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.

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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

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- ➤ Instrumentation and Analytical Principles
- ➤ Proteins and Tumor Markers
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- ➤ Liver Function and Porphyrin Formation
- ➤ Electrolytes and Osmolality
- ➤ Acid-Base Metabolism
- ➤ Endocrinology
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***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.

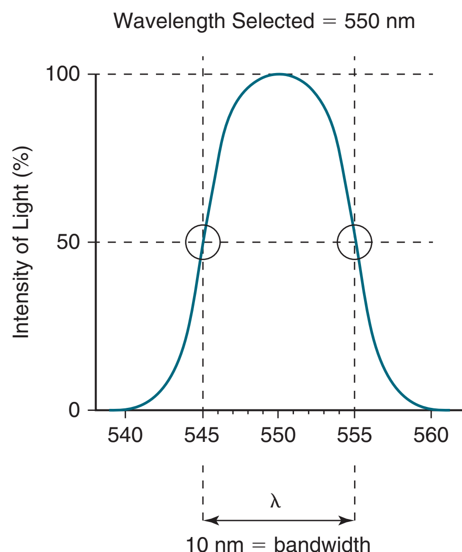

FIGURE 1–1 ■ Determining spectral bandwidth.

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.