
E-mail: bor@ascp.org
Web site: http://www.ascp.org

American Medical Technologists (AMT)
10700 West Higgins Road, Suite 150
Rosemont, IL 60018
847-823-5169 or 800-275-1268
E-mail: MT-MLT@amt1.com
Web site: http://www.americanmedtech.org/

American Association of Bioanalysts (AAB)
906 Olive Street, Suite 1200
St. Louis, MO 63101-1434
314-241-1445
E-mail: aab@aab.org
Web site: http://www.aab.org

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Introduction

If you are currently preparing for a Clinical Laboratory Science/Medical Laboratory Science certification or licensure examination, or if you are a practicing clinical laboratory professional who wants to "brush up" on clinical laboratory information, then this is the review book for you. ***SUCCESS! in Clinical Laboratory Science: Complete Review, Fifth Edition*** is a comprehensive text containing content outlines and more than 2000 questions with paragraph explanations accompanying each answer. Unique to this book is an outline of each content area that concisely summarizes important information. The question and rationale format not only tests your knowledge of the subject matter but also facilitates additional learning. Color plates of 58 full-color pictures are included to help you prepare for national examinations in as realistic a manner as possible. There is a 200-question self-assessment test and a 100-question self-assessment test on the Student Resource Website. Both assessments will assist you in determining your mastery of the material while allowing computer practice for certification examinations.

ORGANIZATION

The book is organized into 17 chapters corresponding to the areas tested on clinical laboratory science/medical laboratory science certification examinations with Chapter 17 being a review exam. The chapters are as follows:

1. Clinical Chemistry
2. Hematology
3. Hemostasis
4. Immunology and Serology
5. Immunohematology
6. Bacteriology

7. Mycology
8. Parasitology
9. Virology
10. Molecular Diagnostics
11. Urinalysis and Body Fluids
12. Laboratory Calculations
13. General Laboratory Principles, Quality Assessment, and Safety
14. Laboratory Management
15. Medical Laboratory Education and Research
16. Computers and Laboratory Information Systems
17. Self-Assessment Test

The chapters are organized into an outline, review questions, and answers with paragraph explanations. A list of references is located at the end of each chapter for further review. The last chapter is a 200-question self-assessment test that should be used to determine overall competency upon completion of the previous chapters. To further synthesize important material, case studies in clinical chemistry, hematology, immunology and serology, immunohematology, and microbiology are included. The Student Resource Website has three types of assessment tools including a 100-question self-assessment test to assist you in preparing for computerized national examinations. In addition, matching puzzles are available to help you to review major points associated with each content area.

QUESTIONS

The style of the questions used adheres to that prevalent in most certification examinations. Each chapter contains questions in a multiple-choice format with a single answer. In some cases, a group of two or more questions may be based on a case study or other clinical situation. Questions are divided among three levels of difficulty: Level 1 questions test recall of information, level 2 questions test understanding of information and application to new situations, and level 3 questions test problem-solving ability. Each of the multiple-choice questions is followed by four choices, with only one of the choices being completely correct. Although some choices may be partially correct, remember that there can only be one best answer.

HOW TO USE THIS BOOK

The best way to use ***SUCCESS! in Clinical Laboratory Science: Complete Review, Fifth Edition*** is to first read through the outline. If you find that some of the material is not fresh in your memory, go to a textbook or recent class notes to review the area in more detail. Then work through short sections of the questions at a time, reading each question carefully, and recording an answer for each. Next, consult and read the correct answers. It is important to read the paragraph explanations for both those questions answered correctly as well as for those missed, because very often additional information will be presented that will reinforce or clarify knowledge

already present. If you answer a question incorrectly, it would be wise to consult the references listed at the end of the chapter.

Lastly, you should take the 200-question self-assessment test as if it was the actual examination. Find a quiet place, free of interruptions and distractions, and allow yourself 3 hours and 30 minutes to complete the self-assessment test. Record your answers; then check the answer key. Review topic areas that seemed difficult. As final preparation, take 2 hours to complete the 100-question computerized test on the Student Resource Website. These tests will give you a more realistic evaluation of your knowledge and your ability to function within a time constraint. It is important that you are comfortable taking a test that is computerized, because the certifying agencies now use either computer-administered or computer-adaptive testing. So be sure to practice on the computer using the Student Resource Website. By the time you have worked through the outlines, the questions and rationales, the two self-assessment tests, case studies, and the matching puzzles, you will have gained a solid base of knowledge.

For students of clinical laboratory science/medical laboratory science and clinical laboratory practitioners, this book has been designed to summarize important information, to test your knowledge, and to explain unfamiliar information through use of the paragraph explanations that accompany each question. Working through the entire book will make you aware of the clinical areas in which you are strong or weak. This review will help you gauge your study time before taking any national certification or state licensure examination. Remember, there is no substitute for knowing the material.

TEST-TAKING TIPS

In addition to studying and reviewing the subject matter, you should also consider the following points:

1. Contact the Sponsoring Agency
   Check the Website of the sponsoring agency that administers the examination and review the general information about the test, including
   - The outline of the test content areas
   - The test question format
   - If it is computer administered or computer adaptive
   - The time allowed to complete the test and the number of test questions to expect
   - The scoring policy

   Note: Because certification examination requirements vary, it is important to read thoroughly all directions published by the sponsoring agency and to read carefully the directions presented on the day of the examination. After completing the computerized examinations, most agencies permit you to return to previously answered questions and

entered responses can be changed. In some cases the sponsoring agency allows you to skip a question and return to it at the end of the exam, whereas other agencies require that you select an answer before being allowed to move to the next question. So know the rules! Checking your answers is a very important part of taking a certification exam. During the exam, check the computer screen after an answer is entered to verify that the answer appears as it was entered.

2. Prepare before Examination Day
   - Study thoroughly prior to taking the exam. Set up a study schedule that allows sufficient time for review of each area. Treat studying like a job.
   - Use this review book to help you to identify your strengths and weaknesses, to sharpen your test-taking skills, and to be more successful with multiple choice examinations.
   - Know the locations of the test center and the parking facilities. If the area is unfamiliar to you, a visit to the site a week before the exam may help to prevent unnecessary anxiety on the morning of the test.
   - Check your calculator (if one is allowed) for proper function and worn batteries. Some agencies allow a nonprogrammable calculator to be used during the exam.
   - Get plenty of rest. Do not cram. A good night's sleep will prove to be more valuable than cramming the night before the exam.
3. On the Examination Day
   - Eat a good breakfast.
   - Take two types of identification with you—your photo identification and another form of identification, with both illustrating your current name and signature, as these are generally required—and your admission letter (if required by the agency).
   - Take a nonprogrammable calculator (if one is allowed) to the test center. Most test centers do not permit any paper, pencils, or study materials in the testing area. In addition, electronic devices such as cell phones, etc. are not permitted in the test center.
   - Allow sufficient time to get to the test center without rushing. Most agencies require that you be at the test center 30 minutes before the start of the exam.
   - Wear a wristwatch in order to budget your time properly.
   - Read the directions thoroughly and carefully. Know what the directions are saying.
   - Read each question carefully. Be sure to answer the question asked. Do not look for hidden meanings.
   - Take particular note of qualifying words such as "least," "not," "only," "best," and "most."
   - Rapidly scan each choice to familiarize yourself with the possible responses.
   - Reread each choice carefully, eliminating choices that are obviously incorrect.
   - Select the one best answer.
   - Enter the correct response in accordance with the directions of the test center.

- Budget your time. If the test has, for example, 100 questions and 2 hours and 30 minutes are allowed for completion, you have approximately 1 minute and 30 seconds for each question.
- Above all, don't panic! If you "draw a blank" on a particular question or set of questions, skip it and go on unless the directions indicate that all questions must be answered when presented. At the end of the exam, if you are permitted, return to review your answers or to complete any skipped questions. Stay calm and do your best.

KEYS TO SUCCESS ACROSS THE BOARDS

Study, review, and practice.
Keep a positive, confident attitude.
Follow all directions on the examination.
Do your best.

Good luck!

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CHAPTER

1 Clinical Chemistry

contents

***Note**: The reference ranges used throughout the book are meant to function as guides to understand and relate to the analytes; each laboratory facility will have established its own reference ranges based on the laboratory's specific instrumentation, methods, population, and so on.*

I. INSTRUMENTATION AND ANALYTICAL PRINCIPLES

A. Spectrophotometry General Information

1. **Electromagnetic radiation** has wave-like and particle-like properties.
   a. **Radiant energy** is characterized as a spectrum from short wavelength to long wavelength: cosmic, gamma rays, X-rays, ultraviolet, visible, infrared, microwaves, and radiowaves.
   b. **Wavelength (λ)** is the distance traveled by one complete wave cycle (distance between two successive crests) measured in **nanometers (nm).**
   c. The **shorter the wavelength**, the **greater the energy** contained in the light, and the greater the number of photons.
   d. Light is classified according to its wavelength: **Ultraviolet (UV) light** has very short wavelengths and **infrared (IR) light** has very long wavelengths. When all **visible wavelengths** of light (400–700 nm) are combined, white light results.
      1) Visible color: wavelength of light transmitted (not absorbed) by an object
2. Particles of light are called **photons.** When an atom absorbs a photon, the atom becomes **excited** in one of three ways: An electron is moved to a higher energy level, the mode of the covalent bond vibration is changed, or the rotation around its covalent bond is changed.
   a. When energy is absorbed as a photon, an **electron** is moved to a higher energy level where it is **unstable.**
      1) An excited electron is not stable and will **return to ground state.**
      2) An electron will **emit** energy in the form of **light** (radiant energy) of a characteristic wavelength.
      3) Absorption or emission of energy forms a **line spectrum** that is characteristic of a molecule and can help identify a molecule.

B. Spectrophotometer

1. In order to determine the concentration of a light-absorbing analyte in solution, a **spectrophotometer measures light transmitted** by that analyte in solution. Such an analyte may absorb, transmit, and reflect light to varying degrees, but always of a characteristic nature for the analyte.
2. **Components of a spectrophotometer**
   a. Power supply
   b. Light source
   c. Entrance slit
   d. Monochromator
   e. Exit slit
   f. Cuvet/sample cell
   g. Photodetector
   h. Readout device

3. The **light source** or **exciter lamp** produces an intense, reproducible, and constant beam of light. A variety of light sources exist to measure within the visible and ultraviolet regions as described further.
   a. Incandescent lamps
      1) **Tungsten (incandescent tungsten or tungsten-iodide):** Most common, used in visible and near-infrared regions. Approximately 15% of radiant energy falls within the visible region, the rest falls in the near-infrared region; therefore, a heat absorbing filter is placed between the lamp and the sample to absorb the infrared radiation. NOTE: This lamp does not provide sufficient energy for measurements in the UV region.
      2) **Deuterium:** Used in the ultraviolet region; provides continuous emission down to 165 nm
      3) **Mercury arc (low, medium, and high pressure):** Used in the ultraviolet region; low-pressure mercury arc lamps (not practical for absorbance measurements) emit sharp ultraviolet and visible line spectra; medium- to high-pressure lamps emit continuum from ultraviolet to the mid-visible region.
      4) **Xenon arc:** Used in the ultraviolet region; provide continuous spectra
   b. **Lasers (light amplification by stimulated emission of radiation):** Lasers produce extremely intense, focused, and nearly nondivergent beam of light.
   c. **Important:** When a lamp is changed in the spectrophotometer, the instrument must be recalibrated because changing the light source changes the angle of the light striking the monochromator.
4. The **monochromator** is a device used to isolate radiant energy of wider wavelengths to a mechanically selected narrow band of desirable wavelengths of light, in other words, it is a wavelength isolator. When a monochromator is set to a particular wavelength, light with a Gaussian distribution of wavelengths emerges from the exit slit. This information can be used to describe **bandpass.** The bandpass, or spectral bandwidth, is defined as the width of the band of light at one-half the peak maximum. The bandpass describes the purity of light emitted from the monochromator, which is a reflection of the resolution capabilities of the instrument. For example, if a spectrophotometer is set to read at 550 nm, light with a Gaussian distribution of wavelengths between 540 and 560 nm emerges from the exit slit. Bandpass may be determined by locating one-half the maximum intensity of light (50%) on the *y*-axis and dropping two vertical lines down to the *x*-axis from these midpoints, 545 and 555 nm. The distance between these two points (545–555) is equal to the bandpass, in other words, 10 nm. See Figure 1–1■. Types of monochromators are described further.
   a. **Filters**
      1) **Glass filters** are used in **photometers;** simple and inexpensive; isolate a relatively wide band of radiant energy and therefore have low transmittance of the selected wavelength; considered less precise.
      2) **Interference filters:** Produce monochromatic light using constructive interference of waves using two pieces of glass; can be constructed to yield a very narrow range of wavelengths with good efficiency.

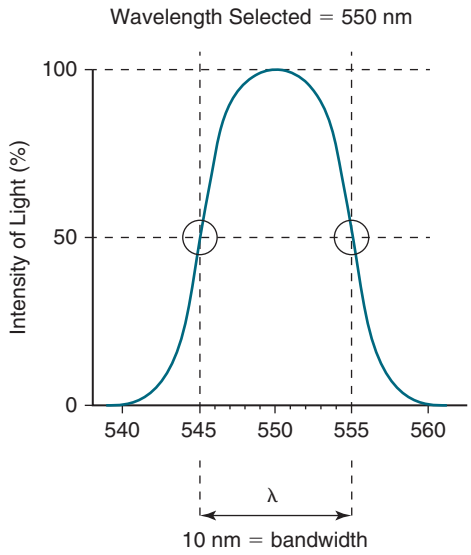

FIGURE 1–1 ■ Determining spectral bandwidth.

b. **Diffraction gratings** are the most commonly used monochromators in **spectrophotometers;** consist of a flat glass plate coated with a thin layer of aluminum-copper alloy with rulings of many parallel grooves into the coating. The more rulings, the better the grating, for example, better diffraction gratings contain 1000–2000 lines/mm.

c. **Prisms:** Used in **spectrophotometers;** separate light through refraction with shorter wavelengths that are bent (refracted) more than longer wavelengths as they pass through the prism.

1) **Wavelength selection:** Entrance slit allows lamp light to enter; slit is fixed in position and size. Monochromator disperses the light into wavelengths. Exit slit selects the bandpass of the monochromator that allows light of the selected wavelength to pass through the cuvet onto the detector.

5. **Photodetectors:** A **detector converts** the **electromagnetic radiation** (light energy) transmitted by a solution **into an electrical signal.** The more light transmitted, the more energy, and the greater the electrical signal that is measured.
6. **Readout devices:** Electrical energy from a detector is displayed on some type of digital display or readout system. The readout system may be a chart recorder or a computer printout.

C. Atomic Absorption Spectrophotometry

1. **Principle: Ground-state atoms absorb light at defined wavelengths.**
   a. **Line spectrum** refers to the wavelengths at which an atom absorbs light; each metal exhibits a specific line spectrum.
   b. The sample is **atomized in a flame** where the atoms of the metal to be quantified are dissociated from its chemical bonds and placed at ground state.

Once in this state, it is at a low-energy level and is now capable of absorbing light corresponding to its own line spectrum.

c. Then a beam of light from a **hollow-cathode lamp** (HCL) is passed through a chopper to the flame.
d. The **ground-state atoms** in the flame **absorb** the same wavelengths of **light** from the HCL as the atoms emit when excited.
e. The **light not absorbed** by the atoms is **measured as a decrease in light intensity by the detector.** The detector (photomultiplier tube) will selectively read the pulsed light from the chopper that passes through the flame and will not detect any light emitted by the excited atoms when they return to ground state.
f. The difference in the amount of light leaving the HCL and the amount of light measured by the detector is **indirectly proportional to the concentration** of the metal analyte in the sample.

2. **Components**

Hollow-cathode lamp ⟶ chopper ⟶ burner head for flame ⟶ monochromator ⟶ detector ⟶ readout device

3. **Hollow-cathode lamp (HCL)**
   a. HCL contains an anode, a cylindrical cathode made of metal being analyzed, and an inert gas such as helium or argon.
   b. **Principle:** Applied voltage causes ionization of the gas, and these excited ions are attracted to the cathode, where they collide with the metal coating on the cathode, knocking off atoms, and causing atomic electrons to become excited. When the electrons of the metal atoms from the cathode return to ground state, the characteristic light energy of that metal is emitted.
   c. Vaporized metal atoms from the sample can be found in the flame. The flame serves as the sample cuvet in this instrument.
   d. The light produced in the HCL passes through a chopper and then to the flame, and the light is absorbed by the metal in the sample. The light not absorbed will be read by the photomultiplier tube.
   e. A **flameless system** employs a carbon rod (graphite furnace), tantalum, or platinum to hold the sample in a chamber. The temperature is raised to vaporize the sample being analyzed. The atomized sample then absorbs the light energy from the HCL. This technique is more sensitive than the flame method.

D. Nephelometry

1. **Definition:** Nephelometry is the **measurement of light scattered** by a particulate solution. Generally, scattered light is measured at an angle to the incident light when small particles are involved; for large molecules, forward light scatter can be measured. The **amount of scatter is directly proportional** to the number and size of particles present in the solution.

2. The **sensitivity of nephelometry** depends on the absence of background scatter from scratched cuvets and particulate matter in reagents.

E. Turbidimetry

1. **Definition:** Turbidimetry **measures light blocked** as a decrease in the light transmitted through the solution; dependent on particle size and concentration.
2. **Turbidimetry uses a spectrophotometer** for measurement, and it is limited by the photometric accuracy and sensitivity of the instrument.

F. Molecular Emission Spectroscopy

1. Types of **luminescence** where **excitation requires absorption of radiant energy**
   a. **Fluorescence** is a process where atoms absorb energy at a particular wavelength (excitation), electrons are raised to higher-energy orbitals, and the electrons release energy as they return to ground state by emitting light energy of a longer wavelength and lower energy than the exciting wavelength. The emitted light has a very short lifetime.
      1) **Fluorometry:** Frequently UV light is used for excitation and is passed through a primary filter for proper wavelength selection for the analyte being measured. The excitation light is absorbed by the atoms of the analyte in solution, which causes the electrons to move to higher-energy orbitals. Upon return to ground state, light is emitted from the fluorescing analyte and that light passes through a secondary filter. The secondary filter and the detector are placed at a right angle to the light source to prevent incident light from being measured by the detector. Whereas fluorometers use filters, spectrofluorometers use prisms or diffraction gratings as monochromators.
      2) **Advantages:** Fluorometry is about 1000 times more **sensitive** than absorption techniques and has increased **specificity** because optimal wavelengths are chosen both for absorption (excitation) and for monitoring emitted fluorescence.
      3) **Limitations:** It changes from the established protocol that affect pH, temperature, and solvent quality; self-absorption; quenching.
   b. **Phosphorescence** is the emission of light produced by certain substances after they absorb energy. It is similar to fluorescence except that the time delay is longer (greater than 10^{-4} seconds) between absorption of radiant energy and release of energy as photons of light.
2. Types of **luminescence** where excitation does **not** require **absorption of radiant energy**
   a. **Chemiluminescence** is the process where the **chemical energy** of a reaction produces excited atoms, and upon electron return to ground state, photons of light are emitted.

b. **Bioluminescence** is the process where an **enzyme-catalyzed** chemical reaction produces light emission. For example, this may occur in the presence of the enzyme luciferase because of oxidation of the substrate luciferin.
   1) **Luminometer** is a generic term for the type of instrument that is used to measure chemiluminescence and bioluminescence.

G. Chromatography

1. **Chromatography** is a technique where solutes in a sample are separated for identification based on **physical differences** that allow their differential distribution between a mobile phase and a stationary phase.
   a. **Mobile phase:** Phase that passes through the column; may be an inert gas or a liquid
   b. **Stationary phase:** Phase bound to the column, therefore, it does not pass through the column; may be silica gel bound to the surface of a glass plate or plastic sheet; may be silica or a polymer that is coated or bonded within a column

H. Thin-Layer Chromatography (TLC)

1. TLC is a type of planar chromatography. The **stationary phase** may be silica gel that is coated onto a solid surface such as a glass plate or plastic sheet. The **mobile phase** is a solvent, where solvent polarity should be just enough to achieve clear separation of the solutes in the sample. TLC is a technique used clinically for **urine drug screening.**
2. **The mobile phase moves through the stationary phase by absorption and capillary action.** The solute components move at different rates because of solubility in the mobile phase and electrostatic forces of the stationary phase that retard solute movement. These two phases work together to provide **solute resolution and separation.**
   a. Solute will stay with the **solvent front** if solvent is too polar for the solute.
   b. Solute will remain at **origin** if solvent is insufficiently polar.
3. Basic steps in performing TLC include sample extraction using a liquid-liquid or column technique; concentration of the extracted sample; sample application by spotting onto the silica gel plate; development of the solute in the sample using the stationary and mobile phases; solute detection using chromogenic sprays, UV light, fluorescence, and heat; and interpretation of chromatographic results utilizing R_f values of solutes in comparison to aqueous standards.
4. **R_f values** are affected by chamber saturation, temperature, humidity, and composition of the solvent.

I. Gas-Liquid Chromatography (GLC)

1. **Gas-liquid chromatograph** components include a carrier gas with a flow-control device to regulate the gas flow, a heated injector, chromatographic column to separate the solutes, heated column oven, detector, and computer to process data and control the operation of the system.

2. **Gas-liquid chromatography** is a technique used to **separate volatile solutes.**
   a. The sample is injected into the injector component of the instrument where the **sample is vaporized** because the injector is maintained approximately 50°C higher than the column temperature.
   b. An **inert carrier gas (mobile phase)** carries the vaporized sample into the column. Carrier gases commonly used include hydrogen, helium, nitrogen, and argon. The **carrier gas flow rate is critical** to maintaining column efficiency and reproducibility of elution times.
   c. The types of **columns (stationary phase)** used are designated as packed or capillary. When the volatile solutes carried by the gas over the stationary phase of the column are eluted, the column effluent is introduced to the detector. The solutes are introduced to the detector in the order that each was eluted.
   d. The **detector** produces a signal for identification and quantification of the solutes. Commonly used detectors include flame ionization, thermal conductivity, electron capture, and mass spectrometer.
   e. Separation of solutes is a function of the relative differences between the vapor pressure of the solutes and the interactions of the solutes with the stationary column. The **more volatile** a solute, **the faster it will elute** from the column; the **less interaction** of the solute **with the column**, the **faster it will elute.**
   f. **Identification** of a solute is based on its **retention time** and **quantification** is based on **peak size** where the amount of solute present is proportional to the size of the peak (area or height of the sample peak is compared to known standards).

J. High-Performance Liquid Chromatography (HPLC)

1. **High-performance liquid chromatograph** components include solvent reservoir(s), one or more pumps to propel the solvent(s), injector, chromatographic column, detector, and computer to process data and control the operation of the system.
2. HPLC is a type of liquid chromatography where the **mobile phase** is a **liquid** that is passed over the **stationary phase** of the **column.** The separation of solutes in a sample is governed by the selective distribution of the solutes between the mobile and stationary phases.
   a. **Solvents** commonly used for the **mobile phase** include acetonitrile, methanol, ethanol, isopropanol, and water.
      1) **Isocratic elution:** Strength of solvent **remains constant** during separation.
      2) **Gradient elution:** Strength of solvent **continually increases** (%/min) during separation.
   b. **Stationary phase** is an **organic material covalently bonded to silica** that may be polar or nonpolar in composition.
      1) **Normal-phase** liquid chromatography: Polar stationary phase and nonpolar mobile phase
      2) **Reversed-phase** liquid chromatography: Nonpolar stationary phase and polar mobile phase

3. The **solvent-delivery system** utilizes a solvent reservoir from which the pump can push the mobile phase through the column. The sample is introduced through a loop injector. A precolumn and guard column function to maintain the integrity of the column and are positioned prior to the sample reaching the main column. The column, which functions as the stationary phase, generally operates at room temperature. The effluent from the column passes to a detector system. The solutes are introduced to the detector in the order that each was eluted.
4. The **detector** produces a signal for identification and quantification of the solutes. Commonly used detectors include spectrophotometer, photodiode array, fluorometer, electrochemical, and mass spectrometer.

K. Mass Spectrometry

1. A **mass spectrometer** is an instrument that uses the principle of **charged particles moving through a magnetic or electric field**, with **ions** being **separated** from other charged particles **according to their mass-to-charge ratios.** In this system, electrons bombard a sample, ionizing the compound into **fragment ions**, which are separated by their mass-to-charge ratios. The **mass spectrum** produced is unique for a compound **(identification)**, and the **number of ions** produced relates proportionally to **concentration** (quantification).
2. **Mass spectrometry** is a high-quality technique for identifying drugs or drug metabolites, amino acid composition of proteins, and steroids. In addition, mass spectrometry has applications in the field of proteomics. The **eluate gas from a gas chromatograph** may be introduced into a mass spectrometer that functions as the detector system, or the **liquid eluate** may be introduced **from** a high-performance liquid chromatograph.
3. **Instrumentation**
   a. **Mass spectrometer** components include ion source, vacuum system, analyzer, detector, and computer.
   b. **Ion source:** Samples enter the ion source and are bombarded by the ionization beam. When the sample is in gas form and introduced from a gas chromatograph, the ion source may be electron or chemical ionization. Other types, such as electrospray ionization and sonic spray ionization, may be used when a high-performance liquid chromatograph is used in conjunction with a mass spectrometer.
   c. **Vacuum system:** Prevents the collision of ions with other molecules when electronic or magnetic separation is occurring.
   d. **Analyzer:** Beam-type and trapping-type
      1) **Beam-type** is a destructive process, where ions pass through the analyzer one time and then strike the detector.
      2) **Quadrupole** is a beam-type analyzer, where mass-to-charge ratios are scanned during a prescribed time period to form a mass spectrum.

e. **Detector** usually detects ions using electron multipliers, such as discrete dynode and continuous dynode electron multipliers.
f. **Computer and software** convert the detector's signal to a digital form. Sample **identification** is achieved because each compound produces a **unique spectrum**, which is analyzed by a database for matching to a computerized reference library.

4. To further improve selectivity and sensitivity, a system known as **tandem mass spectrometers** can be employed, where a gas chromatograph or a high-performance liquid chromatograph is connected to **two** mass spectrometers (GC/MS/MS) or (HPLC/MS/MS). In these systems, ions of a specific mass-to-charge ratio are allowed to continue to the **second mass spectrometer**, where **additional fragmentation** occurs and final analysis is done.

L. Polarography

1. **Polarography** employs an **electrochemical cell.**
   a. Gradually increasing the voltage applied between two electrodes of the cell in contact with a solution containing the analyte
   b. Current measured; voltage change versus current plotted to produce a polarogram
   c. Voltage at which sharp rise in current occurs characteristic of the electrochemical reaction involved
   d. Amount of increase in current (i.e., the wave height) proportional to the concentration of analyte
2. **Anodic stripping voltammetry** is based on polarography.
   a. Negative potential applied to one of the electrodes
   b. Trace metal ions in the solution reduced and **plated onto anodic electrode;** preconcentrating step
   c. Plated electrode used as anode in polarographic cell; **metal stripped off anode**
   d. Current flow during stripping provides polarogram that **identifies** and **quantifies** the analyte being measured (trace metals)
   e. Used to assay heavy metals such as **lead in blood**

M. Potentiometry

1. **Potentiometry** is a technique used to determine the concentration of a substance in solution employing an **electrochemical cell** that consists of two half-cells, where the potential difference between an indicator electrode and a reference electrode is measured.
   a. Half-cell, also called an electrode, composed of single metallic conductor surrounded by solution of electrolyte
   b. Two different half-cells are connected to make a complete circuit; current flows because of potential difference between two electrodes (reference and indicator electrodes)

   c. Salt bridge connection between two metallic conductors and between two electrolyte solutions
   d. Comparison made between the voltage of one half-cell connected to another half-cell
   e. Half-cell potentials compared to potential generated by standard (reference) electrode; desirable to use one half-cell (reference electrode) with known and constant potential, not sensitive to composition of material to be analyzed
   f. Universally accepted standard (reference) half-cells; standard hydrogen electrode, arbitrarily assigned a potential E° of 0.000 volt
   g. Calomel electrode, consisting of mercury covered by a layer of mercurous chloride in contact with saturated solution of potassium chloride; silver-silver chloride (Ag/AgCl) electrode; common type of reference electrode; consists of a silver wire coated with silver chloride dipped in a solution containing silver
   h. Other half-cell **(indicator electrode)** selected on basis of change in its potential with change in concentration of analyte to be measured. Types of indicator electrodes include, glass, solid state, ion-exchange, gas, and polymer
2. A **pH/blood gas analyzer** employs a pH-sensitive glass electrode for measuring blood pH, and it employs PCO_2 and PO_2 electrodes for measuring gases in blood. For measuring pH, the **pH electrode** is a functioning **glass electrode** that is dependent on properties of pH-sensitive glass.
   a. Glass electrode made by sealing thin piece of pH-sensitive glass at the end of glass tubing and filling tube with solution of hydrochloric acid saturated with silver chloride
   b. Glass electrode constructed from specially formulated glasses consisting of a melt of silicon dioxide with added oxides of various metals; membrane thickness ranged from 10 to 100 μm; glass membranes have been made with selectivity for H^+, Na^+, K^+, Li^+, Rb^+, Cs^+, Ag^+, Tl^+, and NH_4^+
   c. Silver wire immersed in tube's solution with one end extending outside the tube for external connection; silver-silver chloride reference electrode sealed within tube with pH-sensitive glass tip
   d. pH-sensitive glass must be saturated with water. Surface of the glass develops a hydrated lattice, allowing exchange of alkaline metal ions in the lattice for hydrogen ions in the test solution. A potential is created between the inside and the outside of the electrode, and that potential is measured.
   e. Glass electrode calibrated by comparison with two primary standard buffers of known pH
   f. Because pH readings are temperature sensitive, the calibration must be carried out at a constant temperature of 37°C.
3. In a pH/blood gas analyzer, the **PCO_2 electrode** for measuring the **partial pressure of carbon dioxide (PCO_2)** in blood is actually a pH electrode immersed in a bicarbonate solution.
   a. The bicarbonate solution is separated from the sample by a membrane that is permeable to gaseous CO_2 but not to ionized substances such as H^+ ions.

   b. When CO_2 from the sample diffuses across the membrane, it dissolves, forming carbonic acid and thus lowering the pH.
   c. The pH is inversely proportional to the log of the PCO_2. Hence, the scale of the meter can be calibrated directly in terms of PCO_2.
4. The **ion-exchange electrode** is a type of potentiometric **ion-selective electrode.**
   a. Consists of liquid ion-exchange membrane made of inert solvent and ion-selective neutral carrier material
   b. Collodion membrane may be used to separate membrane solution from sample solution.
   c. **K^+ analysis:** Antibiotic **valinomycin**, because of its ability to bind K^+, used as a neutral carrier for K^+-selective membrane
   d. **NH_4^+ analysis:** Antibiotics **nonactin** and **monactin** used in combination as neutral carrier for NH_4^+-selective membrane
5. **Sodium analysis:** Ion-selective electrodes based on principle of potentiometry
   a. Utilize **glass membrane electrodes** with selective capability
   b. Constructed from glass that consists of silicon dioxide, sodium oxide, and aluminum oxide

N. Amperometry

Electrochemical technique that measures the amount of current produced through the oxidation or reduction of the substance to be measured at an electrode held at a fixed potential

1. In a pH/blood gas analyzer, the electrode for measuring the **partial pressure of oxygen (PO_2)** in the blood is an electrochemical cell consisting of a platinum cathode and a Ag/AgCl anode connected to an external voltage source. The cathode potential is adjusted to −0.65 V.
2. The cathode and anode are immersed in the buffer. A polypropylene membrane selectively permeable to gases separates the electrode and buffer from the blood sample.
3. When there is no oxygen diffusing into the buffer, there is practically no current flowing between the cathode and the anode because they are polarized.
4. When oxygen diffuses into the buffer from a sample, it is reduced at the platinum cathode.
5. The electrons necessary for this reduction are produced at the anode. Hence a current flows; the current is directly proportional to the PO_2 in the sample. For every molecule reduced at the cathode, four electrons of current will flow.

O. Coulometry

1. A **chloride coulometer** employs a coulometric system based on Faraday's law, which states that in an electrochemical system, the **number of equivalent weights of a reactant oxidized or reduced is directly proportional to the quantity of electricity used in the reaction.** The quantity of electricity is measured in coulombs. The coulomb is the unit of electrical quantity; 1 coulomb of electricity flowing per minute constitutes a current of 1 ampere.

2. If the current is constant, the number of equivalent weights of reactant oxidized or reduced **depends only on the duration of the current.**
3. In the chloride coulometer, the electrochemical reaction is the generation of Ag^+ ions by the passage of a direct current across a pair of silver electrodes immersed in a conducting solution containing the sample to be assayed for chloride. As the Ag^+ ions are generated, they are immediately removed from solution by combining with chloride to form insoluble silver chloride. When all the chloride is precipitated, **further generation of Ag^+ ions** causes an **increase in conductivity** of the solution.
4. The **endpoint** of the titration is indicated by the **increase in conductivity** of the solution. **Amperometry** is used to **measure the increase in conductivity.**

P. Electrophoresis

1. Used clinically to separate and identify proteins, including serum, urine and cerebrospinal fluid (CSF) proteins, lipoproteins, isoenzymes, and so on.
2. **Electrophoresis** is defined as the **movement of charged molecules** in a liquid medium when an electric field is applied.
3. **Zone electrophoresis** is defined as the movement of charged molecules in a porous supporting medium where the **molecules separate as distinct zones.**
4. **Support medium** provides a matrix that allows molecules to separate (e.g., agarose gel, starch gel, polyacrylamide gel, and cellulose acetate membranes).
5. Movement of charged particles through a medium depends on the nature of the particle, including net charge, size and shape, the character of the buffer and supporting medium, temperature, and the intensity of the electric field.
   a. Nature of the charged particle: **Proteins are amphoteric** and may be charged positively or negatively depending on the pH of the buffer solution.
   b. The pH at which negative and positive charges are equal on a protein is the **protein's isoelectric point.**
6. **Buffer solutions of pH 8.6** are generally used for serum protein electrophoresis. Using agarose gel or cellulose acetate at this alkaline pH, **serum proteins** take on a **net negative charge** and will migrate toward the **anode (+)**. Albumin migrates the fastest toward the anode and the gamma-globulins remain closer to the cathode (−).
7. **Visualizing the separated analyte:** Following electrophoresis, treat the support medium with colorimetric stains or fluorescent chemicals. **Amido black B, Ponceau S, and Coomassie brilliant blue stains** are used for visualizing **serum proteins.** Silver nitrate is used for CSF proteins, fat red 7B and oil red O are used for lipoproteins, and nitrotetrazolium blue is used for lactate dehydrogenase isoenzymes.
8. Detection and quantification of the separated protein are accomplished using a **densitometer.**

9. Commonly encountered problems in electrophoresis
   a. **Holes in staining pattern:** Analyte present in too high a concentration
   b. **Very slow migration:** Voltage too low
   c. **Sample precipitates in support:** pH too high or low; excessive heat production
10. **Isoelectric focusing** is a type of zone electrophoresis in which protein separation is based on the **isoelectric point (pI)** of the proteins. This method utilizes polyacrylamide or agarose gel containing a pH gradient formed by ampholytes in the medium. When exposed to an electric field, the ampholytes migrate based on their pI to their respective positions in the gradient. In turn, the serum proteins will migrate in the gel to the position where the gel's pH equals the pI of the respective protein.
11. **Capillary electrophoresis** is based on **electroosmotic flow** (EOF). When an electric field is applied, the flow of liquid is in the direction of the cathode. Thus, EOF regulates the speed at which solutes move through the capillary.

Q. Hemoglobin Electrophoresis

1. **Hemoglobin:** Tetramer composed of **four globin chains**, four heme groups, and four iron atoms
   a. **Hemoglobin A_1:** Two alpha chains and **two beta chains**
   b. **Hemoglobin A_2:** Two alpha chains and **two delta chains**
   c. **Hemoglobin F:** Two alpha chains and **two gamma chains**
2. A number of hemoglobinopathies exist where a **substitution** of one amino acid on either the alpha chain or the beta chain causes the formation of an abnormal hemoglobin molecule.
   a. **Hemoglobin S:** Substitution of valine for glutamic acid in position 6 of the beta chain.
   b. **Hemoglobin C:** Substitution of lysine for glutamic acid in position 6 of the beta chain.
3. Although hemoglobin differentiation is best achieved by use of electrophoresis, hemoglobin F may be differentiated from the majority of human hemoglobins because of its alkali resistance.
4. At **pH 8.6**, hemoglobins have a net negative charge and migrate from the point of application toward the anode. Using **cellulose acetate:**
   a. Hemoglobin A_1 moves the fastest toward the anode, followed by hemoglobin F and hemoglobins S, G, and D, which migrate with the same mobility.
   b. Hemoglobins A_2, C, O, and E have the same electrophoretic mobility and migrate slightly slower than hemoglobins S, G, and D.
5. At **pH 6.2 on agar gel**, hemoglobins exhibit different electrophoretic mobilities in comparison with hemoglobins electrophoresed at pH 8.6 on cellulose acetate.
   a. Order of migration, from the most anodal hemoglobin to the most cathodal hemoglobin, is hemoglobins C and S; followed by hemoglobins A_1, A_2, D, E, and G, which migrate as a group with the same mobility; followed by hemoglobin F.
   b. The different migration patterns seen with cellulose acetate at pH 8.6 and agar gel at pH 6.2 are useful in differentiating hemoglobins that migrate with the same electrophoretic mobility.

R. Automation Parameters/Terminology

1. **Centrifugal analysis:** Centrifugal force moves samples and reagents into cuvet areas for simultaneous analysis.
2. **Discrete analysis:** Each sample reaction is compartmentalized. This may relate to an analyzer designed to assay only one analyte (e.g., glucose) or an analyzer capable of performing multiple tests where the sample and reagents are in a separate cuvet/reaction vessel for each test.
3. **Random access:** Able to perform individual test or panel, and allows for stat samples to be added to the run ahead of other specimens
4. **Batch analysis:** Samples processed as a group
5. **Stand-alone:** Instrument from a single discipline with automated capability
6. **Automated stand-alone:** Instrument from a single discipline with additional internal automated capability (e.g., autorepeat and autodilute)
7. **Modular workcell:** At least two instruments from a single discipline with one controller
8. **Multiple platform:** Instrument able to perform tests from at least two disciplines
9. **Integrated modular system:** At least two analytical modules supported by one sample and reagent processing and delivery system
10. **Pneumatic tube system:** Transports specimens quickly from one location to another
11. **Throughput:** Maximum number of tests generated per hour
12. **Turnaround:** Amount of time to generate one result
13. **Bar coding:** Mechanism for patient/sample identification; used for reagent identification by an instrument
14. **Dead volume:** Amount of serum that cannot be aspirated
15. **Carry-over:** The contamination of a sample by a previously aspirated sample
16. **Reflex testing:** Use of preliminary test results to determine if additional tests should be ordered or canceled on a particular specimen; performed manually or automated
17. **Total laboratory automation:** Automated systems exist for laboratories where samples are received, centrifuged, distributed to particular instruments using a conveyor system, and loaded into the analyzer without operator assistance. This kind of automation is seen in large medical center laboratories and commercial laboratories where the volume of testing is high.

S. Principles of Automation

1. Automated instruments use robotics and fluidics to replicate manual tasks.
2. **Specimen handling:** Some instruments have **level-sensing probes** that detect the amount of serum or plasma in the tube. Some systems have a reading device that **allows bar-coded sample tubes** to be loaded onto the instrument. Although not as common, other instruments require the operator to **manually enter** the position of the patient sample.

3. **Reagents**
   a. **Dry reagents** can be packaged as **lyophilized powder** or **tablet form** that must be reconstituted with a buffer or reagent-grade water. Reconstituting of reagents may need to be done manually and then the reagents placed on an analyzer for use, or reconstituting the reagents may be part of the total automation process as employed by the Dimension® analyzer.
   b. **Dry reagents** can be spread over a support material and assembled into a **single-use slide.** This technique is employed by the Vitros® analyzer.
   c. **Liquid reagents** are pipetted by the instrument and mixed with the sample.
4. **Testing phase**
   a. Mixing of sample and reagents occurs in a vessel called a **cuvet.** Some instruments have permanent, nondisposable cuvets made of quartz glass. Other cuvets are made of plastic and are disposable.
   b. **Reaction temperatures and times** vary for each analyte. The most common reaction temperatures are 37°C and 30°C.
   c. **Kinetic assays:** Determination of sample concentration is based on **change in absorbance** over time.
   d. **Endpoint/colorimetric assays:** Incubated for a specific time, absorbance determined, absorbance related to calibrators for calculation of sample concentration
   e. A spectrophotometer is built within the system to read absorbances for kinetic and colorimetric assays. These systems may use a diffraction grating or a series of high-quality filters. Some automated analyzers incorporate fluorometry or nephelometry.
5. **Data management**
   a. The **computer module** of most automated instruments has a data management system that allows analysis of quality control (QC) materials and assessment of patient values (e.g., delta check) before releasing patient results.
   b. Instruments/laboratory information systems (LISs) also archive patient results and QC values. These archived results are stored by the laboratory for various lengths of time.

T. Point-of-Care Testing (POCT)

1. **Definition:** Defined as performing diagnostic tests outside the main laboratory and at or near patient care areas.
2. **Applications:** POCT is designed to provide immediate laboratory test results for immediate patient assessment and determination of appropriate treatment. POCT may be used in neonatal intensive care, coronary care, intensive care, or the emergency department.
3. **Operators:** Only waived laboratory tests can be performed using point-of-care instruments. Clinical laboratory technicians and clinical laboratory scientists must operate instruments that perform complex or high-complexity laboratory tests.

4. **Point-of-care (POC) instrument evaluations:** All POC instruments must be evaluated in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). The values obtained from POC instruments must correlate with values obtained from larger laboratory instruments. Linearity testing, calculation of control ranges, correlations of sample data, and reference range validations/verifications must be done for each instrument.
5. **Training:** All POC instrument operators must be trained and training must be documented.
6. **Quality control:** All effective quality control systems must be set up for each POC instrument. The program must use appropriate standards and controls, statistical analyses, and a proficiency testing system. This information must be documented.

U. Immunochemical Techniques

1. **Immunoassays** encompass a number of immunochemical techniques used to detect an extremely small amount of analyte (functions as antigen) by reacting it with an antibody (functions as reagent) to form an **antigen-antibody complex.** The signal measured has a relationship to the **label** used, and the label may be attached to either a reagent antigen or a reagent antibody.
   a. **Detection limits:** Immunochemical techniques detect very small amounts of substances. Monoclonal antibodies increase the specificity of the procedure.
   b. **Polyclonal antiserum:** Antibodies produced in an animal from many cell clones in response to an immunogen; heterogeneous mixture of antibodies
   c. **Monoclonal antiserum:** Antibodies produced from a single clone or plasma cell line; homogeneous antibodies
   d. **Used to quantify:** Hormones, tumor markers, drugs, and other analytes present in small concentrations
2. **Methods**
   a. **Competitive-binding immunoassays** are based on the **competition between an unlabeled antigen** (sample analyte) and a **labeled antigen** for an antibody. In this type of assay, the unlabeled antigen (sample analyte) is an unknown concentration and varies from sample to sample, whereas the labeled antigen concentration and the antibody concentration are constant for a particular method.
      1) As the assay proceeds, there will be some free labeled antigen remaining that does not bind to antibody.
      2) The concentration of the antibody binding sites is limited with respect to total antigens (unlabeled and labeled) present, which leads to less-labeled antigen bound to antibody when sample analyte concentration is high.

3) It is then necessary to measure either the free labeled antigen or the labeled antigen-antibody complex and relate it to the concentration of analyte in the sample. Depending on the method, it may be necessary to separate the free labeled antigen from the labeled antigen-antibody complex.
   a) **Heterogeneous** assays require that free labeled antigen be physically removed from the labeled antigen bound to antibody. Radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and immunoradiometric assay (IRMA) are examples of this technique.
   b) **Homogeneous** assays do **not** require physical removal of free labeled antigen from bound-labeled antigen.
4) The **original labels** used for immunoassays were **radioactive isotopes** (e.g., I^{125}); thus the term **radioimmunoassay.** Most immunoassays in use today use nonradioactive labels. **Enzyme** (e.g., alkaline phosphatase), **fluorophore** (e.g., fluorescein), and **chemiluminescent** (e.g., acridinium ester) **labels** are commonly used for immunoassays.

b. **Enzyme multiplied immunoassay technique (EMIT)** is a **homogeneous** immunoassay where the **sample** analyte (functions as **unlabeled antigen**) competes with the **enzyme-labeled antigen** for the binding sites on the antibody. The more analyte (unlabeled antigen) present in the mixture, the less binding of enzyme-labeled antigen to the antibody. The unbound enzyme-labeled antigen will react with substrate because the enzyme is in a conformational arrangement that allows for substrate to bind at the active site of the enzyme. The product formed is read spectrophotometrically. The more product formed, the greater the concentration of analyte in the sample.

c. **Fluorescent polarization immunoassay (FPIA)** is based on measuring the degree to which fluorescence intensity is greater in one plane than in another **(polarized versus depolarized).** FPIA is based on the amount of polarized fluorescent light detected when the fluorophore label is excited with polarized light.
   1) FPIA is a **homogeneous** technique where the **sample** analyte (functions as **unlabeled antigen**) competes with the **fluorophore-labeled antigen** for the binding sites on the antibody. The more analyte (unlabeled antigen) present in the mixture, the less binding of fluorophore-labeled antigen to the antibody.
   2) The **free fluorophore-labeled antigen** has **rapid** rotation and emits **depolarized light.** The **fluorophore-labeled antigen-antibody complex** rotates more **slowly;** light is in the vertical plane **(polarized light)** and is detected as **fluorescence polarization.**
   3) The greater the concentration of analyte in the sample, the less binding between antibody and fluorophore-labeled antigen (bound complex emits polarized light), the greater the amount of free fluorophore-labeled antigen (emits depolarized light), and thus the lesser amount of polarization sensed

by the detector. The amount of **analyte** in the sample is **inversely proportional** to the amount of fluorescence polarization. That is, the greater the concentration of analyte, the less the amount of polarized light detected.

4) It is used to measure hormones, drugs, and fetal pulmonary surfactant to assess fetal lung maturity.

d. **Chemiluminescent immunoassay** is a technique between antigen and antibody that employs a chemiluminescent indicator molecule such as isoluminol and acridinium ester as labels for antibodies and haptens. In the presence of hydrogen peroxide and a catalyst, isoluminol is oxidized, producing light emission at 425 nm. In such an assay, the chemiluminescent signal is proportional to the concentration of analyte in the serum sample.

e. **Luminescent oxygen channeling immunoassay (LOCI™)** is a homogeneous technique that is an adaptation of the chemiluminescent immunoassay.

1) Antigen (from serum sample) links to two antibody-coated particles. The first is an antibody-coated sensitizer particle containing a photosensitive dye (singlet oxygen source), and the second is an antibody-coated particle (singlet oxygen receptor) containing a precursor chemiluminescent compound and a fluorophore.

2) Irradiation of the immunocomplex produces singlet oxygen at the surface of the sensitizer particle that diffuses to the second particle being held in close proximity.

3) Singlet oxygen reacts with the precursor chemiluminescent compound to form a chemiluminescent product that decays and emits light. This light energy is accepted by a fluorophore, which results in light emission of a longer wavelength.

4) In this assay, the chemiluminescent signal is enhanced by the resulting fluorescent signal, which is proportional to the concentration of analyte in the serum sample.

f. **Electrochemiluminescence immunoassay** uses an indicator label such as **ruthenium** in sandwich and competitive immunoassays. Following a wash procedure to remove unbound label, label bound to magnetic beads at an electrode surface undergoes an electrochemiluminescent reaction with the resulting light emission measured by a photomultiplier tube.

II. PROTEINS AND TUMOR MARKERS

A. Characteristics of Proteins

1. Proteins are macromolecules made of **amino acids**, with each amino acid being linked to another via a **peptide bond.**

a. **Peptide bond** is formed when the **carboxyl** ($-COOH$) group of one amino acid links to the **amino** ($-NH_2$) group of another amino acid with the loss of a water molecule.

b. **N-terminal:** End of protein structure with a free amino group

c. **C-terminal:** End of protein structure with a free carboxyl group
d. **Nitrogen content:** Proteins consist of 16% nitrogen, which differentiates proteins from carbohydrates and lipids.

2. Protein structure
   a. **Primary structure:** The amino acids are linked to each other through covalent peptide bonding in a specific sequence to form a polypeptide chain.
   b. **Secondary structure:** The polypeptide chain winds to form alpha helixes and beta sheets through the formation of hydrogen bonds between CO and NH groups of the peptide bonds.
   c. **Tertiary structure:** The coiled polypeptide chain folds upon itself to form a three-dimensional structure through the interactions of the *R* groups of the amino acids. Such interactions include the formation of disulfide linkages, hydrogen bonds, hydrophobic interactions, and van der Waals forces.
   d. **Quaternary structure:** Two or more folded polypeptide chains bind to each other through hydrogen bonds and electrostatic interactions to form a functional protein.

B. Classification of Proteins

1. **Simple proteins:** Polypeptides composed of only amino acids
   a. **Globular proteins:** Symmetrical, compactly folded polypeptide chains (e.g., albumin)
   b. **Fibrous proteins:** Elongated, asymmetrical polypeptide chains (e.g., troponin and collagen)
2. **Conjugated proteins:** Composed of protein (apoprotein) and nonprotein (prosthetic group) components; **prosthetic groups** are commonly metal, lipid, and carbohydrate in nature
   a. **Metalloproteins:** Protein with a metal prosthetic group (e.g., ceruloplasmin)
   b. **Lipoproteins:** Protein with a lipid prosthetic group (e.g., cholesterol, triglyceride)
   c. **Glycoproteins:** Protein with 10–40% carbohydrates attached (e.g., haptoglobin)
   d. **Mucoproteins:** Protein with >40% carbohydrates attached (e.g., mucin)
   e. **Nucleoproteins:** Protein with DNA or RNA nucleic acids attached (e.g., chromatin)

C. Protein Functions

1. **Energy production:** Proteins can be broken down into amino acids that can be used in the citric acid cycle to produce energy.
2. **Water distribution:** Proteins maintain the colloidal osmotic pressure between different body compartments.
3. **Buffer:** The ionizable *R* groups of the individual amino acids of a protein provide buffering capacity by binding or releasing H^+ ions as needed.

4. **Transporter:** Binding of proteins to hormones, free hemoglobin, lipids, drugs, calcium, unconjugated bilirubin, and so on, allows movement of these and other molecules in the circulation.
5. **Antibodies:** Defined as proteins that protect the body against "foreign" invaders
6. **Cellular proteins:** These function as receptors for hormones so that the hormonal message can activate cellular components; some hormones are protein in nature [e.g., adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH)].
7. **Structural proteins:** Collagen is the fibrous component that maintains the structure of body parts such as skin, bone, cartilage, and blood vessels.
8. **Enzymes:** Catalysts that accelerate chemical reactions

D. Plasma Total Protein

1. **Regulation**
   a. The **liver synthesizes** most of the **plasma proteins. Plasma cells synthesize** the **immunoglobulins.**
      1) Proteins are synthesized from amino acids, with one amino acid linked to another through the formation of a peptide bond.
      2) When proteins **degrade**, their constituent amino acids undergo **deamination** with the **formation of ammonia**, which is **converted to urea** for excretion in the urine. The liver and kidneys play a key role in the process of amino acid deamination as they are responsible for ridding the body of potentially toxic levels of ammonia.
   b. Some **cytokines** released at the site of injury or inflammation cause the **liver** to **increase synthesis** of the **acute-phase reactant proteins.** This is a nonspecific response to inflammation that may be caused by autoimmune disorders or infections, as well as a nonspecific response to tissue injury from tumors, myocardial infarctions, trauma, or surgical procedures. On the other hand, some proteins will decrease in concentration and are referred to as **negative acute-phase proteins**, including prealbumin (transthyretin), albumin, and transferrin.
   c. Immunoglobulins are humoral antibodies produced in response to foreign antigens for the purpose of destroying them.
   d. **Reference ranges:** Total protein 6.5–8.3 g/dL; albumin 3.5–5.0 g/dL
2. In general, changes in total protein concentration are associated with:
   a. **Hypoproteinemia** caused by urinary loss, gastrointestinal tract inflammation, liver disorders, malnutrition, inherited immunodeficiency disorders, and extensive burns
   b. **Hyperproteinemia** caused by dehydration, increased protein production associated with monoclonal and polyclonal gammopathies, and chronic inflammatory diseases associated with paraprotein production

E. Clinical Significance of the Major Proteins

1. **Prealbumin** (also termed transthyretin): Act as indicator of nutritional status and is one of the proteins that transports thyroid hormones.
   a. **Decreased** in liver disorders, inflammation, malignancy, and poor nutrition
   b. **Increased** in steroid therapy, chronic renal failure, and alcoholism
2. **Albumin** is synthesized in the liver and has the **highest concentration of all plasma proteins.** Albumin is a nonspecific binder of many analytes **for transport** in blood, including unconjugated bilirubin, steroids, and ions such as calcium and magnesium, fatty acids, and drugs, and it significantly contributes to plasma **osmotic pressure.**
   a. **Decreased** in liver disorders because of decreased production, gastrointestinal disease associated with malabsorption, muscle-wasting diseases, severe burns caused by loss, renal disease caused by loss (nephrotic syndrome, glomerulonephritis), starvation, and malnutrition
   b. **Increased** in dehydration (relative increase)
3. **α_1-Antitrypsin** is an acute-phase reactant and a protease inhibitor that neutralizes trypsin-type enzymes that can damage structural proteins.
   a. **Decreased** in emphysema-associated pulmonary disease and severe juvenile hepatic disorders that may result in cirrhosis
   b. **Increased** in inflammatory disorders
4. **α_1-Fetoprotein (AFP)** is synthesized during gestation in the yolk sac and liver of the fetus, peaking at 13 weeks and declining at 34 weeks. Normally, adult levels are very low.
   a. **Maternal serum AFP** is measured between 15 and 20 weeks of gestation and is reported as **multiples of the median** (MoM).
      1) **Increased** AFP level in maternal serum: neural tube defects, spina bifida, and fetal distress
      2) **Decreased** AFP level in maternal serum: Down syndrome, trisomy 18
   b. **In adults, increased** levels of AFP can be indicative of hepatocellular carcinoma and gonadal tumors.
5. **α_1-Acid glycoprotein (orosomucoid):** Acute-phase reactant; binds to basic drugs
   a. **Increased** in inflammatory disorders such as rheumatoid arthritis, pneumonia, and conditions associated with cell proliferation
   b. **Decreased** in nephrotic syndrome
6. **Haptoglobin:** α_2-globulin that binds free hemoglobin and is an acute-phase reactant
   a. **Increased** in inflammatory conditions, burns, trauma
   b. **Decreased** in intravascular hemolysis because of formation of a haptoglobin-hemoglobin complex for removal by the liver
7. **Ceruloplasmin** is an acute-phase reactant that is an α_2-globulin, **copper-containing protein** with enzymatic activity. Approximately 90% of serum copper is bound in ceruloplasmin.
   a. **Increased** in pregnancy, inflammatory disorders, malignancies, and with intake of oral estrogen and oral contraceptives
   b. **Decreased** in **Wilson disease**, malnutrition, malabsorption, and severe liver disease

8. α_2**-Macroglobulin:** It is a proteolytic enzyme inhibitor that inhibits thrombin, trypsin, and pepsin.
   a. **Increased** in nephrotic syndrome, contraceptive use, pregnancy, and estrogen therapy
   b. **Decreased** slightly in acute inflammatory disorders and prostatic cancer; decreased markedly in acute pancreatitis
9. **Transferrin:** β-globulin that **transports iron**
   a. **Decreased** in infections, liver disease, and nephrotic syndrome
   b. **Increased** in iron-deficiency anemia and pregnancy
10. **C-reactive protein (CRP):** β-globulin that is an acute-phase reactant
    a. Increased in tissue necrosis, rheumatic fever, infections, myocardial infarction, rheumatoid arthritis, and gout
    b. The American Heart Association (AHA) and the Centers for Disease Control and Prevention (CDC) have defined risk groups for future coronary events according to hs-CRP levels. $<$ 1.0 mg/L = low risk, 1.0–3.0 mg/L = average risk, and $>$ 3.0 mg/L = high risk.
11. **Immunoglobulins:** Antibodies
    a. Five major classes: IgA, IgD, IgE, IgG, and IgM
       1) Synthesized in **plasma cells** as an immune response
       2) One of the immunoglobulins will be increased in a **monoclonal gammopathy** (e.g., **multiple myeloma**). Such disorders are generally associated with an increase in IgG, IgA, or IgM; seldom is the increase associated with IgD or IgE.
    b. **IgG** can cross the placenta.
       1) **Increased** in liver disorders, infections, and collagen disease
       2) **Decreased** in the presence of increased susceptibility to infection and when a monoclonal gammopathy is associated with an increase in another immunoglobulin
    c. **IgA** levels increase after birth.
       1) **Increased** in liver disorders, infections, and autoimmune diseases
       2) **Decreased** in inhibited protein synthesis and hereditary immune disorders
    d. **IgM** cannot cross the placenta; it is made by the fetus.
       1) **Increased** in various bacterial, viral, and fungal infections and **Waldenström macroglobulinemia**
       2) **Decreased** in renal diseases associated with protein loss and immunodeficiency disorders
    e. **IgD** is increased in liver disorders, infections, and connective tissue disorders.
    f. **IgE** is increased in allergies, asthma, and hay fever, and during parasitic infections.
12. **Fibronectin:** Fetal fibronectin is used to **predict risk of premature birth.** It is a normal constituent in the placenta and amniotic fluid. In cases of stress, infection, or hemorrhage these can cause leakage of fibronectin into the cervicovaginal secretions, increased fibronectin is suggestive of risk for premature birth.

F. Methodology for Serum Total Protein, Albumin, and Protein Fractionation

1. **Refractometry** is based on the change in velocity of light (light is bent) as light passes through the boundary between air and water, which function as two transparent layers. In protein analysis, the light is bent and such change is proportional to the concentration of the solutes (proteins) present in the water (serum).
2. The **biuret method** is based on cupric ions complexing with peptide bonds in an alkaline medium to produce a purple-colored complex. The amount of purple complex produced is directly proportional to the number of peptide bonds present and reflects protein concentration.
3. **Dye binding** techniques allow proteins to bind to a dye, forming a protein-dye complex that results in a shift of the maximum absorbance of the dye (e.g., **Coomassie brilliant blue**). The increase in absorbance is directly proportional to protein concentration.
4. The **Kjeldahl** technique for the determination of total protein is too cumbersome for use in routine testing. It is considered the **reference method** of choice to validate materials used with the biuret method. The Kjeldahl technique is based on the quantification of the nitrogen content of protein.
5. **Electrophoresis**
   a. **Serum protein electrophoresis:** Serum is applied at the cathode region of an agarose gel or cellulose acetate plate saturated with a buffer of pH 8.6. At a pH of 8.6, serum proteins have a **net negative charge** and migrate toward the **anode**, with **albumin traveling the farthest**, followed by α_1-globulins, α_2-globulins, β-globulins, and γ-globulins. The proteins are fixed in the medium, stained, and then quantified using a densitometer.
   b. **High-resolution protein electrophoresis** is a modified technique that uses agarose gel, a higher voltage, a cooling system, and a more concentrated buffer to separate proteins into as many as 12 zones.
   c. **Isoelectric focusing** is a type of zone electrophoresis in which protein separation is based on the **isoelectric point (pI)** of the proteins.
6. **Immunochemical methods**
   a. Homogeneous and heterogeneous immunoassays
   b. Immunonephelometry
   c. Immunoelectrophoresis
   d. Radial immunodiffusion (RID)
   e. Electroimmunodiffusion
   f. Immunofixation
7. **Test methodology for albumin: Dye binding** techniques using **bromcresol green** and **bromcresol purple** dyes allow albumin to be positively charged for binding to the anionic dye, forming an albumin-dye complex that results in a shift of the maximum absorbance of the dye. The increase in absorbance is directly proportional to the albumin concentration.

8. **Test methodology for globulins:** The direct measurement of total globulins is not generally performed. The concentration of the globulins is determined by calculation. **Globulins = Total Protein − Albumin**

G. Proteins in Other Body Fluids

1. **Urinary proteins: Quantification** performed on 24-hour urine specimens
   a. **Test methods:** Sulfosalicylic acid, trichloroacetic acid, benzethonium chloride (turbidimetric), and Coomassie brilliant blue (spectrophotometric)
   b. **Reference range urine total protein:** 1–14 mg/dL; <100 mg/day
   c. Clinical significance of proteinuria
      1) **Increased** protein in urine may result from tubular or glomerular dysfunction, multiple myeloma, Waldenström macroglobulinemia, and nephrotic syndrome.
      2) **Bence Jones protein** may be found in urine of patients with multiple myeloma.
      3) Glomerular membrane can be damaged in diabetes, amyloidosis, and collagen diseases.
      4) Glomerular dysfunction can be detected in its early stages by measuring albumin in urine. **Albuminuria** is a condition where the quantity of albumin in the urine is greater than normal, yet it is not able to be detected by the urine dipstick method. The presence of albuminuria in a diabetic individual is a concern because it **generally precedes nephropathy.**
         a) **Methods** for quantification: Enzyme immunoassays and immunonephelometric assays
         b) **Reference range for urine albumin:** <30 mg/day
2. **Cerebrospinal fluid (CSF) proteins**
   a. CSF is an **ultrafiltrate of plasma** formed in the ventricles of the brain.
   b. **Test methods** include sulfosalicylic acid, trichloroacetic acid, benzethonium chloride, and Coomassie brilliant blue.
   c. **Reference range:** 15–45 mg/dL
   d. **Clinical significance**
      1) **Increased** in viral, bacterial, and fungal meningitis, traumatic tap (bloody), multiple sclerosis, herniated disk, and cerebral infarction
      2) **Decreased** in hyperthyroidism and with central nervous system leakage of CSF

H. Tumor Marker Utilization

1. In general, tumor markers used today are **not very useful in diagnosis**, but they are **useful** in tumor staging, monitoring therapeutic responses, predicting patient outcomes, and detecting cancer recurrence. **Ideal characteristics** for tumor markers include:
   a. Measured easily
   b. High analytical sensitivity of assay method
   c. High analytical specificity of assay method
   d. Cost-effective
   e. Test results contribute to patient care and outcome

2. **Prostate-specific antigen (PSA)**
   a. **Function**
      1) Produced by epithelial cells of the **prostate gland** and secreted into seminal plasma
      2) Glycoprotein protease that functions in liquefaction of seminal coagulum
   b. **Forms of PSA found in blood**
      1) **Enveloped** by protease inhibitor, α_2-macroglobulin; **lacks** immunoreactivity
      2) **Complexed** to another protease inhibitor, α_1-antichymotrypsin; **immunologically detectable**
      3) **Free PSA**, not complexed to protease inhibitor; **immunologically detectable**
      4) **Total PSA** assays measure complexed and free PSA forms, as both are immunologically detectable.
   c. **Specificity**
      1) PSA is a tissue-specific marker but **not** tumor specific.
      2) Small amounts present in serum normally
      3) Lacks specificity because serum level of PSA is increased in benign prostate hypertrophy as well as in adenocarcinoma of the prostate
   d. **Prostate cancer detection**
      1) Early-detection guidelines endorse lower cutoff of **PSA up to 2.5 ng/mL**.
      2) PSA >2.5 ng/mL perform biopsy
      3) **PSA velocity** is measurement of the **rate of change per year.**
         a) Biopsy recommended when PSA rises more than 0.75 ng/mL/year even when PSA is <2.5 ng/mL
      4) **Free PSA:** Men with **prostate cancer** tend to have **lower % free PSA** (free PSA/total PSA) than men with benign disease. Lower % free PSA is associated with a higher risk of prostate cancer.
      5) PSA is used to monitor therapeutic response and to follow radical prostatectomy.
   e. Methods used to measure serum levels of PSA include fluorescence immunoassay, enzyme immunoassay, and chemiluminescence immunoassay.
3. **α_1-Fetoprotein (AFP)**
   a. Oncofetal glycoprotein antigen
      1) Synthesized in liver, yolk sac, and gastrointestinal (GI) tract of **fetus**
      2) Fetal serum AFP peaks at 12–15 weeks of gestation with levels of 2–3 mg/mL.
      3) At birth, levels fall to 50 μg/mL, and at 2 years of age only trace amounts are present.
      4) **Adult levels <20 ng/mL**
   b. **Clinical significance**
      1) **Increased** levels of AFP in adults are associated with hepatocellular carcinoma, testicular and ovarian teratocarcinomas, pancreatic carcinoma, and gastric and colonic carcinomas.

2) **Increased** levels of AFP in adults are also seen in **nonmalignant** disorders, including viral hepatitis and chronic active hepatitis.
3) It is useful in monitoring therapeutic response of cancer patients to treatment protocols.
4) In **pregnancy, increased** maternal serum levels are associated with **spina bifida, neural tube defects**, and **fetal distress. Decreased** levels of maternal serum AFP are associated with increased incidence of **Down syndrome.**

c. Enzyme immunoassay methods are used for measurement.

4. **Carcinoembryonic antigen (CEA)**
   a. Oncofetal glycoprotein antigen
   b. Normally found in epithelial cells of the **fetal** GI tract
   c. **Clinical significance in adults**
      1) **Increased** levels of CEA are associated with adenocarcinoma of digestive tract and colorectal carcinoma.
      2) Elevations are seen in other malignancies and noncancerous disorders.
      3) CEA is useful in monitoring therapeutic response of cancer patients to treatment protocols.
   d. Enzyme immunoassay methods are used for measurement.
5. **Human chorionic gonadotropin (hCG)**
   a. hCG is a glycoprotein composed of α- and β-subunits. The **β-subunit is unique** and not common to other hormones; α-subunit is common to other hormones.
   b. Normally secreted by the trophoblast cells of the placenta.
   c. **Increased** secretion is associated with **trophoblastic tumors**, choriocarcinoma, nonseminomatous testicular tumors, and ovarian tumors.
   d. It is useful for monitoring the progress of patients.
   e. Immunoassay measures **β-hCG**.
6. **CA 15-3**
   a. Mucin glycoprotein antigen
   b. It is useful for monitoring therapeutic response and for detecting recurrence of **breast cancer** in patients previously treated.
   c. Elevated levels are observed in nonmalignant diseases such as chronic hepatitis, tuberculosis, and systemic lupus erythematosus.
   d. Immunoassay methods are used for measurement.
7. **CA 125**
   a. Mucin glycoprotein antigen
   b. Marker for **ovarian** and endometrial cancer
   c. It is useful for monitoring the progress of patients.
   d. Immunoassay methods are used for measurement.
8. **CA 19-9**
   a. Glycolipid blood group antigen-related marker; sialylated derivative of the Lewis blood group system, known as Le^{xa}

b. Marker for **pancreatic, colorectal**, lung, and gastric carcinomas
c. It is useful for monitoring the progress of patients.
d. Immunoassay methods are used for measurement.

III. NONPROTEIN NITROGENOUS COMPOUNDS

A. Urea

1. **Regulation:** Urea is the **major nitrogen-containing compound** in the blood. It results from protein catabolism and is synthesized in the liver from the deamination of amino acids. Urea is **excreted by the kidneys.**
2. **Clinical significance:** Abnormal serum urea levels may be due to prerenal, renal, or postrenal disorders.
   a. **Increased serum urea:** Renal failure, glomerular nephritis, urinary tract obstruction, congestive heart failure, dehydration, and increased protein catabolism
   b. **Decreased serum urea:** Severe liver disease, vomiting, diarrhea, and malnutrition
3. Blood urea nitrogen (BUN) is an older term still in use, and the terminology was based on previous methodology where nitrogen was measured. To convert BUN to urea: BUN $\times$ 2.14 = Urea.
4. **Test methodology**
   a. **Kinetic method: Urease/GLDH coupled enzymatic method**

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \alpha\text{-ketoglutaric acid} + \text{NADH} \xrightarrow{\text{GLDH}} NAD^+ + \text{Glutamic acid}$$

   b. **Chemical method**

$$\text{Diacetyl monoxime} + H_2O \longrightarrow \text{diacetyl} + \text{hydroxylamine}$$

$$\text{Diacetyl} + \text{urea (acid medium)} \longrightarrow \text{diazine derivative (yellow)}$$

5. **Reference range:** 6–20 mg/dL

B. Creatinine

1. **Regulation:** Creatinine is a waste product of muscle contraction that is formed from phosphocreatine, a high-energy compound. Creatinine levels are **regulated by kidney excretion.** Measurements of creatinine in serum and urine (creatinine clearance) are used to assess the glomerular filtration rate (GFR). Creatinine levels are not changed by diet or rate of urine flow. Creatinine is not reabsorbed by renal tubules.
2. **Clinical significance**
   a. **Increased serum creatinine:** Renal disease, renal failure
3. **Test methodology**
   a. **Jaffe method**

$$\text{Creatinine} + \text{picric acid (alkaline solution)} \rightarrow \text{creatinine picrate (red tautomer)}$$

b. **Enzymatic method: Creatininase (creatinine amidohydrolase)**

$$\text{Creatinine} + H_2O \xrightarrow{\text{creatinine amidohydrolase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{creatinase}} \text{sarcosine} + \text{urea}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{\text{sarcosine oxidase}} \text{glycine} + \text{formaldehyde} + H_2O_2$$

$$H_2O_2 + \text{indicator (reduced)} \xrightarrow{\text{peroxidase}} \text{indicator (oxidized)} + 2H_2O$$

4. **Reference ranges:** Male, 0.9–1.3 mg/dL; female, 0.6–1.1 mg/dL
5. **Creatinine clearance** is used to **assess** the **GFR.** Testing requires a plasma sample and a 24-hour urine collection.
   a. **P:** plasma creatinine mg/dL, **U:** urine creatinine mg/dL, **V:** urine flow in mL/min, and **SA:** body surface area; 1.73 m^2 = average body surface area
   b. **Creatinine clearance formula:**

$$\text{CrCl (mL/min)} = \frac{U \times V}{P} \times \frac{1.73\ m^2}{SA}$$

   c. **Reference ranges:** Differ according to age and sex; values decrease with age
   Creatinine clearance (males): 105 ± 20 mL/min/1.73 m^2
   Creatinine clearance (females): 95 ± 20 mL/min/1.73 m^2
   d. **Estimated glomerular filtration rate (eGFR)** uses a **serum creatinine** or **serum cystatin C** in conjunction with multiple equations including, CKD-EPI creatinine equation (2009), CKD-EPI creatinine-cystatin equation (2012), CKD-EPI cystatin C equation (2012), and the **MDRD** (Modification of Diet in Renal Disease) equation. The National Kidney Foundation recommends using the CKD-EPI Creatinine equation (2009) to estimate GFR.
      1) CKD-EPI creatinine equation (2009) is expressed as a single equation:

$$\text{eGFR} = 141 \times \min(\text{SCr}/\kappa, 1)^{\alpha} \times \max(\text{SCr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if African American}]$$

      2) Recommended method for estimating GFR in adults; designed for use with laboratory creatinine values that are standardized to IDMS; estimates GFR from serum creatinine, age, sex, and ethnicity; more accurate than the MDRD Study equation, particularly in people with higher levels of GFR; based on the same four variables as the MDRD Study equation, but uses a two-slope "spline" to model the relationship between estimated GFR and serum creatinine, and a different relationship for age, sex, and ethnicity.

         Some clinical laboratories are still reporting GFR estimates using the MDRD study equation. The National Kidney Foundation has recommended that clinical laboratories should begin using the CKD-EPI equation to report estimated GFR in adults.
      3) Results only reported as a number if <60 mL/min/1.73 m^2

C. Uric Acid

1. **Regulation:** Uric acid, the major waste product of purine (adenosine and guanine) catabolism, is synthesized in the liver. Uric acid **elimination** from the blood is **regulated by the kidneys** through glomerular filtration, and some uric acid is excreted through the GI tract.
2. **Clinical significance**
   a. **Increased serum uric acid:** Gout, renal disorders, treatment of myeloproliferative disorders, lead poisoning, lactic acidosis, toxemia of pregnancy, and Lesch-Nyhan syndrome
   b. **Decreased serum uric acid:** Severe liver disease as a secondary disorder, tubular reabsorption disorders, and drug induced
3. **Test methodology**
   a. **Chemical method**

$$\text{Uric acid} + \text{phosphotungstic acid} + O_2 \rightarrow \text{allantoin} + CO_2 + \text{tungsten blue}$$

   b. **Enzymatic uricase method:** Decrease in absorbance monitored at 293 nm when only the first reaction is employed; otherwise, the coupled enzymatic reactions yield a colored product.

$$\text{Urate} + O_2 \xrightarrow{\text{uricase}} \text{allantoin} + H_2O_2 + CO_2$$

$$2H_2O_2 + \text{phenol} + \text{4-aminophenazone} \xrightarrow{\text{peroxidase}} \text{quinine-monoimine dye} + 2H_2O$$

4. **Reference ranges:** Male, 3.5–7.2 mg/dL; female, 2.6–6.0 mg/dL

D. Ammonia

1. **Regulation**
   a. Ammonia produced from **deamination of amino acids**.
   b. Hepatocytes convert ammonia **to urea** for excretion.
   c. With severe liver cell malfunction, blood levels of ammonia **increase**.
   d. Ammonia is neurotoxic.
2. **Type of specimen and storage**
   a. Venous blood free of hemolysis; **place on ice immediately**
   b. Blood collected in ethylenediaminetetra-acetic acid (EDTA) may be used.
   c. Centrifuge sample within 20 minutes of collection and remove plasma.
   d. Plasma stable up to 3½ hours in ice bath; stable several days frozen
3. **Clinical significance:** Increased plasma ammonia levels seen in hepatic failure and Reye syndrome
4. **Test methodology**

$$NH_4^+ + \alpha\text{-ketoglutaric acid} + \text{NADH} \xrightarrow{\text{GLDH}} NAD^+ + \text{glutamic acid}$$

5. **Interferences**
   a. Incorrect handling of blood sample
   b. Ammonia contamination
6. **Reference range:** 11–32 μmol/L

IV. CARBOHYDRATES

A. Glucose Metabolism

1. During a fast, the blood glucose level is kept constant by mobilizing the **glycogen** stores in the liver.
2. During long fasts, **gluconeogenesis** is required to maintain blood glucose levels because glycogen stores are used up in about 24–48 hours.
3. An individual with a **fasting blood glucose level >100 mg/dL** is referred to as **hyperglycemic.** An individual with a **fasting blood glucose level <50 mg/dL** is referred to as **hypoglycemic.**

B. Hormones Affecting Blood Glucose Levels

1. **Insulin:** Produced by the beta cells of the pancreatic islets of Langerhans; promotes the entry of glucose into liver, muscle, and adipose tissue to be stored as glycogen and fat; inhibits the release of glucose from the liver, resulting in a decreased glucose level
2. **Somatostatin:** Synthesized by delta cells of the pancreatic islets of Langerhans; inhibits secretion of insulin, glucagon, and growth hormone, resulting in an increase in plasma glucose level
3. **Growth hormone** and adrenocorticotropic hormone (**ACTH**)**:** Hormones secreted by the anterior pituitary that raise blood glucose levels
4. **Cortisol:** Secreted by the adrenal glands; stimulates glycogenolysis, lipolysis, and gluconeogenesis
5. **Epinephrine** is secreted by the medulla of the adrenal glands. It stimulates glycogenolysis and lipolysis; it inhibits secretion of insulin. Physical or emotional stress causes increased secretion of epinephrine and an immediate increase in blood glucose levels.
6. **Glucagon:** Secreted by the α cells of the pancreatic islets of Langerhans; increases blood glucose by stimulating glycogenolysis and gluconeogenesis
7. **Thyroxine:** Secreted by the thyroid gland; stimulates glycogenolysis and gluconeogenesis; increases glucose absorption from the intestines

C. Renal Threshold for Glucose

1. Glucose is filtered by the glomeruli, reabsorbed by the tubules, and normally **not** present in urine. If the blood glucose level is elevated, glucose appears in the urine, a condition known as **glucosuria.**
2. An individual's **renal threshold** for glucose varies between **160 and 180 mg/dL.** When blood glucose reaches this level or exceeds it, the renal tubular transport mechanism becomes saturated, which causes glucose to be excreted into the urine.

D. Abnormal Carbohydrate Metabolism

1. **Classification of diabetes mellitus**
   a. **Type 1 diabetes mellitus**
      1) This has been characterized by **insulinopenia**, a deficiency of insulin.
      2) Individuals require **treatment with insulin** to sustain life.
      3) Most individuals exhibit it as an autoimmune disorder where β cells of the islets of Langerhans are destroyed by the body.

4) Peak incidence is in childhood and adolescence, but it may occur at any age.
5) Primary symptoms include polyuria, polydipsia, and weight loss.
6) **Ketosis-prone:** It can produce excess ketones, resulting in diabetic ketoacidosis; this is a result of the breakdown of fatty acids for energy.

b. **Type 2 diabetes mellitus**
1) This is characterized by defect in insulin secretion, insulin action, and cellular resistance to insulin.
2) Individuals are **not dependent on treatment with insulin.** Individuals generally respond to dietary intervention and oral hypoglycemic agents, but some may require insulin therapy.
3) It is associated with obesity and sedentary lifestyle; symptoms include polyuria, polydipsia, and weight loss.
4) Although associated with individuals over the age of 40, type 2 diabetes mellitus is becoming a significant problem in children and adolescents.
5) **Nonketosis-prone:** Without exogenous insulin or oral hypoglycemic medication, these individuals will have an elevated glucose but will not go into diabetic ketoacidosis.

c. **Gestational diabetes mellitus (GDM)**
1) GDM is the onset of diabetes mellitus during **pregnancy**.
2) After childbirth, the individual generally returns to normal metabolism. However, there is an increased chance that type 2 diabetes mellitus may develop later in life.

2. **Inherited disorders of carbohydrate metabolism**
a. **Glycogen storage diseases**, of which there are 10 types, are inherited diseases involving the deficiency of particular enzymes; these deficiencies cause defects in the normal metabolism of glycogen.
1) **von Gierke, type I:** Glucose-6-phosphatase deficiency
2) **Pompe, type II:** α-1,4-glucosidase deficiency
3) **Cori, type III:** Amylo-1,6-glucosidase deficiency

b. **Galactosemia**
1) This is characterized by a deficiency or absence of galactokinase, galactose 1-phosphate uridyl transferase, or uridyl diphosphate glucose-4-epimerase; the enzyme defect prevents metabolism of galactose. Galactose is found in milk as a component of lactose, with galactosemia generally identified in infants.
2) Most commonly, **galactose 1-phosphate uridyl transferase** is deficient, which leads to excessive galactose in blood and excretion in urine.

E. Laboratory Diagnosis

1. **Normal** fasting plasma glucose (FPG) **<100 mg/dL**
2. **Impaired fasting glucose (IFG)** is defined as a fasting plasma glucose level that **ranges between 100 and 125 mg/dL.**
3. **Provisional diagnosis of diabetes mellitus** is made when **FPG ≥126 mg/dL.** The diagnosis **must be confirmed** by one of the three methods described in the following outline section.

4. **Diagnosis of diabetes mellitus**
   a. A plasma glucose analysis that yields **any one** of the following results is **diagnostic for the presence of diabetes mellitus**, provided that unequivocal hyperglycemia is apparent. If unequivocal hyperglycemia is not apparent, the **glucose result must be confirmed** by repeat analysis on a subsequent day using any one of the following three methods. However, the American Diabetes Association does **not** recommend the oral glucose tolerance test (OGTT) for routine clinical use.
      1) An individual expressing physical symptoms and a **casual or random plasma glucose (RPG) level of ≥200 mg/dL**
      2) **Fasting plasma glucose** level that is **≥126 mg/dL** (fasting defined as no caloric intake for minimum of 8 hours)
      3) Plasma glucose level of **≥200 mg/dL at 2-hour point** of an **OGTT** using a 75-g glucose load, as described by the World Health Organization (WHO)
      4) A1c ≥ 6.5%; performed with a method traceable to the Diabetes Control and Complications Trial (DCCT)
   b. **Gestational diabetes mellitus (GDM)**
      1) A woman at **high risk** for GDM should have an **initial screening early** in the pregnancy. If she is not found to have GDM during the initial screening, the woman should be **retested at 24–28 weeks** of gestation. For women of **average risk**, testing should be performed at 24–28 weeks of gestation.
      2) Screening and diagnosis of GDM
         a) One-step approach: 75-g OGTT with plasma glucose measurement at fasting, 1 and 2 hours, performed at 24–28 weeks of gestation in women not previously diagnosed with overt diabetes.
         b) The OGTT should be performed in the morning after an overnight fast of at least 8 hours.
         c) The diagnosis of GDM is made when any of the following plasma glucose values are met or exceeded, as recommended by the International Association of the Diabetes and Pregnancy Study Groups (IADPSG):
            - Fasting: 92 mg/dL (5.1 mmol/L)
            - 1 hour: 180 mg/dL (10.0 mmol/L)
            - 2 hours: 153 mg/dL (8.5 mmol/L)
         d) Two-step approach: 50-g (nonfasting) screen followed by a 100-g OGTT for those who screen positive
            Step 1: Perform a 50-g glucose loading test (GLT) nonfasting, with plasma glucose measurement at 1 hour, at 24–28 weeks of gestation in women not previously diagnosed with overt diabetes; plasma glucose level measured 1 hour after the load; glucose ≥ 140 mg/dL (some experts recommend ≥ 130 mg/dL), proceed to Step 2.
            Step 2: 100-g OGTT performed when the patient is fasting; glucose measured at fasting, 1, 2, and 3 hours postload.
            The diagnosis of GDM is made if at least two* of the following four plasma glucose levels (measured fasting and 1, 2, and 3 hours during OGTT) are met or exceeded.

	Carpenter-Coustan	OR	NDDG
Fasting	95 mg/dL		105 mg/dL
1 hour	180 mg/dL		190 mg/dL
2 hours	155 mg/dL		165 mg/dL
3 hours	140 mg/dL		145 mg/dL

5. **Oral glucose tolerance test** based on the criteria published by the World Health Organization (WHO). NOTE: American Diabetes Association does **not** recommend the OGTT for routine clinical use.
   a. Timed measurements of plasma glucose before and after ingesting a specific amount of glucose
   b. **Patient preparation:** It includes unrestricted carbohydrate-rich diet for 3 days before the test with physical activity, restrict medication on the test day, 12-hour fast required, no smoking.
   c. Adult patient ingests 75 g of glucose in 300–400 mL of water and children 1.75 g/kg up to 75 g of glucose. For assessment of GDM, 50, 75, or 100 g of glucose may be used (see previous description for details).
   d. Plasma glucose specimen is collected fasting at 10 minutes before glucose load and at 120 minutes after ingestion of glucose. Urine glucose may be measured.
   e. **Interpretation of OGTT results** is based on the criteria published by the WHO.
      1) **Impaired fasting glucose (IFG)** is diagnosed when fasting plasma glucose ranges between 110 and 125 mg/dL.
      2) The following two criteria must be met for diagnosis of **impaired glucose tolerance (IGT):** Fasting plasma glucose level must be ≤126 mg/dL and the 2-hour plasma glucose level of the OGTT must fall between 140 and 199 mg/dL.
      3) **Diabetes mellitus** is diagnosed when the fasting plasma glucose level is ≥126 mg/dL or the 2-hour glucose is ≥200 mg/dL.
6. **Glycated/glycosylated hemoglobin**
   a. Hemoglobin A is composed of three forms, HbA_{1a}, HbA_{1b}, and HbA_{1c}, which are referred to as **glycated** or **glycosylated hemoglobin. HbA_{1c}** represents the **main form.**
   b. Glycated hemoglobin is formed from the **nonenzymatic, irreversible attachment of glucose** to hemoglobin A_1.
   c. Measurement of glycated hemoglobin **reflects blood glucose levels for the past 2–3 months.** It is useful in monitoring effectiveness of treatment and compliance of diabetic individual to treatment protocol.
   d. **Measured** by affinity chromatography, ion-exchange chromatography, and high-performance liquid chromatography
   e. **Specimen collection:** Nonfasting blood drawn in EDTA tubes
   f. **Reference range:** 4–6% HbA_{1c}; effective treatment range <7% HbA_{1c}

7. **Fructosamine**
   a. Ketoamine linkage forms between glucose and protein, mainly represented by albumin.
   b. **Clinical significance:** Measurement of fructosamine **reflects blood glucose levels for 2–3 weeks** before sampling.
   c. **Measured** by spectrophotometric/colorimetric methods, affinity chromatography, and high-performance liquid chromatography
   d. **Reference range:** 205–285 μmol/L
8. Measurement of albumin excretion is useful for patients with **renal complications of diabetes mellitus.** Performed on random urines, microalbumin analysis always requires the simultaneous analysis of creatinine, and it is reported as an albumin/creatinine ratio. Abnormal values **(albuminuria)** will be ≥**30 mg albumin/g creatinine.**

F. Measurement of Plasma Glucose

1. **Glucose oxidase method**

$$\beta\text{-D-Glucose} + H_2O + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen (colored)} + H_2O$$

2. **Hexokinase method**

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + NADP^+ \xrightarrow[\text{dehydrogenase}]{\text{glucose-6-phosphate}} \text{6-phosphogluconate} + NADPH + H^+$$

3. **Clinical significance:** An increase in the blood glucose level is the hallmark of diabetes mellitus, but it can also be indicative of other hormonal disorders such as Cushing disease.
4. **Reference range:** Adult fasting, 74–99 mg/dL

G. Lactate

1. The normal end-product of glucose metabolism is pyruvate; however, lactate is produced under conditions of oxygen deficit (anaerobic metabolism). The production and accumulation of lactate in the blood and its measurement aid in assessing the degree of oxygen deprivation that is occurring. Change in the blood lactate level precedes a change in blood pH. Lactate is metabolized by the liver via gluconeogenesis.
2. **Test methodology**

$$\text{Lactate} + O_2 \xrightarrow{\text{lactate oxidase}} \text{pyruvate} + H_2O_2$$

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen (colored)} + H_2O$$

3. **Clinical significance: Type A lactic acidosis** is caused by depressed oxygen levels that may occur in acute myocardial infarction, congestive heart failure,

shock, pulmonary edema, and so on. **Type B lactic acidosis** is caused by metabolic processes that may occur in diabetes mellitus, renal disorders, liver disease, ingestion of toxins (salicylate overdose and excess ethanol), and so on.

4. **Special specimen handling** is required and includes the following: Avoid using a tourniquet because venous stasis will falsely raise blood lactate levels; place the specimen on ice and immediately transport to the laboratory; centrifuge the specimen and remove the plasma (additives NaF and $K_2C_2O_4$) as soon as possible.
5. **Reference range (venous):** 0.5–1.3 mmol/L

V. LIPIDS AND LIPOPROTEINS

A. Lipid Structure

1. **Fatty acids** exist as short, medium, and long chains of molecules that are major constituents of triglycerides and phospholipids. Minimal amounts of fatty acids are bound to albumin and circulate free (unesterified) in plasma.
2. **Triglyceride**
   a. Triglyceride is formed from one glycerol molecule with three fatty acid molecules attached via ester bonds.
   b. Triglycerides comprise 95% of all fats stored in adipose tissue.
   c. Triglycerides are **transported** through the body by **chylomicrons** and **VLDL** (very-low-density lipoprotein).
   d. Metabolism involves releasing the fatty acids to the cells for energy, then recycling the glycerol into triglyceride.
   e. Lipase, lipoprotein lipase, epinephrine, and cortisol breakdown triglycerides.
3. **Cholesterol**
   a. It is an unsaturated steroid alcohol; exists in the **esterified** form, where a fatty acid forms an ester bond at carbon-3, and the **free** (unesterified) form.
   b. Acts as **a precursor for synthesis** of bile acids, steroid hormones, and vitamin D.
   c. Low-density lipoprotein (LDL) is the primary carrier of cholesterol.
4. **Phospholipid**
   a. Composed of one glycerol molecule and two fatty acid molecules attached via ester bonds
   b. Found on the surface of lipid layers, they are major constituents of cell membranes and outer shells of lipoprotein molecules.

B. Classification of Lipoproteins

1. **Lipoproteins** are molecules that combine water-insoluble dietary lipids and water-soluble proteins (apolipoproteins) so that lipids can be transported throughout the body. Micelles are spherical and have an inner core of neutral fat.
2. **Chylomicrons** are the largest lipoproteins and have the lowest density. They are formed in the intestines and transport **triglycerides** after a meal, giving serum a turbid appearance. Because of their low density, chylomicrons will float to the top and form a creamy layer when plasma is stored overnight. **Chylomicrons** are composed of **86% triglyceride**, 5% cholesterol, 7% phospholipid, and