Clinical Hematology

Theory and Procedures

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Foreword by

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To my husband and parents
who have always encouraged me
in my pursuits

Foreword

The clinical laboratory sciences have undergone revolutionary changes since the enactment of a variety of health and related legislation beginning in 1982. The effects to all scientific and nonscientific disciplines within the clinical laboratory sciences have been staggering. Laboratory and hospital administrations have had to creatively redefine the roles of all clinical laboratory practitioners, including those of phlebotomists, clinical laboratory technicians, clinical laboratory scientists, and pathologists.

The resulting challenge to educators to produce graduates who are able to fulfill the needs of managers and effectively practice in this newly regulated health care environment is indeed great. Graduates must be able to work in hospitals, independent laboratories, urgent care centers, surgical care centers, physician offices, small group physician laboratories, reference laboratories, research centers, educational institutions, and commercial industrial organizations. Their competencies must be compatible with the needs and resources of small rural 50-bed hospitals employing 1 to 5 full-time personnel and those of major university medical centers, which may employ 200 to 300 clinical laboratory professionals. They must be able to think creatively, to solve problems, to make sound decisions, and to participate as an effective member of the health care team.

Educators can be successful in preparing graduates who are capable of functioning in these new roles and environments if they are

supported by textbooks that reflect the comprehensive nature of the related scientific disciplines and give them practice in the resolution of problems. *Clinical Hematology* is such a text. Its organization is particularly effective in presenting a lot of material in a very logical sequence. The inclusion of an outline, a set of clear multilevel objectives, a chapter summary, and a set of multiple choice review questions for each chapter provides a superb format for student learning and self-assessment. The use of case studies and accompanying questions throughout the book provides appropriate practice in problem solving and decision making.

The inclusion of selected topics in immunology as they relate to leukemias and lymphomas provides a foundation for students who have not yet learned the concepts of T and B cell function and other basic immunological theory. The discussion of viruses as they relate to leukemia and the applications of electron microscopy further supports the interrelationships of laboratory disciplines. The incorporation of basic principles of cytogenetics, including karyotyping and cell development, introduces students to an important and rapidly developing discipline in clinical laboratory science.

It is indeed rare to find a textbook that addresses the needs of students in multiple levels of clinical laboratory science. Because of its highly organized format and its ability to present basic concepts and to build upon them by applying them to the etiology, diagnosis, and pathophysiology of disease processes, *Clinical Hematology* is useful for the education of both CLT and CLS level students. The chapter on clinical laboratory procedures is useful to both levels of students as representative examples of procedures currently used in a variety of test settings.

Dr. Turgeon has provided a much-needed text for educators and students in the clinical laboratory sciences. Her background as a medical technologist and an educator rings throughout her book. It is relevant, current, appropriate, and reflects the competencies that are required for today's clinical laboratory practitioners. I am truly proud to have had the opportunity to provide this foreword to *Clinical Hematology*. It will undoubtedly be a book that will find its way to the bookshelves of many hematology/coagulation students, educators, and practitioners.

Lucy J. Randles, M.A., CLS/CLDir

Preface

Clinical Hematology: Theory and Procedures has been written primarily for the undergraduate student in a clinical laboratory science curriculum. This text is intended to fulfill the needs of both medical laboratory technology (CLT) and medical technology (CLS) students and their instructors for a concise and understandable written presentation of the theory and practice of hematology and coagulation. Students and practitioners in other allied health disciplines, nursing, and medicine can utilize the text as a basic reference book.

The purpose of this book is to introduce students to fundamental concepts in hematology and to directly apply this knowledge to the clinical environment. In order to correlate past learning in college biology and chemistry with this professional medical discipline, topics in cellular biology and genetics are reviewed before presenting related hematological concepts. Throughout the text an integrated approach to learning, rather than rote memorization of facts is emphasized. Case studies are presented in Chapters 3 through 9. The use of case studies enhances the integration of conceptual and factual knowledge because the student has an immediate opportunity to apply new learning to clinical examples of patient diagnosis. The reader is introduced to contemporary topics in instrumentation including the principles and use of laser technology in hematology. New areas of interest, such as hypercoagulation and the role of protein C and protein S, are presented in the chapter on blood coagulation.

In order to accommodate major student

learning styles, a variety of instructional strategies have been included in the book. A topical outline and chapter objectives are presented at the beginning of each chapter. This outline assists the student in the organization of content and may be of convenience to instructors in preparing lectures. Illustrations and diagrams are included in order to visually clarify cellular descriptions or conceptual themes. An additional feature of this book is the inclusion of a comprehensive set of multiple choice examination questions at the end of each chapter. Laboratory procedures have been separated from the text of the book to permit ease of use. The format of the procedures is consistent with the style recommended by the National Commission on Clinical Laboratory Standards and will familiarize the student with the type of procedures manual used in a working clinical laboratory.

Most books in the subject area of hematology are intended for the advanced rather than the beginning professional student. No attempt has been made to replace either specialty books in the field or comprehensive cell atlases. This text should provide the student with a basic foundation during a one-term course. Because the book is intended for both CLT and CLS students, some portions of the book may not be applicable to CLT students.

M.L.T.

Acknowledgments

My primary objective in writing this book was to translate technological concepts into understandable ideas, to provide current information in the discipline, and to share my experience as a medical technologist and educator. Special thanks go to those at Little, Brown who supported this sustained creative effort: Nancy L. Coon, who guided the project through the process of development, Lynne Herndon, Katherine Arnoldi, Kristina Johnson, Mark Del Franco, and Jim Fitch.

I want to thank the peer reviewers, Shirley E. Anderson, Shoreline Community College, Seattle; Thomas L. Heilman, Texas Southmost College, Brownsville; Barbara E. Martin, Northeastern University, Boston; Ann E. Neely, Medical College of Georgia, Augusta; Yasmen Simonian, Weber State College, Ogden; and Ferne Zabezensky, Phoenix College, Phoenix. I would like to thank my former students at Corning Community College as well as the Class of '86 of the Robert Packer Hospital-Guthrie Medical Center, School of Medical Technology, Sayre, Pennsylvania with whom I shared this book as a work in progress. The illustrators, John Benjamin, Judy Swimelar, Rich Howard, and Don Turgeon, enriched the book with their artistic contributions. I would also like to acknowledge the technical support offered by Dr. Ron Searcy and William Starkweather, Sigma Diagnostics; Laurie Weiss, Rose Padgett, and Michael Hickerson, Coulter Electronics, Inc.; Susan Singer, Ortho Diagnostic Systems; and Jane G. Lenahan, Organon Teknika Corp.

Finally, the year of this book's publication marks the twenty-fifth anniversary of my graduation from Presbyterian–St. Luke's Hospital, School of Medical Technology, Chicago, Illinois. My thanks to the faculty who introduced me to the profession of medical technology.

Contents

An Introduction to Hematology

Chapter 1

Objectives

At the conclusion of this chapter, the reader will be able to:

I. An overview of the hematology laboratory
- Describe the role of the medical technologist or medical technician in providing quality patient care.
- List at least four functions of the hematology laboratory.
- Explain the purpose and contents of a laboratory safety manual.
- Describe five essential safety practices.
- Explain the basic techniques in the prevention of disease transmission.
- Name two essential components of the laboratory policies document.
- Describe the contents of the laboratory procedure manual.

II. Blood collection supplies and equipment
- Name the major potential type of error in specimen collection.
- Name the three anticoagulants most commonly used in hematology and briefly explain their mode of action.
- Compare the color codes of evacuated tubes with the additive contained in the tube.
- Describe the equipment used for venous blood collection.

III. Blood collection techniques
- Describe the proper technique for the collection of a venous blood specimen.
- Name and describe the solutions to eight typical phlebotomy problems.
- Describe the proper technique for the collection of a capillary blood specimen.

IV. Preparation of a blood smear
- Describe the procedure for preparing a push-wedge blood smear.
- List the characteristics of a good push-wedge smear.
- Explain the factors that influence the preparation of a high-quality push-wedge blood smear.
- Describe the coverslip method of blood film preparation.

V. Special collection procedures
- Describe the purpose and use of the Unopette system.
- Name the appropriate sites for

bone marrow aspiration in adults and children.
- Explain the proper technique for preparing bone marrow specimens.

VI. Routine staining of peripheral blood films
- Explain the principle of the Wright stain.
- Cite the reasons Romanowsky-type stains produce too red or too blue an appearance upon microscopic examination of blood cells.
- Describe the manual procedure of the Wright stain, including sources of error in the technique.

VII. Quality assurance in the hematology laboratory
- List and explain the eight essential nonanalytical factors in quality assurance.
- Define the terms used in describing quality assurance.
- Name the five functions of a quantitative quality control program.
- Define a variety of basic statistical terms.
- Describe the terms and state the formulae for the standard deviation, coefficient of variation, and Z score.
- Describe the use of a Levey-Jennings quality control chart.
- Explain the three types of changes that can be observed in a quality control chart.
- Briefly describe computer-based control systems.

VIII. Using statistical data in the hematology laboratory
- Explain the various methods for organizing frequency distributions.
- Name and describe two applications of histograms.

An Overview of the Hematology Laboratory

Hematology, the study of blood, is a basic medical science. In this discipline, the fundamental concepts of biology and chemistry are applied to the medical diagnosis and treatment of various disorders or diseases related to the blood. The field of hematology includes the study of the appearance and physiological activities of the cellular elements of the bone marrow and circulating blood and organs (e.g., lymph nodes). The study of blood coagulation is included in the field of hematology.

Functions of the Hematology Laboratory

Medical technologists (clinical laboratory scientists) and medical laboratory technicians (clinical laboratory technicians) play a major role in patient care. The results of assays and examinations performed by technologists and technicians in the hematology laboratory can

1. Confirm a physician's clinical impression of a possible hematological disorder
2. Establish a diagnosis or rule out a diagnosis
3. Detect an unsuspected disorder
4. Monitor the effects of radiation or chemotherapy

Although the complete blood count (CBC) is the most frequently requested procedure, the technologist or technician must be familiar with the theory and practice of a wide variety of automated and manual tests performed in the laboratory in order to provide quality patient care.

The clinical laboratory is a unique environment. To enable the reader to become acquainted with the laboratory, in which the goal is excellence in patient care through accurate, efficient, and cost-effective testing, general topics as well as blood collection and preparation procedures are presented in this chapter. Medical terms used in this text are defined in the Glossary (Appendix A), and basic medical terminology information is presented in Appendix B.

Important Laboratory Practices and Documents

LABORATORY SAFETY

The clinical medical laboratory can be a dangerous place. The purpose of safety guidelines is to protect laboratory personnel and patients.

Each laboratory *must* have an up-to-date safety manual. This manual should contain a comprehensive listing of acceptable practices and precautions. Specific regulations that conform to current state and federal requirements such as the Occupational Safety and Health Administration (OSHA) regulations must be included in the manual. Other sources of mandatory and voluntary standards include the Joint Commission on Accreditation of Hospitals (JCAH), the College of American Pathologists (CAP), and the Centers for Disease Control (CDC) guidelines.

In addition to the safety practices common to all laboratory situations, such as the proper storage of flammable materials, certain procedures are *mandatory* in a medical laboratory. Proper procedures for handling and disposing of toxic, radioactive, or potentially carcinogenic materials must be included in the safety manual. Information regarding the hazards of particular substances must be included as a safety practice and to comply with the legal right of workers to know about the hazards associated with these substances.

In the clinical laboratory, it is particularly important to safely handle and dispose of specimens. All specimens should be handled as potentially hazardous. *Essential* safety practices in the hematology laboratory include the following:

1. Implement universal blood and body fluid precautions for all patients. Gloves should be worn for performing venipunctures and capillary blood collections and for processing specimens. Gloves should be properly discarded and hands washed after completion of each individual venipuncture or following specimen handling. Masks, protective eyewear, and aprons or gowns should be worn during procedures that are likely to generate droplets of blood or other body fluids.
2. Properly dispose of needles in a puncture-proof container used solely for such disposal. These containers should be as close as practical to the use area. Needles should *not* be bent, reinserted into their original plastic sheath, removed by hand from syringes, or otherwise manipulated by hand because this can produce an accidental needle wound. The most significant occupational hazard to laboratory personnel is exposure to hepatitis B virus due to accidental needle sticks!
3. Immediately report all accidents such as needle punctures or glassware cuts to the supervisor. Allow this type of wound to bleed freely and then rinse with alcohol.
4. Wipe off the outside of containers that are visibly contaminated with blood or body fluids with a 1:10 dilution of household bleach before handling.
5. All work areas should be kept clean and well organized. Clean up spilled specimens or chemicals immediately. It is equally important to clean and disinfect work areas frequently during the work day as well as before and after the work day. The World Health Organization (WHO) recommends the use of a strong chlorine solution containing 10,000 parts per million of available chlorine for the cleanup of blood spills and for routine cleaning of work areas. Food and drinks should not be stored or consumed in the same work areas or refrigerators as specimens.
6. Slowly and carefully open rubber-stoppered test tubes in order to minimize direct droplet contact. Tubes should be pointed away from the face as well as from other workers.
7. Use auto-dilutors or safety bulbs for pipetting. Pipetting *by mouth* of any clinical material must be strictly forbidden.
8. Use a sodium azide-free diluent in cell-

counting systems requiring an isotonic diluent. Several violent explosions have occurred due to the waste disposal hazard associated with sodium azide. These explosions occurred in plumbing containing brass, copper, lead, or alloy metals that react with sodium azide to form heavy metal azides; these azides are unusually sensitive to mechanical shock, which can occur during maintenance procedures such as clearing a clogged drain.

9. Store xylene and other volatile chemicals in an adequately ventilated area or directly under a laboratory hood. Store alcohol in metal safety cans. Care must be taken to store and use acetone away from an open flame.
10. Decontaminate nondisposable equipment, such as counting chambers used for spinal fluid cell counts, by soaking overnight in a strong disinfectant solution and rinse with methyl alcohol and water before reuse. Disposable glassware or supplies that have come in contact with blood should be autoclaved or incinerated.

Prevention of Disease Transmission

Implementation of the CDC universal blood and body fluid precautions for *all* patients eliminates the need for separate isolation procedures for patients known or suspected to be infected with blood-borne pathogens. The application of universal blood and body fluid precautions also eliminates the need for warning labels on specimens. This policy maintains that all patients should be considered as potentially infectious.

Adherence to infection-control precautions, such as wearing gloves, and so forth, minimizes the risk of exposure to diseases transmitted by blood or other body fluids. Proper disposal of gloves and other potentially contaminated items, washing of hands after completing laboratory activities, and removal of laboratory coats before leaving the laboratory should reduce disease transmission.

Additional isolation precautions should be used as necessary if associated conditions, such as infectious diarrhea or tuberculosis, are diagnosed or suspected. In some cases, reverse isolation technique is used in order to protect the patient (e.g., burn victims) from infectious agents. Specific isolation techniques are also employed in the nursery because newborn and premature infants are at a high risk of infection.

Laboratory Policies

Laboratory policies should be included in a laboratory reference manual that is available to all hospital personnel. This manual should contain information regarding patient preparation for laboratory tests. Approved policies regarding the reporting of abnormal values should be clearly stated in this document.

Laboratory Procedures

The procedure manual should be a current and complete document of laboratory procedures, including safety rules and approved policies for the reporting of results. The laboratory procedure manual should detail each procedure performed in the hematology laboratory. This manual should comply with the National Commission for Clinical Laboratory Standards (NCCLS) format standards for a procedure manual. Minimally, the manual should include the name of the test method; the principle of the test and its clinical applications; specimen collection and storage; quality control; special chemical reagents, equipment, or supplies; the procedural protocol; normal values; and technical sources of error. The procedural format found in Chapter 10 of this text follows these guidelines.

In order to support a quality control program, methods for documenting laboratory results should be included in the procedure manual. Proper documentation ensures that control specimens have been properly monitored.

Blood Collection Supplies and Equipment

A properly collected blood specimen is essential to quality performance in the laboratory. Strict adherence to the rules of specimen collection is critical to the accuracy of any test. Identification errors, either of the patient or of the specimen, are major potential sources of error.

For hematological studies, anticoagulated blood is the type of specimen most frequently used. When fresh whole blood is mixed with substances that prevent blood clotting, *anticoagulants*, the blood can be separated into *plasma*, a straw-colored fluid, and the cellular components: erythrocytes, leukocytes, and platelets (thrombocytes). Whole blood that is allowed to clot normally produces the straw-colored fluid *serum*.

Anticoagulants

Three types of anticoagulants are commonly used in the hematology laboratory: tripotassium ethylenediamine tetraacetate (EDTA), heparin, and sodium citrate. Each of the anticoagulant types prevents the coagulation of whole blood in a specific manner. The proper proportion of anticoagulant to whole blood is important in order to avoid the introduction of errors into test results. The specific type of anticoagulant needed for a procedure should be stated in the laboratory procedure manual.

Another anticoagulant, ammonium-potassium oxalate (double oxalate), is no longer in general use in the hematology laboratory. Among the disadvantages of ammonium-potassium oxalate is the fact that it rapidly causes crenation of erythrocytes and vacuolization or distortion of leukocytes on blood smears.

EDTA (K3 EDTA) is tripotassium ethylenediamine tetraacetate. It may be referred to as Versene or Sequestrene. EDTA is used in concentrations of 1.5 mg per 1 mL of whole blood. The mode of action of this anticoagulant is that it removes ionized calcium (Ca^{2+}) through a process referred to as chelation. This process forms an insoluble calcium salt that prevents blood coagulation. EDTA is the most commonly used anticoagulant in hematology for tests such as the CBC or any of its component tests (hemoglobin, packed cell volume – microhematocrit, total leukocyte count, and leukocyte differential count), and platelet count. The proper ratio of EDTA to whole blood is important because some test results will be altered if the ratio is incorrect. Excessive EDTA produces shrinkage of erythrocytes, thus affecting tests such as the manually performed packed cell volume – microhematocrit.

Heparin is used as an anticoagulant in a concentration of 0.2 mL of saturated heparin per 1 mL of whole blood. It acts as an antithrombin, or substance that inactivates the blood clotting factor thrombin. This inactivation of thrombin is caused by the interaction of heparin with the antithrombin III molecule, which can inhibit the action of thrombin and the subsequent formation of blood clots. Heparin is the preferred anticoagulant for the osmotic fragility test and is used to coat micro (capillary blood) collection tubes. It is an inappropriate anticoagulant for many hematological tests, including Wright-stained blood smears, because the smear will stain too blue.

Sodium citrate in the concentration of a 3.2% solution has been adopted by the International Committee for Standardization in Hematology and the International Society for Thrombosis and Hemostasis as the appropriate concentration. Tubes containing 3.2% trisodium citrate *replace* those with 3.8% solution previously used for coagulation studies. Sodium citrate removes calcium from the coagulation system by precipitating it into an unusable form. This anticoagulant is used for many coagulation assays and for the Westergren erythrocyte sedimentation rate (ESR). The correct ratio of one part anticoagulant to nine parts of whole blood in blood collection tubes is critical. An excess of anticoagulant can alter the expected dilution of blood and produce errors in the results. Because of the dilution of anticoagulant to blood, sodium citrate is generally unacceptable for most other hematology tests.

Blood Collection Equipment

Evacuated Blood Collection Tubes

Evacuated tubes are the most widely used system for collecting venous blood samples. This