

Jean Jorgenson Linné and Karen Munson Ringsrud

BASIC TECHNIQUES FOR THE MEDICAL LABORATORY

Second Edition

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Jean Jorgenson Linné, B.S., M.T. (ASCP)

Karen Munson Ringsrud, B.S., M.T. (ASCP)

DEPARTMENT OF LABORATORY MEDICINE AND PATHOLOGY
UNIVERSITY OF MINNESOTA

Second Edition

McGraw-Hill Book Company • New York • St. Louis • San Francisco • Auckland
Bogotá • Düsseldorf • Johannesburg • London • Madrid • Mexico • Montreal • New Delhi
Panama • Paris • São Paulo • Singapore • Sydney • Tokyo • Toronto

BASIC TECHNIQUES FOR THE MEDICAL LABORATORY

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1 2 3 4 5 6 7 8 9 0 D O D O 7 8 3 2 1 0 9 8

This book was set in Press Roman by Hemisphere Publishing Corporation. The editor was Stuart D. Boynton; the designer was Hermann Strohbach; the production supervisor was Milton J. Heiberg.

Library of Congress Cataloging in Publication Data

Linné, Jean Jorgenson.

Basic techniques for the medical laboratory.

First ed. (c1970) published under title: Basic laboratory techniques for the medical laboratory technician.

Bibliography: p.

1. Diagnosis, Laboratory—Laboratory manuals.

I. Ringsrud, Karen Munson, joint author. II. Title.

[DNLM: 1. Technology, Medical. QY25.3 L578b]

RB37.L67 1979 616.07'5 78-12209

ISBN 0-07-037948-3

TO

David and Peter — our husbands

David, Erik, and Jonathan — our children

PREFACE TO THE SECOND EDITION

The first edition of this text, "Basic Laboratory Techniques for the Medical Laboratory Technician," has met with favorable response. Because of this success, and in order to update and to generally improve and enhance the material covered in the first edition, this second edition was written. The goals of the book remain the same as those for the first edition.

Significant advances in laboratory medicine have demanded the rather extensive revision seen in the second edition. Much of the information contained in the first edition remains unchanged, but a significant amount of information has been added, including new chapters covering serology, hemostasis and coagulation, examination of extravascular fluid, and examination of the feces. One chapter on basic metabolic rate and electrocardiography tests has been deleted.

It is anticipated that this basic general information will be applicable to persons at various levels of clinical laboratory training. As in the first edition, the clinical laboratory specialties of hematology, urinalysis, and chemistry are stressed, and these chapters have been expanded considerably.

The reader will notice that a change in the title of the textbook has also been made. The new title, "Basic Techniques for the Medical Laboratory," is less cumbersome and conveys the general intent of the second edition.

Several new illustrations have been added to enhance the written word. Photomicrograph reproductions of various blood cells and urinary sediment structures have also been included to

visually describe these common microscopic findings.

Since publication of the first edition of this text, the authors have been involved in the teaching of basic laboratory techniques to medical students at the University of Minnesota under the direction of Dr. G. Mary Bradley. We want to thank Dr. Bradley for her inspiration, practical knowledge, and clinical expertise, which we hope has been conveyed in this revision.

In the preparation of this second edition, the authors were assisted once again by many interested persons in the Department of Laboratory Medicine and Pathology at the University of Minnesota. We extend another word of thanks to Dr. Ellis S. Benson, professor and head of the Department of Laboratory Medicine and Pathology, for his continued support. In addition to those persons mentioned in the preface to the first edition, we wish to thank Dr. G. Mary Bradley, Sandra Carter, Marilyn Cavanaugh, Helen Hallgren, Mary Damron, Dolores Harvey, Grace Mary Ederer, Patricia Johnson, and Karen Viskochil.

A special thanks to Ruth Hovde and Verna Rausch who originated the medical laboratory assistant course at the University of Minnesota. Without their foresight, this textbook would never have been written.

Several new illustrations have been added, thanks to the fine artwork of Mr. Martin Finch and Mrs. Linda Richter. We want to thank Drs. Robert McKenna and Richard Brunning for their advice in preparing several of the photomicro-

graphs of blood cells and the urinary sediment. A very special thanks to Peter Ringsrud for the many days he spent in the darkroom processing these photomicrographs in addition to his photography, which is included in the text. Thanks also to Drs. Patrick Ward and G. Mary Bradley and to the University of Minnesota Medical School for permission to use several photomicrographs of the urinary sediment from slide collections of the Department of Laboratory Medicine and Pathology.

We also wish to thank Dorothy Lekson and her

fellow instructors in the medical laboratory assistant course at the St. Paul Technical Vocational Institute for their helpful cooperation and suggestions during the writing of this second edition.

Revising a textbook requires a great deal of time. The authors wish to thank their families—husbands and children—for their patience and support throughout the revision process, which was a time of personal disruption. Erik and Peter Ringsrud and Jonathan, David, and David Linné were a continual source of encouragement and survival.

Jean Jorgenson Linné
Karen Munson Ringsrud

PREFACE TO THE FIRST EDITION

In the modern medical laboratory a wide variety of analyses ranging from simple to complex are utilized by the physician and are essential to him in the management of his patients. Laboratory personnel, to qualify for such work, require specific training and education. Usually the laboratory is under the direction of a medical doctor, the pathologist, who is a physician specializing in pathology, the study of disease. Under the direction of the pathologist is the medical technologist, who is educated to perform the complex laboratory procedures, to engage in teaching activities, and to handle supervisory and administrative duties. To assist the medical technologist, the medical laboratory technician is specifically trained for routine tasks.

Although the laboratory technician has proper understanding of basic fundamentals and techniques so as to be flexible in the use of his training and skills, he is not qualified to make technical and administrative decisions.

It takes a special type of person to work in a clinical laboratory. He must understand the need for accuracy, be conscientious, and above all, want to be of service to the patient.

The materials for this textbook have been developed from the lectures given students in the medical laboratory assistant program at the University of Minnesota. It is intended to provide

the student technician with basic information in the departments of the clinical laboratory where technicians will work; these departments include chemistry, hematology, urinalysis, blood banking, microbiology, electrocardiography, and basal metabolism. In addition to these chapters, a discussion of basic laboratory fundamentals is included.

The authors gratefully acknowledge the contributions of Ruth Hovde, Verna Rausch, Margaret Ohlen Hanson, Elizabeth Lundgren, Ruth Brown Anderson, Marilyn Scovil Cavanaugh, and Mary Lou Kuefner Carlson, people who formerly were associated with the instruction and the administration of the medical laboratory assistant program from which the material for this book evolved.

The authors express their appreciation to Mary Damron, Barbara Merritt, Grace Mary Ederer, Patricia Hanauer Bordewich, Donna Blazevic, Sandra Benson, and Margaret Halsted for their assistance in reviewing the various sections of the book during its preparation, and to Mr. Martin Finch for his assistance in the preparation of the illustrations.

The authors thank Dr. Ellis S. Benson, Professor and head of the Department of Laboratory Medicine, for his encouragement and support of this project.

Jean Jorgenson Linné
Karen Munson Ringsrud

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ONE

FUNDAMENTALS OF THE CLINICAL LABORATORY

The aim of this chapter is to give general information that applies to most laboratory work. The use of certain laboratory equipment (the microscope, the photometer, glassware, and the centrifuge, for example) is discussed, as well as laboratory calculations,

units of measurement, quality control programs, laboratory safety, the proper preparation of reagents, and the cleaning of laboratory glassware. Certain general techniques are also discussed, such as weighing, pipetting, and titration. The proper

collection of laboratory specimens, including the procedure for collecting blood, is covered. A knowledge of these subjects is basic information for those who engage in the major areas of laboratory work covered in the chapters that follow.

SAFETY IN THE LABORATORY

The importance of laboratory safety and correct first-aid procedures cannot be overemphasized to anyone working in the medical laboratory. Students as well as laboratory personnel should be constantly reminded of safety precautions. Most accidents do not just happen—they are caused by carelessness. For this reason, safety should be foremost in the mind of anyone involved in doing laboratory work of any kind.

Most laboratory accidents are preventable by the exercise of good technique and by the use of common sense. There are many potential hazards in the laboratory, but they can be controlled by taking simple precautions. In every medical institution, the administration supplies the laboratory with safety devices for equipment and personal

use, but it is up to the individual to make use of them. Safety is personal, and its practice must be a matter of individual desire and accomplishment. Real appreciation for safety requires a built-in concern for the other person, for an unsafe act may harm the bystander without harming the person who performs the act.

To ensure that workers have safe and healthful working conditions, the United States government created a system of safeguards and regulations under the Occupational Safety and Health Act of 1970.¹ This system touches almost every person working in the United States today. It is especially relevant to discuss the meaning of the act in any presentation concerning safety in the clinical laboratory. In this setting there are

special problems with respect to potential safety hazards, and diseases or accidents associated with preventable causes cannot be tolerated in the busy laboratory today.

The Occupational Safety and Health regulations apply to all businesses with one or more employees and are administered by the U.S. Department of Labor through the Occupational Safety and Health Administration (OSHA). The program deals with many aspects of safety and health protection, including compliance arrangements, inspection procedures, penalties for noncompliance, complaint procedures, duties and responsibilities for administration and operation of the system, and how the many standards are set. Responsibility for compliance is placed on both the administration of the institution and the employee. The maximum fine for noncompliance with the OSHA regulations is \$1000 per day.

A person who understands the potential hazards in a laboratory and knows the basic safety precautions can prevent accidents. The Occupational Safety and Health Act requires a safety program in every clinical laboratory. Identification of potential hazards is a most important part of any such program. In each department in the laboratory, the type of hazard is slightly different. However, many hazards are commonly found throughout the laboratory.

One safeguard that can be taken is to see that all containers are properly labeled. Labeling may be the simplest, single important step in the proper handling of hazardous substances. Because many hazards of the clinical laboratory are unique, a special term, *biohazard*, was devised. This word is posted throughout the laboratory to denote infectious materials or agents that present a risk or even a potential risk to the health of humans or animals in the laboratory. The potential risk can be either through direct infection or through the environment. Biological infections are frequently caused by accidental aspiration of infectious material orally through a pipet, accidental inoculation with contaminated needles or syringes, animal bites, sprays from syringes, and

centrifuge accidents. Some other sources of laboratory infections are cuts or scratches from contaminated glassware, cuts from instruments used during animal surgery or autopsy, and spilling or spattering of pathogenic samples on the work desks or floors. People working in laboratories on animal research or other research involving biologically hazardous materials are most susceptible to the problems of biohazards. The symbol shown in Fig. 1-1 is used to denote the presence of biohazards. A label for a container should include a date and the chemical contents of the container. When the contents of one container are transferred to another container, this information should also be transferred to the new container. Proper labeling of containers is discussed further under Quantitative Transfer in this chapter.

The fact that clinical laboratories present many potential hazards simply because of the nature of the work done there cannot be overemphasized; open flames, electrical equipment, glassware, chemicals of varying reactivity, flammable solvents, biohazards, and toxic fumes are but a few. One serious hazard in laboratory work is the

Fig. 1-1. Biohazard symbol.



potential for fire and explosion when flammable solvents such as ether and acetone are used. These materials should always be stored in special safety cans or other appropriate storage devices. Even with proper storage of these materials, there is always some release of flammable vapors in a working laboratory. A good ventilation system in the room and vent sites for the storage area will help to eliminate some of the potential hazard. When using flammable materials, proper precautions must be taken; for instance, flammable liquids should be poured from one container to another slowly, they should never be used when there is an open flame in the room, and they should be kept in closed containers when they are not being used.

Other sources of injury in the laboratory are poisonous, volatile, caustic, or corrosive reagents such as strong acids or bases. Chemicals and reagents can present different types of hazards. Some are dangerous when inhaled (sulfuric acid), some are corrosive to the skin (phenol), some are caustic (acetic acid), some are volatile (many solvents), and some combine these hazards. Acids and bases should be stored separately in well-ventilated storage units. When not in use, all chemicals and reagents should be returned to their storage units. Bottles of particularly volatile substances should not be left open for extended periods. Some chemicals that must be handled with care and some potential hazards in their use are:

Sulfuric acid: At a concentration above 65% may cause blindness; may produce burns on the skin; if taken orally may cause severe burns, depending on the concentration.

Nitric acid: Gives off yellow fumes that are extremely toxic and damaging to tissues; over-exposure to vapor can cause death, loss of eyesight, extreme irritation, smarting, itching, and yellow discoloration of the skin; if taken orally can cause extreme burns, may perforate the stomach wall, can cause death.

Acetic acid: Severely caustic; continuous exposure to vapor can lead to chronic bronchitis.

Hydrochloric acid: Inhalation of vapors should be avoided; any on the skin should be washed away immediately to prevent a burn.

Sodium hydroxide: Extremely hazardous in contact with the skin, eyes, and mucous membranes (mouth), causing caustic burns; dangerous even at very low concentrations; any contact necessitates immediate care.

Phenol (a disinfectant): Can cause caustic burns or contact dermatitis even in dilute solutions; wash off skin with water or alcohol.

Carbon tetrachloride: Damaging to the liver even at an exposure level where there is no discernible odor.

Trichloroacetic acid: Very severely caustic; respiratory tract irritant.

Ethers: Cause depression of central nervous system.

When using any potentially hazardous solution or chemical, protective equipment for the eyes, face, head, and extremities, as well as protective clothing or barriers, should be used. Volatile or fuming solutions should be used under a fume hood. In case of accidental contact with a hazardous solution or a contaminated substance, quick action is essential. The laboratory should have a safety shower where quick, all-over decontamination can take place immediately. Another safety device that is essential in all laboratories is a face or eye washer that streams aerated water directly onto the face and eyes to prevent burns and loss of eyesight. Any action of this sort must be undertaken immediately, so these safety devices must be present in the laboratory area.

In case of fire hazards, various types of fire-extinguishing agents must be available and their use must be understood. Fire in clothing should be smothered with a fire blanket or heavy towel; the flame should be beaten out; it should not be flooded with water. Everyone in the laboratory should know the correct use of the fire alarm.

It is a generally accepted rule that all pipetting must be done by mechanical means and not by using mouth suction. For pipetting use either

mechanical suction or aspirator bulbs. This procedure safeguards against burning the mouth with caustic reagents and against contamination by pathogenic organisms in samples. All specimens of human origin that are used in the laboratory (blood, urine, spinal fluid, stools, etc.) should be considered potentially infectious. Hepatitis B virus is a serious biohazard found in the laboratory.

One well-known virus, which causes type B viral hepatitis, can be transmitted through the blood of a patient with that virus. In hospitals where renal transplants are done, this problem is especially serious. Those most heavily exposed to blood from renal patients—for example, through accidental inoculation, ingestion of blood, or inhalation of blood aerosols while doing laboratory work on these samples—run the greatest risk of infection. Laboratories must exert every effort to make this risk factor as low as possible. This can start with prevention of contamination while the specimens are collected and delivered to the laboratory (see under Collection, Preservation, and Preparation of Laboratory Specimens). A large percentage of the specimens sent to the laboratory contain blood, and their safe collection and transportation must take top priority in any discussion of safety in the laboratory. Disposal of contaminated needles and blades used in collecting the specimens must also be done properly. For example, they may be discarded in a special wax-lined, heavy cardboard box, coded with red stripes.² When these specially labeled and coded boxes are full, they can be taped shut, marked *contaminated*, and autoclaved before being incinerated.

To further eliminate the risk of transmitting type B hepatitis virus, those working in the laboratory with blood specimens must take precautions. Washing the hands frequently is one of the most important ways of preventing contamination. At least one sink in the laboratory should be equipped with a foot pedal for operating the faucets, and it should also have a foot pedal dispenser with an antiseptic solution for the hands. Wearing disposable rubber gloves and a

special protective laboratory coat while handling blood, serum, or any biological specimen from a patient is another preventive measure. Such coats are worn only in the laboratory and never leave the laboratory except when they are specially bagged for the laundry.

More recently, attention has been drawn to chemical agents that are carcinogenic. A carcinogen is any agent that can produce cancer. Many carcinogens are not noticed in a normal inspection for hazards in the laboratory. OSHA has published a list of carcinogenic agents, which should be found in a prominent location in all laboratories.³ Where chemical carcinogens are being used, several extra precautions must be taken: protective clothing should be worn; protective equipment such as a face mask or a respirator should be worn; a shower should be taken immediately after exposure to a carcinogen; there should be no eating, drinking, or smoking in the area; no oral pipetting should be done; and all personnel should wash their hands after completing procedures involving the use of any carcinogen. If proper techniques are used, safeguards against most of the carcinogens found in the clinical laboratory are provided.

Biohazards are generally treated with great respect in the clinical laboratory. The effects of pathogenic substances on the body are well documented. The presence of pathogenic organisms is not limited to the culture plates in the microbiology laboratory. Aerosols can be found in all areas of the laboratory where human specimens are used. Substances can become airborne when the cork is popped off a blood-collecting container, a serum sample is poured from one tube to another, or a serum tube is centrifuged. Another step that should be taken to lessen the hazard from aerosols is to exercise caution in handling pipets and other equipment used to transfer human specimens, especially pathogenic materials. These materials should be discarded properly and carefully.

The preferred method for decontamination and disinfection of toxic or infectious materials is

autoclaving. Autoclaving depends on humidity, temperature, and time. Under pressure, steam becomes hotter than boiling water and kills bacteria much more quickly. Autoclaves must be used with caution.

To clean up the work area, a strong bleach solution can be used for any spills of biological materials. Desk tops can be cleaned daily with a dilute solution of bleach. Any contaminated laboratory ware that must be reused cannot be cleaned with bleach because it corrodes stainless steel containers and coagulates proteins. A strong detergent solution such as 3% phenolic detergent can be used before autoclaving. Contaminated pipets should be placed in long horizontal covered trays deep enough to minimize the chance of spilling when they are transported to the autoclave.

Shocks from the various pieces of electrical apparatus in the clinical laboratory are a common source of injury if one is not aware of the potential hazard. This may be one of the most serious hazards in the laboratory. The important thing to understand with respect to danger to the human body is the effect of an electrical current. Current flows when there is a difference in potential between two points, and this knowledge is used in determining the approach to safety in the use of electrical equipment. Grounding of all electrical equipment is essential. If there is no path to ground, such a path might be established through the human in contact with the apparatus, resulting in serious injury. Attempts to repair or inspect a disabled electrical device should be left to someone who is trained to do it.

The use of many kinds of glassware is basic to anyone working in the clinical laboratory. Caution must be used to prevent unnecessary or accidental breakage. Some types of glassware can be repaired, but most glassware used today is discarded when it is broken. Any broken or cracked glassware should be discarded in a special container for broken glass, and not thrown into the regular waste containers. Common sense

should be used in storing glassware, with heavy pieces placed on the lower shelves and tall pieces placed behind smaller pieces. Shelves should be placed at reasonable heights; glassware should not be stored out of reach. Broken or cracked glassware is the cause of many lacerations, and care should be taken to avoid this laboratory hazard.

General rules for safety in the clinical laboratory

1. Know where the fire extinguishers are located, the different types for specific types of fires, and how to use them properly.
2. Pipet *all* solutions by using mechanical suction or an aspirator bulb. Never use mouth suction.
3. Handle all flammable solvents and fuming reagents under a fume hood. Store in a well-ventilated cabinet.
4. Use an explosion-proof refrigerator to store ether. Never use ether near an open flame, as it is highly flammable.
5. Do not use *any* flammable substance near an open flame.
6. Wear gloves when handling infectious substances or toxic substances such as bromine or cyanide.
7. Mercury is poisonous. Clean up spilled mercury immediately.
8. If glass tubing is to be cut, hold the tubing with a towel to prevent cuts of the hands. This precaution also applies to putting a piece of glass tubing through a rubber stopper.
9. Use extreme caution when handling laboratory glassware. Broken glass is probably the greatest source of injury in the laboratory. Immediately discard cracked or broken glassware in a separate container, not with other waste.
10. If strong acids or bases are spilled, wipe them up immediately, using copious amounts

of water and great care. Keep sodium bicarbonate on hand to assist in neutralizing acid spillage.

11. Plainly label all laboratory bottles, specimens, and other materials. When a reagent bottle is no longer being used, store it away in its proper place.
12. Put away safely or cover any equipment that is not being used.
13. Replace covers, tops, or corks on all reagent bottles as soon as they are no longer being used. Never use a reagent from a bottle that is not properly labeled.
14. If water is spilled on the floor, wipe it up immediately. Serious injuries can result from falls caused by slipping on a wet floor.
15. Never taste any chemical. Smell chemicals only when necessary and then only by fanning the vapor of the chemical toward the nose.
16. When handling blades or needles, use extreme caution to avoid cuts and infections. Dispose of all blades and needles properly.
17. Always pour acid into water for dilution. Never pour water into acid. Pour strong acids or bases slowly down the side of the receiving vessel to prevent splashing.
18. Use caution when pipetting any specimen from a patient. Handle blood, serum, plasma, cerebrospinal fluid, urine, or any other patient specimen with care, as it may be contaminated. Severe infections and illnesses can result from handling such specimens carelessly.
19. Wash hands frequently while working in the laboratory, especially after handling patient specimens or reagents. *Always* wash hands before leaving the laboratory.
20. Wear safety goggles when preparing reagents with strong chemicals (such as the dichromate acid cleaning solution used to clean laboratory glassware, or aqua regia, another cleaning solution). Some states (e.g., Minnesota) have enacted laws that require students, teachers, and visitors in educational institutions who are participating in or observing activities in eye-protection areas (areas where work is performed that is potentially hazardous to the eyes) to wear devices to protect their eyes.
21. In case of severe fire or burns, know where the safety shower is located and how to operate it.
22. Know the location of a fire blanket, which is used to smother flames in case of fire.
23. Most hospitals and teaching institutions have some type of warning signal and a procedure to follow in the event of a fire. This procedure should be understood thoroughly by anyone working in that institution, whether as a student or an employee. Such institutions also have disaster plans, with which every worker must be thoroughly familiar.
24. When using burners and other heating devices, keep them far enough away from the working area that there is no possibility that anything will catch fire.
25. Never lean over an open flame. Extinguish flames when they are no longer being used.
26. Learn the procedure used in the laboratory for discarding hazardous substances such as strong acids and bases.
27. Never pour volatile liquids down a sink.
28. To free a frozen glass stopper, run hot water over it, tap it lightly with a towel wrapped around it, or grasp it with a rubber glove or tourniquet.
29. Wear gloves when cleaning glassware in case there is broken glass in the sink or soaking bucket.
30. If blood or another body specimen comes in contact with the mouth, spit it out immediately into the sink, rinse with mouthfuls of tap water, never swallowing but discarding each mouthful of rinse water. The most important thing is to spit out the blood or other specimen immediately without swallowing it.
31. Handle all hot objects with tongs, not hands.

Extremely hot objects are to be handled with asbestos gloves.

32. If contaminated materials such as human specimens or bacterial agents are spilled on the work area, discard the contaminated material properly and wipe off the work area with phenol, bleach solution, or another laboratory disinfectant.
33. Cover all centrifuges to avoid flying broken glass. Do not open centrifuges before they have stopped. Do not stop the centrifuge head by hand.
34. Be familiar with the Occupational Safety and Health rules and regulations and be ready for an inspection by OSHA.

Every clinical laboratory should have at its disposal a safety reference library. This library should be available at all times to all technical personnel, students, and employees. It should include books and manuals that will be helpful in preventing unsafe conditions and that provide a guide to safe procedures to be employed in the event of an accident in the laboratory. The following list includes some of the more valuable references for a safety library:

1. U.S. Department of Labor, Occupational Safety and Health Administration, "Guide for Applying Safety and Health Standards," 29 CFR 1910, Government Printing Office, Washington, D.C., 1972.
2. U.S. Department of Labor, Occupational Safety and Health Administration, Occupational Safety and Health Standards, *Fed. Regist.*, vol. 29, no. 125, part II, 1974.
3. "Laboratories in Health-Related Institutions," National Fire Protection Association, Boston, 1973.
4. Norman V. Steere (ed.), "Handbook of Laboratory Safety," 2d ed., Chemical Rubber Company, Cleveland, 1971.
5. "A Laboratory Safety Guide," California Association of Public Health Laboratory Directors, April 1976.
6. National Institutes of Health, "Biohazards

Safety Guide," Government Printing Office, Washington, D.C., 1974.

Basic first-aid procedures

Since there are so many potential hazards in a clinical laboratory, it is easy to understand why a basic knowledge of first aid should be an integral part of any educational program in laboratory medicine. The first emphasis should be on removal of the accident victim from further injury; the next involves definitive action or first aid to the victim. By definition, first aid is "the immediate care given to a person who has been injured or suddenly taken ill." Any person who attempts to perform first aid before professional treatment by a physician can be arranged should remember that such assistance is only a stopgap—an emergency treatment to be followed until the physician arrives. Stop bleeding, prevent shock, then treat the wound—in that order.

A rule to remember in dealing with emergencies in the laboratory is to keep calm. This is not always easy to do, but it is very important to the well-being of the victim. Keep crowds of people away and give the victim plenty of fresh air.

Because so many of the possible injuries are of such an extreme nature and because in the event of such an injury immediate care is most critical, application of the proper first-aid procedures must be thoroughly understood by every person in the medical laboratory. A few of the more common emergencies and the appropriate first-aid procedures are listed below. These should be learned by every student or person working in the laboratory.

1. *Alkali or acid burns on the skin or in the mouth.* Rinse thoroughly with large amounts of running tap water. If the burns are serious, consult a physician.
2. *Alkali or acid burns in the eye.* Wash out thoroughly with running water for a minimum of 15 minutes. Help the victim by holding the