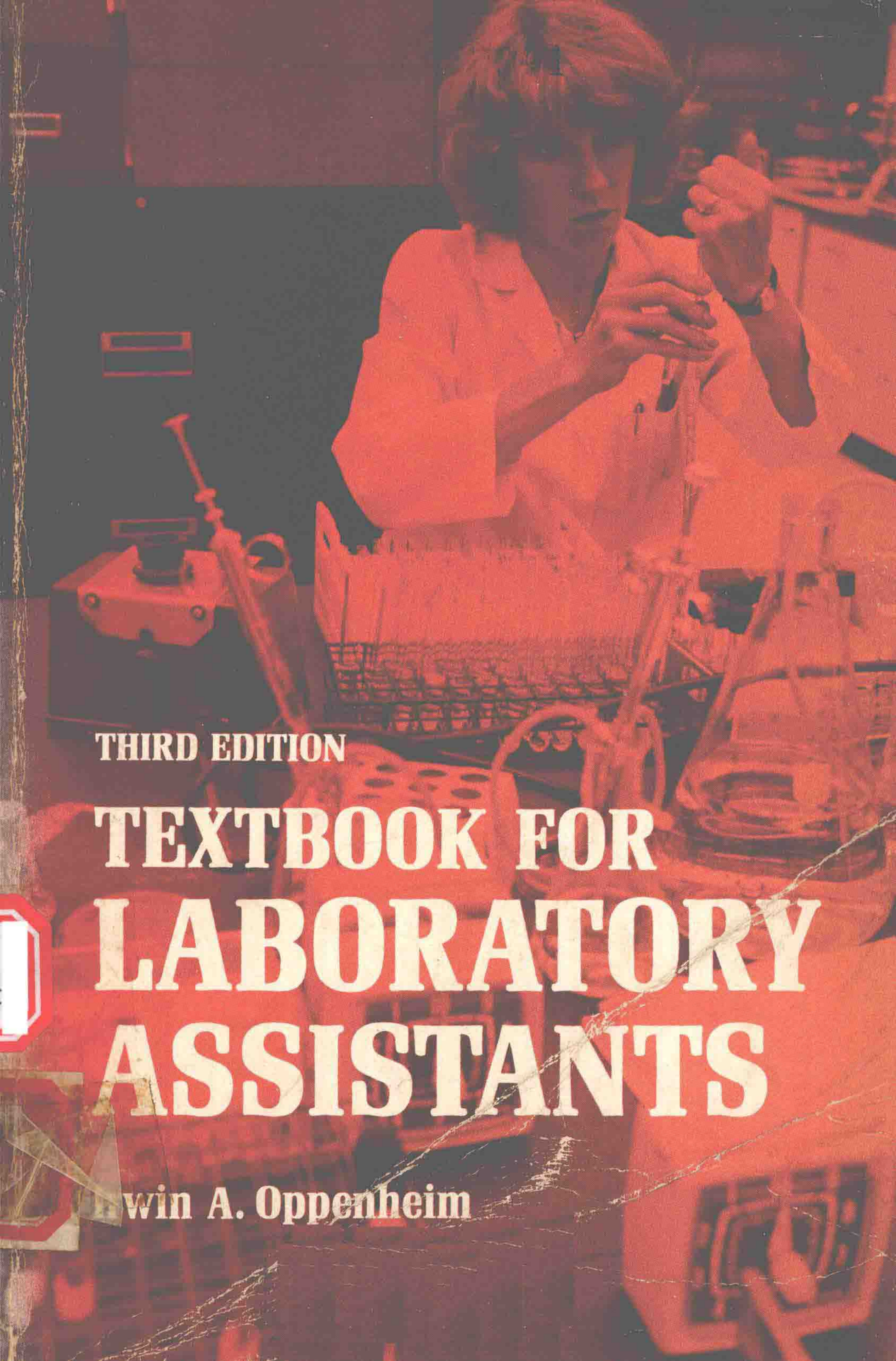

THIRD EDITION

TEXTBOOK FOR LABORATORY ASSISTANTS

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TEXTBOOK FOR LABORATORY ASSISTANTS

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TEXTBOOK FOR

LABORATORY ASSISTANTS

To

my students, my teachers, my family

Preface

Significant advances in technology and laboratory medicine have occurred since the first edition of this book. New techniques in chemistry such as enzyme-linked immunoassay, revised taxonomy in microbiology, additional applications of immunologic procedures, and many other changes in the state of the art have required this revised edition of the original text. To keep to my original intent to provide a compact, relevant primer for the new student, some procedures no longer of general interest have been deleted, while others of sufficient historical value for the student's understanding have been retained.

In planning this revision, I have made a sincere effort to bring basic updated information into focus regardless of whether the new student is entering at the level of certified laboratory assistant (C.L.A.), medical laboratory technician (M.L.T.), or medical technologist (M.T.). Also, a reference bibliography, which should be of value to the student seeking additional information, has been added to this edition. Adaptation to the International System of Units (SI) has been made whenever possible.

Several new illustrations by Diane L. Nelson have been added. Others have been reproduced by the kind permission of Ames, Division of Miles Laboratories, Inc.

Irwin A. Oppenheim

Contents

1 □ The clinical laboratory: its organization and operation

THE MEDICAL LABORATORY STAFF: TRAINING AND QUALIFICATIONS

Medical technology consists of the services rendered to patients by the performance of tests in a medical laboratory. Although some aspects of medical technology require research and investigation, this text is concerned with established and routine medical tests. Nevertheless, the career medical laboratory worker will keep abreast of newer and better methods of testing as they evolve.

The newcomer to the medical laboratory soon becomes aware of the staff organization needed to turn out a quality product—the accurate test report. The physician's dependence on the medical laboratory for information necessary for diagnosis and treatment is also quickly apparent.

Medical laboratories had their inception during the 1920s, when physicians found they could no longer simultaneously attend their patients and perform laboratory tests in their offices. The physicians began to delegate the laboratory work to persons whom they trained. As the volume and scope of testing became even greater, pathologists, physicians specializing in laboratory medicine, found themselves establishing schools and training and directing the new paramedical personnel. In 1928 the Board of Registry of the American Society of Clinical Pathologists was established to certify qualified *medical technologists* (M.T.). Standards of training for approved schools were introduced. M.T. students are expected to complete 4 years of training at the college level and pass a Registry examination. In 1967 a second category of trainees to assist technologists was developed and designated *certified laboratory assistants* (C.L.A.). After high school and 1 year of training in an approved school, the laboratory assistant would take a certifying examination. In 1970 a third and intermediate category of *medical laboratory technicians* (M.L.T.) was developed, which requires 2 years of college-level training. So great has been the demand for qualified laboratory workers that most universities, colleges, and junior colleges and many independent schools (usually affiliated with hospitals) offer some type of training program in medical technology.

In addition, various other organized schools provide training for the *medical assistant* at the noncollegiate level. Such schools are usually more oriented toward the training of personnel for the medical practitioner's office and office laboratory. Medical technology schools that offer the C.L.A., M.L.T., and M.T. programs are evaluated for their maintenance of high standards and accredited on a voluntary basis by the Committee on Allied Health Education and Accreditation (CAHEA) of the American Medical Association on recommendation by the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS). These programs, as they exist in the United States, provide the major portion of trained general medical technicians at three distinct levels of competency. Recently, details have been worked out for upward career mobility. Through the Board of Registry, mechanisms also exist for categorical certification in chemistry (C), hematology (H), histologic technic (HT), microbiology (M), cytotechnology (CT), and nuclear medicine technology (NM). Specialist certification is available in chemistry (SC), hematology (SH), microbiology (SM), and blood banking (SBB). Other registries certifying laboratory workers are NCA (CLT and CLS), ICMT, RMT, and AMT.

The medical technologist, technician, laboratory assistant, and others collectively form a team led by the laboratory director, often a pathologist who has spent many years of training in medical school, internship, and residency. By virtue of this training the pathologist is able to communicate with the attending physicians. Like the conductor of a symphony orchestra, who coordinates the musicians for the quality performance desired by the audience, the laboratory director–pathologist coordinates the medical laboratory staff to meet the testing needs of patient and physician. If the laboratory director is a pathologist, there is additional responsibility for mak-

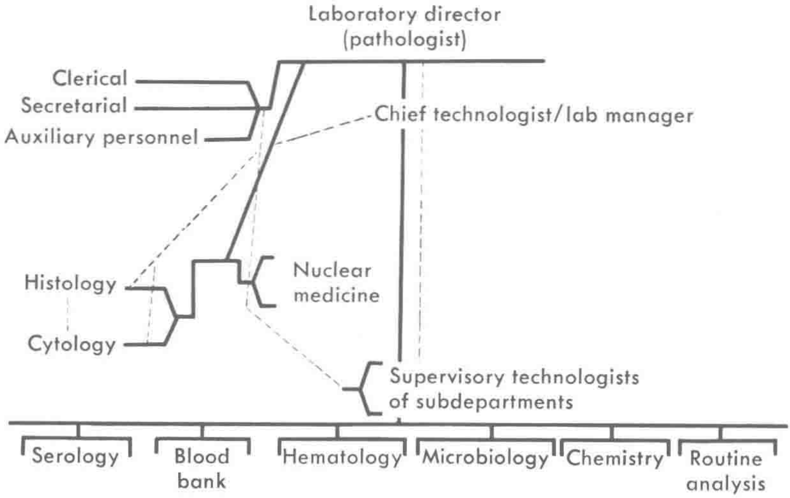

Fig. 1. Organization of the laboratory.

ing diagnoses on body tissues and fluids. Pathologists have been called "doctor's doctors" because of their key role in diagnosis. Coordination of laboratory functions is facilitated by a chief technologist and/or laboratory manager. Several clerical and secretarial workers assist the laboratory director and technical staff.

All staff members are bound by the highest ethical principles to respect the patient's privacy. Reports of tests and overheard information are treated confidentially, and the work of the laboratory is conducted with courteous and professional attitudes. Neophytes as well as experienced laboratory workers must constantly be on guard that the stress and strain of daily work do not reduce their comportment to nonprofessional levels. Each must try continuously to improve skills and knowledge.

A typical organizational plan of a medical laboratory, as shown in Fig. 1, will be helpful in understanding the next section.

DEPARTMENTS OF CLINICAL PATHOLOGY

Microbiology

Historically, microbiology began to develop in the seventeenth century when van Leeuwenhoek experimented with homemade microscopes and observed minute organisms. Louis Pasteur's studies in the nineteenth century formed the scientific foundation of microbiology. The field's development was accelerated by Robert Koch's introduction of culture media, leading to the isolation and identification of infective organisms.

Microbiology includes bacteriology, parasitology, mycology, virology, and certain aspects of serology.

Bacteriology is concerned with the detection and identification of pathogenic (disease-producing) bacteria. Under certain conditions, commensals (harmless bacteria) may become pathogens and require laboratory study; therefore interpretation of bacteriologic studies requires information about the source of the specimen, the conditions under which it was obtained, and the features of the illness.

Bacteria are microscopic unicellular organisms that are often arranged in chains, groups, or filaments. Under the proper conditions they multiply quickly by simple cell division. Some are rod shaped; others are spherical, comma shaped, spiral, or filamentous. Certain bacteria develop resistant forms called spores. Some species possess whiplike extensions called flagella that provide motility. As in other cells the cytoplasm of bacteria contains ribonucleic acid (RNA), and the invisible nucleus contains deoxyribonucleic acid (DNA), the genetic material essential to the life and reproduction of the organism. Bacteria also contain enzymes useful in their identification: those that liquefy gelatin, ferment sugars, and cause oxidation, reduction, hydrolysis, and splitting of proteins.

Complete bacteriologic laboratories usually include a subdivision in *mycology* for diagnostic determination of fungi. In the larger institutions, work may also be done in *parasitology* and *virology* on the identification of parasites and viruses, respectively.

Fungi are studied in much the same way as bacteria. They have two main morphologic forms: (1) branching hyphae (filaments) which can interlace to form a mycelium (meshwork) and reproduce by means of spores developed in special "fruiting organs" and (2) the yeast form, which is usually round or oval and multiplies by "budding."

Parasitology differs from bacteriology and mycology in that parasites are identified mostly on the basis of their morphologic structure and the appearance of their ova rather than by cultural and biochemical characteristics. Parasites are often transmitted from one animal or species to another by means of vectors. An example of a vector is the *Anopheles* mosquito responsible for the transmission of malaria parasites.

Special tissue culture and serologic techniques, which are beyond the scope of most hospital laboratories, are required for the identification of viruses.

Serology

Serology is a laboratory subdivision closely related to microbiology, hematology, and blood banking. The common denominator is investigation for the presence of antigens and antibodies. Their presence is evidence of current, recent, or past infectious disease. Serologic tests are also useful in the diagnosis of infectious mononucleosis, rheumatoid arthritis, rheumatic fever, immunodeficiency, organ transplantation, and autoimmunity. Several serologic techniques are available: complement fixation, neutralization, hemagglutination, inhibition-agglutination, radioimmunoassay, fluorescent antibody, immunoelectrophoresis, and gel diffusion.

Hematology

Hematology encompasses examination of blood cells and the clotting mechanism of blood. Blood is composed of erythrocytes (red blood cells), leukocytes (white blood cells), and plasma (fluid). It also contains small structures called thrombocytes (platelets) that are necessary for coagulation. Developing blood cells can be studied in specimens of bone marrow and lymphoid tissue. Erythrocytes are responsible for transporting oxygen, and leukocytes have an important role in defending the body against infectious disease. Plasma contains nutritive materials absorbed from the intestine during digestion; it transports them throughout the body to nourish cells and tissues. Plasma also carries metabolic waste material to the kidneys for excretion. Other materials transported in the blood plasma include antibodies, hormones, and chemicals that are needed for body processes. A blood count is performed routinely when a patient is admitted to a hospital. Anemias are thus quickly discovered by examination of red blood cells and their hemoglobin content. Diagnosis of infections and leukemia is facilitated by examination of the white blood cells. Coagulation tests for deficient blood-clotting factors may be performed as well.

Blood banking

Closely related to hematology is the field of blood banking. The blood volume of the average healthy person is 8% of the total body weight and must be maintained at adequate levels. When blood transfusion is required, the specific blood groups of the recipient and donor must be matched. The main blood-grouping system is the ABO system, discovered by Landsteiner in 1900. Other systems such as the Rh system have been discovered since then. Since blood groups are inherited according to Mendel's laws, blood banking has become an exact science. Undesirable transfusion reactions may be caused by factors other that mismatch, but mismatch must be carefully avoided in all cases. The inexperienced laboratory assistant depends significantly on the knowledge of the senior blood banking technologist for investigation of those cases in which immune or nonimmune types of antibodies interfere with transfusion.

Chemistry

Fundamental concepts in chemistry such as the basic rules of valence, atomic and molecular weights, laws of chemical combination, and types or reactions should be well understood by students before they enter the field of medical technology. With this background they can embark on the application of quantitative analytic chemistry, using the techniques of volumetric, gravimetric, and spectrophotometric analyses. Of the three, spectrophotometric analysis is the most important and widely employed technique in the clinical laboratory today. Additional techniques used in the modern clinical laboratory include flame photometry, amperometric titration, fluorometry, nephelometry, and electrophoresis. Many chemical tests have been automated, and tedious manual manipulations have been partially or completely replaced by sophisticated instrumentation.

In practice, chemical determinations in the laboratory may be categorized for convenience as follows: electrolytes, including sodium, potassium, chloride, bicarbonate, and calcium; enzymes, such as alkaline and acid phosphatases, transaminases, amylase, and lactic dehydrogenase; liver battery, comprising bilirubin, total protein and albumin, and certain enzyme determinations; a miscellaneous group concerned with metabolism, including blood urea nitrogen (BUN), urea clearance, glucose, glucose tolerance, creatinine, and uric acid; and special chemistries, such as electrophoresis, pH and blood gases, poisons, drugs, and radioimmunoassay (RIA).

Routine analyses

Under the broad category of routine analyses, laboratory subdepartments are set up to do urinalysis, gastric analysis, cerebrospinal fluid examination, and examination of feces. In most hospitals urinalysis is performed routinely on all patients admitted.

Spinal fluids may be split and examined for cell count and for chemicals such as sugar, protein, and chloride; some of the sample is also sent to the bacteriology and serology laboratories. The diagnosis of meningitis and other serious conditions affecting the central nervous system depends on examination of spinal fluid.

Detection of occult blood in the feces by simple chemical tests is often an early clue to malignancy, ulcer, or inflammatory disease in the intestinal tract. Fecal fat content can be analyzed for evidence of bowel malabsorption.

Histology and cytology

Although histology and cytology are in the domain of technologists with specialized training, the entire laboratory staff should know something about the activities of these departments and the handling of specimens that are processed there.

On arrival in the histology laboratory, each tissue removed in surgery is numbered sequentially, and an entry is made in the acquisition book. The assigned number is carried through all procedures. The specimens are examined and described by the pathologist, who takes appropriate samples for processing and stores the remainder of the specimen for future study if necessary.

The first step in processing is *fixation*, which may be done in the operating room, or in the laboratory after the pathologist's examination. The purpose of fixation is to prevent autolysis and putrefaction, to preserve tissue details, and to harden the tissues. Common fixatives are formalin (40% formaldehyde diluted 1:10), dilute mercuric chloride, dichromate, and alcohol. Bone and calcified specimens have to be softened by decalcification in weak acid after fixation.

The next step is *dehydration*, which is carried out by passing the tissues through alcoholic solutions of increasing strength. Then the tissues are *cleared* with xylol to remove the alcohols. Clearing is followed by *embedding*, impregnation of the specimens with paraffin wax heated to 45° to 60° C. This includes *blocking* the tissues in a formed rectangular mold filled with liquid paraffin and placing the surface to be cut at the face of the block. Most of these steps, except blocking, are carried out by automatic tissue processors that mechanically transfer tissues from reagent to reagent.

After blocking, the sections are cut into thin slices on a microtome. Sections 6 to 8 micrometers (μm) thick are acceptable. The cut sections are flattened and smoothed on a warm water bath, mounted on a slide, dried, and then stained with the appropriate dyes. The *staining* process also requires processing through several solutions to *deparaffinize*, *rehy-*