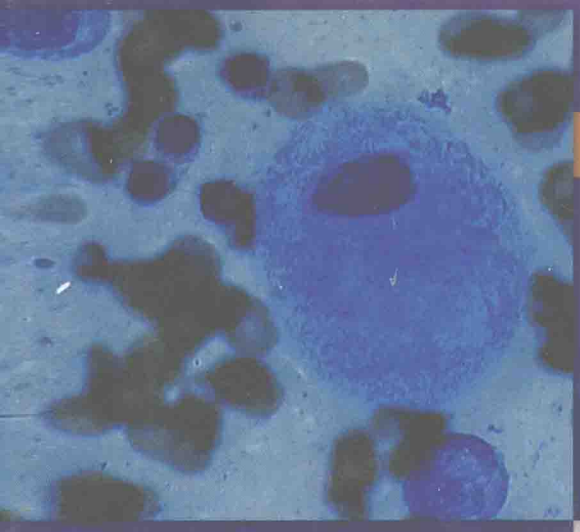
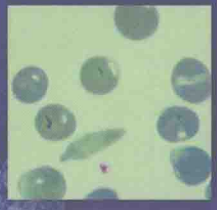
# Clinical Hematology

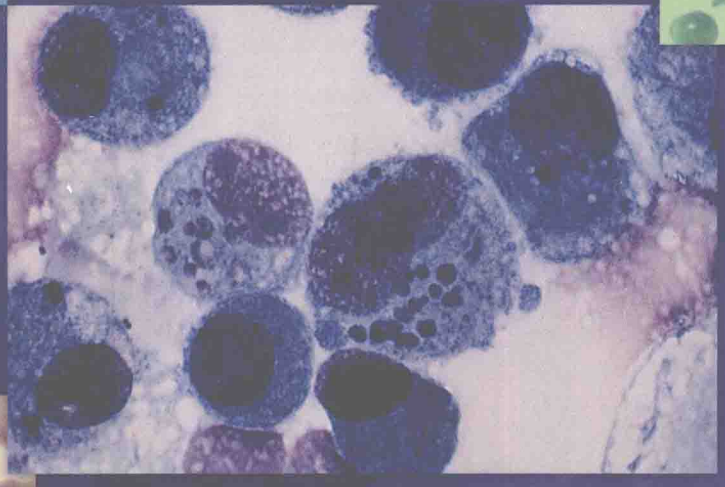
## THEORY & PROCEDURES

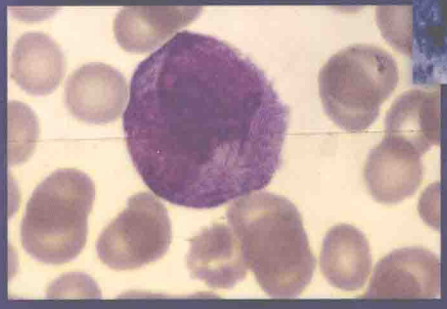
FIFTH EDITION

Mary Louise Turgeon









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## Theory and Procedures

FIFTH EDITION

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Fifth Edition


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Printed in China

**Library of Congress Cataloging-in-Publication Data**
Turgeon, Mary Louise.
Clinical hematology : theory and procedures / Mary Louise Turgeon. — 5th ed.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-60831-076-0
1. Hematology. I. Title.
[DNLM: 1. Hematologic Diseases. 2. Hematology—methods. WH 100]
RB145.T79 2010
616.1'5—dc22

2010031295

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*Namaste*
*To my husband, Dick Mordaunt*
*May we continue to fulfill our dreams of*
*adventure and learning*

# PREFACE

It is a pleasure to author the 5th edition of *Clinical Hematology*. Since the 1st edition was published in 1988, each edition has included exciting changes in clinical hematology and posed challenges to learn more and teach more in a fixed time frame. The 5th edition retains the pedagogy that set the standard for clinical laboratory science textbooks since it was introduced in the 1st edition. *Clinical Hematology* now features integrated four-color images, tables, and boxes throughout the book for ease of learning. New online ancillaries include PowerPoint presentations, a quiz bank for students, and more than 800 unique test questions for instructors (see Additional Resources, below, for more information).

Each chapter in this edition capitalizes on the strengths of previous editions; up-to-date information presented at conferences and published in the professional literature; and comments received from students, faculty, faculty reviewers, and working professionals from around the globe. *Clinical Hematology* has been classroom and laboratory "field tested" by medical laboratory technician (MLT) and medical laboratory science (MLS) students, instructors, and the author. Hands-on presentation of the information and techniques discussed in *Clinical Hematology* underscores the importance of clarity, conciseness, and continuity of information for the entry-level student. Sole authorship of this textbook ensures a smooth transition from chapter to chapter without unnecessary redundancy or changes in writing style.

## THE AUDIENCE

*Clinical Hematology,* 5th edition, is primarily intended to fulfill the needs of medical laboratory science (MLS) and medical laboratory technician (MLT) students and faculty as a time-tested book. MLT students may omit some portions of the book depending on the length of the curriculum. Other health professionals can use the book as an instructional or reference guide.

## WHAT IS NEW IN THIS EDITION

The 5th edition continues with the innovative expansion of exciting molecular discoveries that assumed importance in the 4th edition—for example, p53 function in DNA repair and mechanisms of apoptosis. The book includes knowledge recognized by the Nobel Prize in Physiology or Medicine in 2009 for discoveries of telomere structure and maintenance and covers other genetic irregularities relevant to the pathophysiology and treatment of hematologic disease—for example, genetic abnormalities leading to ribosome dysfunction in Diamond-Blackfan anemia and genetic abnormalities in Fanconi anemia. The expansion of classifications found in the recent World Health Organization Classifications appears in this edition. The treatment of many hematology disorders, particularly effective therapy for chronic myelogenous leukemia and chronic lymphocytic leukemia, clearly focuses research on understanding the molecular aspects of diagnosis and treatment of many other blood disorders.

Numerous new discoveries associated with red blood cells have been reported since the 4th edition. New discoveries are related to diagnosis and treatment of hemoglobin defects—that is, hemoglobinopathies. This information has a direct application to the laboratory, where the importance of global population migration creates new or an increased number of patients with disorders that were not commonly seen in clinical hematology before. In addition, this book describes exciting discoveries in iron metabolism and the relationship of iron physiology to anemia of chronic disorders.

Beginning with the 1st edition of *Clinical Hematology*, safety has been an important consideration. The 5th edition covers the latest safety information associated with the importance of immune status—that is, screening and recommended vaccinations of employees, and proper removal of disposable gloves. ISO 15189, quality and preanalytical error management issues, and a Spanish-English Phlebotomy guide (see Appendix D) are also included. The newest specimen-related information in this edition includes additional types of evacuated tubes, environmental factors that influence evacuated tubes, order of draw of multiple evacuated tubes collection, and order of draw of capillary specimens.

Hematology instrumentation continues to expand the menu of available assays. This edition presents the latest comparative instrument product information for cell counting and identification, and blood coagulation testing. The manual procedures chapter (Chapter 26) has been streamlined, with older techniques moved to a web-based repository. The format of the procedures continues to comply with Clinical Laboratory Standards Institute (CLSI) standards. The 1st edition of this book was the first clinical laboratory science textbook to institute standardization of procedures using the CLSI protocol.

## ORGANIZATIONAL PHILOSOPHY

The six-part organization of *Clinical Hematology* follows the original profile for a logical combination of textbook, cellular morphology atlas, and procedure manual. Part 1, The Principles of Hematology, discusses the newest fundamental concepts including safety, quality assessment, and specimen collection. Chapter 3, Molecular Genetics and Cellular Morphology, continues to be of extreme importance in understanding the pathophysiology and diagnosis of many

blood disorders and related therapy. The last chapter in this part, Chapter 4, presents the normal development of blood cells in humans. This is essential basic information.

Parts 2 and 3 of *Clinical Hematology* focus on erythrocytes and leukocytes, respectively. The content of the chapters in each of these parts progresses from normal structure and function to specific abnormalities in each grouping.

In Part 4, Additional Groups of Clonal Disorders, is in focus. Each of the two chapters investigates multiple disorders that share a common clonal origin.

Part 5, Principles and Disorders of Hemostasis and Thrombosis, presents a distinct specialty in hematology: blood coagulation. An abundance of new knowledge about platelets and coagulation factors continues to emerge.

The final part, Part 6, focuses on hematological analysis. This section includes diversified types of analysis including body fluid analysis, manual procedures, and instrumentation. This part is conveniently located at the end of the book for easy reference when reading other parts of the book.

Handy appendices include answers to review questions, medical terminology basics, SI units, a list of English-Spanish medical phrases for the phlebotomist, the newest evacuated tube pictorial directory, and a sample Material Safety Data Sheet (MSDS). A glossary at the end of the book defines all the key words bolded throughout the text.

## CHAPTER STRUCTURE AND FEATURES

Each chapter of *Clinical Hematology* provides the following elements to enhance the usability of the text:

- **Learning objectives** provide a quick overview of the content to be covered.
- **Case studies** reinforce concepts with real-world applications.
- **Procedure boxes** provide step-by-step information for key processes.
- **Key terms** that emphasize important concepts are italicized and defined in the end-of-book glossary.
- **Review questions** reinforce the student's understanding of key concepts and aid in test preparation.
- **Chapter highlights** enable a quick review of material learned in each chapter.

## ADDITIONAL RESOURCES

*Clinical Hematology* includes additional resources for both instructors and students that are available on the book's companion Web site at http://thePoint.lww.com/Turgeon5e.

### Instructor Resources

Approved adopting instructors will be given access to the following additional resources:

- Two test banks—one contains more than 800 unique questions; the other contains all the review questions from the book
- PowerPoint slides for each chapter
- An image bank of all the figures and tables in the book

### Student Resources

Students who have purchased *Clinical Hematology,* 5th edition have access to the following additional resources:

- A quiz bank of 270 questions
- A lab manual of additional procedures

In addition, purchasers of the text can access the searchable Full Text On-line by going to the *Clinical Hematology* Web site at http://thePoint.lww.com/Turgeon5e.

## ACKNOWLEDGMENTS

My objective in writing *Clinical Hematology,* 5th edition, continues to be to share basic scientific concepts, procedural theory, and clinical applications with fellow teachers and students. Because the knowledge base and technology in hematology continues to expand, writing and revising a book that addresses the need of teachers and students at multiple levels in the clinical sciences continue to be a challenge. In addition, this book continues to provide me with the opportunity to learn and share my working and teaching experience, and insight as an educator, with others.

Special thanks to John Goucher for initiating the project and to Meredith Brittain for her organizational efforts in the process of turning the manuscript into a four-color book. An additional thank you is extended to Christine Selvan and her team at SPi for their excellent performance in the preparation of the manuscript for publication.

Comments from instructors and students are welcome at m.turgeon@neu.edu.

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# CONTENTS

PART ONE

# The Principles of Hematology

CHAPTER 1

# Safety and Quality in the Hematology Laboratory

## OBJECTIVES

### An overview of the hematology laboratory

- Explain the role of the hematology laboratory staff in providing quality patient care.
- List five basic functions of the hematology laboratory.

### Safety in the hematology laboratory

- Explain the basic techniques in the prevention of disease transmission.
- Compare the features of general safety regulations governing the clinical laboratory, including components of the Occupational Safety and Health Administration (OSHA)-mandated plans for chemical hygiene and for occupational exposure to bloodborne pathogens, and the importance of the laboratory safety manual.
- List and describe the basic aspects of infection control policies and practices, including how and when to use personal protective equipment or devices (e.g., gowns, gloves, goggles), and the reasons for using standard precautions.
- Explain the purpose and correct procedure of handwashing.
- Describe the contents of the laboratory procedures manual.

### Quality Assessment and quality control in the hematology laboratory

- Summarize the essential nonanalytical factors in quality assessment.
- Briefly describe computer-based control systems.
- Define terms used in quality control and basic statistical terms.
- Describe the basic terms and state the formulas for the standard deviation, coefficient of variation, and *z* score.
- Describe the use of a Levey-Jennings quality control chart.
- Compare three types of changes that can be observed in a quality control chart.
- Explain the most frequent application of a histogram.

## AN OVERVIEW OF THE HEMATOLOGY LABORATORY

Hematology, the discipline that studies the development and diseases of blood, is an essential medical science. In this field, the fundamental concepts of biology and chemistry are applied to the medical diagnosis and treatment of various disorders or diseases related to or manifested in the blood and bone marrow.

### The Study of Hematology

Basic procedures performed in the hematology laboratory, such as the complete blood cell count (CBC), which includes the measurement and examination of red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes), and the erythrocyte sedimentation rate (ESR), frequently guide the primary care provider in establishing a patient's differential diagnosis. Molecular diagnostics, flow cell cytometry, and digital imaging are modern techniques that have revolutionized the laboratory diagnosis and monitoring of many blood disorders, for example, acute leukemias and inherited blood disorders. The field of hematology encompasses the study of blood coagulation—hemostasis and thrombosis.

### Functions of the Hematology Laboratory

Medical laboratory scientists, medical laboratory technicians, laboratory assistants, and phlebotomists employed in the hematology laboratory play a major role in patient care.

The assays and examinations that are performed in the laboratory can do the following:

- Establish a diagnosis or rule out a diagnosis
- Confirm a physician's clinical impression of a possible hematological disorder

- Detect an unsuspected disorder
- Monitor the effects of therapy
- Detect minimal residual disease following therapy

Although the CBC is the most frequently requested procedure, a laboratory professional must be familiar with the theory and practice of a wide variety of automated and manual tests performed in the laboratory to provide quality patient care. Continuing education is a necessity to keep up with continually changing knowledge and instrumentation in the field.

## SAFETY IN THE HEMATOLOGY LABORATORY

The practice of safety should be uppermost in the mind of all persons working in a clinical hematology laboratory. Accidents do not just happen; they are caused by carelessness, lack of attention to detail, or lack of proper communication. Most laboratory accidents are preventable by exercising good technique, staying alert, and using common sense.

Safety standards for patients and clinical laboratories are initiated, governed, and reviewed by governmental agencies and professional organizations (see Box 1.1). The Joint Commission (www.jointcommission.org) has established National Patient Safety Goals. One of the goals of particular interest to laboratory professionals addresses the issue of critical laboratory assay values, the high and low boundaries of the life-threatening values of laboratory test results (see "Quality Assessment in the Hematology Laboratory"). Urgent clinician notification of critical results is the responsibility of the laboratory.

### The Safety Officer

A designated safety officer is a critical part of a laboratory safety program. This individual has many duties affecting staff including compliance with existing regulations affecting the laboratory and staff, for example, labeling of chemicals and providing supplies for the proper handling and disposal of biohazardous materials.

**BOX 1.1**

#### Safety Agencies and Organizations

- U.S. Department of Labor's Occupational Safety and Health Administration (OSHA)
- Clinical and Laboratory Standards Institute (CLSI)
- CDC, part of the U.S. Department of Health and Human Services (DHHS), Public Health Service
- College of American Pathologists (CAP)
- The Joint Commission (The Joint Commission on Accreditation of Healthcare Organizations)

### Occupational Safety and Health Administration Acts and Standards

To ensure safe and healthful working conditions for workers, the US federal government created a system of safeguards and regulations under the Occupational Safety and Health Act of 1970. In 1988, the Act expanded the Hazard Communication Standard to apply to hospital staff. The programs deal with many aspects of safety and health protection and places responsibility for compliance on management and employees.

The Occupational Safety and Health Administration (OSHA) standards include provisions for warning labels or other appropriate forms of warning to alert all workers to potential hazards, suitable protective equipment, exposure control procedures, and implementation of training and education programs. The primary purpose of OSHA standards is to ensure safe and healthful working conditions for every US worker.

OSHA and the Centers for Disease Control and Prevention (CDC) have published numerous safety standards and regulations that are applicable to clinical laboratories (e.g., 1988 OSHA Hazard Communication Standard). Ensuring safety in the clinical laboratory includes the following measures:

- A formal safety program
- Specifically mandated plans (e.g., chemical hygiene, bloodborne pathogens)
- Identification of various hazards (e.g., chemical, biological)

#### Chemical Hygiene Plan

In 1991, OSHA mandated that all clinical laboratories must implement a chemical hygiene plan (CHP) and an exposure control plan. As part of the CHP, a copy of the material safety data sheet (MSDS) must be readily accessible and available to all employees at all times. This document ensures that laboratory workers are fully aware of the hazards associated with chemicals in their workplaces. The MSDS describes hazards, safe handling, storage, and disposal of hazardous chemicals. The information is provided by chemical manufacturers and suppliers about each chemical and accompanies the shipment of each chemical.

On September 30, 2009, OSHA published the long-awaited Proposed Rule to modify the Hazard Communication Standard (HCS) to conform with the United Nations' (UN's) Globally Harmonized System (GHS) of Classification and Labeling of Chemicals. OSHA has made a preliminary determination that the proposed modifications will improve the quality and consistency of information provided to employers and employees regarding chemical hazards and associated protective measures.

The proposed modifications to the chemical hazard communication (HAZCOM) standard include:

- Revised criteria for classification of chemical hazards
- Revised labeling provisions that include requirements for use of standardized signal words, pictograms, hazard statements, and precautionary statements
- A specified format for safety data sheets (currently known as material safety data sheets)

- Related revisions to definitions of terms used in the standard and requirements for employee training on labels and safety data sheets

OSHA is also proposing to modify provisions of a number of other standards, including standards for flammable and combustible liquids, process safety management, and most substance-specific health standards, to ensure consistency with the modified HCS requirements. OSHA currently anticipates a 2-year phase-in period for new hazard communication training requirements and a 3-year phase-in period for overall implementation once the Final Rule is published.

### "Right to Know" Laws

Legislation on chemical hazard precautions, such as state "right to know" laws, and OSHA document 29 CFR 1910 set the standards for chemical hazard communication (HAZCOM) and determine the types of documents that must be on file in a laboratory. For example, a yearly physical inventory of all hazardous chemicals must be performed, and MSDSs should be made available in each department for use. Each institution should also have at least one centralized area where all MSDSs are stored.

### Occupational Exposure to Bloodborne Pathogens

The OSHA-mandated program, Occupational Exposure to Bloodborne Pathogens, became law in March 1992. This regulation requires that laboratories develop, implement, and comply with a plan that ensures the protective safety of laboratory staff to potential infectious bloodborne pathogens, hepatitis B virus (HBV), and human immunodeficiency virus (HIV). The law further specifies the rules for managing and handling medical waste in a safe and effective manner.

The CDC also recommends safety precautions concerning the handling of all patient specimens, known as standard precautions. The CLSI has also issued guidelines for the laboratory worker in regard to protection from bloodborne diseases spread through contact with patient specimens. In addition, the CDC provides recommendations for treatment after occupational exposure to potentially infectious material.

## Avoiding Transmission of Infectious Diseases

### History of Infectious Disease Prevention

The recognition of HIV-1 generated new policies from the CDC and mandated regulations by the OSHA. Current safety guidelines for the control of infectious disease are based on the original CDC publication, "Recommendations for Prevention of HIV Transmission in Health-Care Settings" (*MMWR*, Suppl 2S, 1987). Clarifications of safety practices appear in the 1988 CDC clarifications of the original guidelines (*MMWR*, 37(24), 1988); in the Department of Labor, OSHA's "Occupational Exposure to Bloodborne Pathogens": Part 1910 to title 29 of the Code of Federal Regulations, 64175–64182, (Fed Reg, 56(235), 1991); and in the U.S. Department of Health and Human Services' "Regulations for Implementing the Clinical Laboratory Improvement Amendments of 1988: A Summary" (*MMWR*, 41(RR-2), 1992). Laboratory personnel must remain alert to further updates of these policies.

The purpose of the standards for bloodborne pathogens and occupational exposure is to provide a safe work environment. OSHA mandates that an employer does the following:

- Educate and train all healthcare workers in standard precautions and in preventing bloodborne infections
- Provide proper equipment and supplies, for example, gloves
- Monitor compliance with the protective biosafety policies

HIV has been isolated from blood and body fluids, for example, semen, vaginal secretions, saliva, tears, breast milk, cerebrospinal fluid (CSF), amniotic fluid, and urine, but only blood, semen, vaginal secretions, and breast milk have been implicated in transmission of HIV to date. Recently, sperm cells themselves have been discovered to be capable of transmitting HIV. Evidence for the role of saliva in the transmission of virus is unclear, but standard precautions do not apply to saliva uncontaminated with blood.

### Preventing Occupational Transmission of HBV and HIV

Blood is the single most important source of HIV, HBV, and other bloodborne pathogens in the occupational setting.

#### Needlestick Prevention

The CDC estimates that more than 380,000 needlestick injuries occur in US hospitals each year; approximately 61% of these injuries are caused by hollow-bore devices. Blood is the most frequently implicated infected body fluid in HIV and HBV exposure in the workplace.

An occupational exposure is defined as a percutaneous injury, for example, needlestick or cut with a sharp object, or contact by mucous membranes or nonintact skin (especially when the skin is chapped, abraded, or affected with dermatitis), or the contact is prolonged or involves an extensive area with blood, tissues, blood-stained body fluids, body fluids to which standard precautions apply, or concentrated virus.

Among healthcare personnel with documented occupationally acquired HIV infection, prior percutaneous exposure is the most prevalent route of infection. Certain percutaneous injuries carry a higher risk of infection. Risk of infection is greater with:

- A deep injury
- Late-stage HIV disease in the source patient
- Visible blood on the device that caused the injury
- Injury with a needle that had been placed in a source patient's artery or vein

There are a small number of instances when HIV has been acquired through contact with nonintact skin or mucous membranes (i.e., splashes of infected blood in the eye or

aerosols). The risk of infection not only varies with the type of exposure but also may be influenced by:

- Amount of infected blood in the exposure
- Length of contact with infectious material
- Amount of virus in the patient's blood or body fluid or tissue at the time of exposure

On November 6, 2000, the Needlestick Safety and Prevention Act became law. The provisions of the new law include:

- Requires healthcare employers to provide safety-engineered sharp devices and needleless system to employees to reduce the risk of occupational exposure to HIV, hepatitis C, and other bloodborne disease.
- Expands the definition of engineering controls to include devices with engineered sharps injury protection.
- Requires that exposure control plans document consideration and implementation of safer medical devices designed to eliminate or minimize occupational exposure. These plans must be reviewed and updated at least annually.
- Requires each healthcare facility to maintain a sharps injury log with detailed information regarding percutaneous injuries.
- Requires employers to solicit input from healthcare workers when identifying and selecting sharps and document process.

The good news is that most occupational exposures do not result in infection. The average risk for HIV transmission after exposure to infected blood is low—about 3 per 1,000 injuries.

### Sharps Prevention

The most widespread control measure required by OSHA and CLSI is the use of puncture-resistant sharps containers. (Fig. 1.1). The primary purpose of using these containers is to eliminate the need for anyone to transport needles and other sharps while looking for a place to discard them. Sharps containers are to be located in the patient areas as well as conveniently placed in the laboratory.


**FIGURE 1.1** Puncture-resistant sharps containers. (Courtesy of Becton Dickinson, Franklin Lakes, New Jersey.)

Phlebotomists should carry these red, puncture-resistant containers in their collection trays. Needle containers should not project from the top of the container. Use of the special sharps container permits quick disposal of a needle without recapping as well as of other sharp devices that may be contaminated with blood. This supports the recommendation against recapping, bending, breaking, or otherwise manipulating any sharp needle or lancet device by hand. Most needlestick accidents have occurred during recapping of a needle after a phlebotomy. Injuries also can occur to housekeeping personnel when contaminated sharps are left on a bed, concealed in linen, or disposed of improperly in a waste receptacle. Most accidental disposal-related exposures can be eliminated by the use of sharps containers. To discard sharps, containers are closed and placed in the biohazard waste. A needlestick injury must be reported to the supervisor or other designated individual.

## Issues Related to HBV, HIV, and HCV Transmission

Medical personnel must be aware that HBV and HIV are totally different viruses. Exposure to HIV is uncommon, but cases of occupational transmission to healthcare personnel with no other known high-risk factors have been documented. Although HIV is an unlikely work-related hazard, it cannot be underrated because it can be fatal. The most feared hazard of all, the transmission of HIV through occupational exposure, is among the least likely to occur, if proper safety practices are followed. The transmission of HBV can also be fatal and is more probable than transmission of HIV.

HBV can be present in extraordinarily high concentrations in blood, but HIV is usually found in lower concentrations. HBV may be stable in dried blood and blood products at 25°C for up to 7 days. HIV retains infectivity for more than 3 days in dried specimens at room temperature and for more than 1 week in an aqueous environment at room temperature.

### HBV Vaccination

Before the advent of the hepatitis B vaccine, the leading occupationally acquired infection in healthcare workers was hepatitis B. Although the number of cases of hepatitis B in healthcare workers has sharply declined since hepatitis B vaccine became widely available in 1982, approximately 800 healthcare workers still become infected with HBV each year following occupational exposure. The likelihood of infection after exposure to blood infected with HBV or HIV depends on additional factors:

1. Concentration of HBV or HIV; viral concentration is higher for HBV than for HIV.
2. Presence of skin lesions or abrasions on the hands or exposed skin of the healthcare worker.
3. Immune status of the healthcare worker for HBV.

OSHA issued a federal standard in 1991 mandating employers to provide the hepatitis B vaccine to all employees who have or may have occupational exposure to blood or other potentially infective materials. The vaccine is to be offered at no expense to the employee, and if the employee refuses the vaccine, a declination form must be signed.