DIAGNOSTIC PROCEDURES

for Bacterial, Mycotic and Parasitic Infections

Fifth Edition, 1970

DIAGNOSTIC PROCEDURES

for Bacterial, Mycotic and Parasitic Infections

TECHNICS FOR THE
LABORATORY DIAGNOSIS AND CONTROL OF THE
COMMUNICABLE DISEASES

FIFTH EDITION

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FOREWORD TO FIFTH EDITION

In the short span of five years it became necessary to plan a new edition of this volume. This fact attests to the success of its predecessor, edited by Albert H. Harris and Marion B. Coleman. Since the present volume is a companion to *Diagnostic Procedures for Viral and Rickettsial Infections,* its title has been changed to *Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections.* Preparation for the new edition made it possible to include new information in this rapidly changing field. Appreciation is expressed to Howard L. Bodily for his willingness to serve as Editor, a challenging task, indeed, for a busy microbiologist, and to Elaine L. Updyke, Coeditor, and James O. Mason, Associate Editor, who have all contributed with distinction. We are indebted, also, to the members of their Committee, who served so effectively in this important task. Sincere thanks are due to the many authors, who devoted much of their valuable time to the preparation of the various chapters. It is hoped that the Fifth Edition will continue to be of value to students, as well as to microbiologists, physicians, and public health workers.

Erwin Neter, M.D., *Chairman*

Coordinating Committee on
Laboratory Methods

June 1969

PREFACE TO FIFTH EDITION

The Fifth Edition of *Diagnostic Procedures and Reagents* is being published under the new title, *Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections,* as a companion volume to the Fourth Edition of *Diagnostic Procedures for Viral and Rickettsial Infections.* The two volumes constitute a collection of laboratory procedures for the diagnosis and control of infectious diseases. The two volumes have been coordinated to provide for proper placement of subject areas and to avoid duplication wherever possible. However, both volumes deal with the mycoplasma, though not in complete duplication. In future editions, this and other subject areas now treated in *Diagnostic Procedures for Viral and Rickettsial Infections* may be more properly placed in this volume.

One of the purposes of this volume is to approach, as nearly as is presently possible, a set of recommended procedures for use in the public health laboratory and other diagnostic laboratories concerned with infectious diseases. To this end, support was sought and generously received from the National Communicable Disease Center. Hence, many of the authors are from the staff of the Center. Their assistance is of particular value, since many have already developed recommended methods for themselves and for state public health laboratories. In addition, the National Communicable Disease Center, with assistance from professional microbiologists from several sources, developed recommended specifications for diagnostic biological reagents used in tests throughout the nation. Recommended methods were compiled by the National Communicable Disease Center staff for the standardization of these reagents. Thus, an effort has been made to relate, where applicable, the methods included in this volume to those recommended for the testing of biological reagents ultimately used in diagnostic procedures.

This volume is primarily a working manual for the diagnostic or public health laboratory. Detail of method is emphasized. The reader is referred to other textbooks for historical or epidemiological information or data concerning the causal agents. Literature references at the end of each chapter have been selected to provide the key references on the subject.

The volume has been divided into three major parts: namely, administrative practices, recommended methods, and media and test procedures. Whenever media or test procedures were peculiar to one chapter only, these were included in the chapter, while those common to several chapters were placed in the section on media and test procedures (Part Three). Formulations of commercially available media have not been included in this volume, since information concerning them is readily available from the manufacturer.

The editors wish to extend special thanks to Dr. U. Pentti Kokko, Chief, Laboratory Division, National Communicable Disease Center, and to the scientific staff of the Center for their generous support and cooperation in making this volume possible. The assistance of many who reviewed the individual chapters is also appreciated. Special appreciation is given to those who were primarily responsible for the preparation of the volume for the printer, among them Helen F. Hough and Marilla C. Bodily.

This volume has been a truly cooperative effort. It is hoped that the volume will justify the enormous amount of voluntary work devoted to its preparation. If it brings about a recognition of the better diagnostic procedures and a continued improvement of methodology and performance of laboratory work, it will have served its purpose.

HOWARD L. BODILY, PH.D., *Editor*
ELAINE L. UPDYKE, SC.D., *Coeditor*
JAMES O. MASON, M.D., *Associate Editor* and *CCLM Reviewer*

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CHAPTER I

GENERAL ADMINSTRATION OF THE LABORATORY

by Howard L. Bodily, Ph.D.†

INTRODUCTION

The public health laboratory is an integral part of the health team whether it is operated at the national, state or local level. Its primary function is to support the health department's activities. Direct support is expected and is given for a wide variety of epidemiologic and environmental health investigations and control activities of the department. In addition, the public health laboratory may be engaged in setting standards for and evaluating performance of local public and private laboratories. It may serve as a reference, consultation and training resource for the local laboratories. In most states, the state and local public health laboratories provide diagnostic laboratory assistance to the medical community, supplementing the work of the private or hospital medical laboratory. In many states, public health laboratories play an important role in research activities of a department and may, indeed, constitute the major research activity of the department, contributing to the newer knowledge of infectious diseases and to the methods for studying the environmental health problems.

It is important that the laboratory be provided equipment and personnel commensurate with the work expected from it. Modern equipment and laboratory space are necessary, and well-trained scientists are essential. High standards for the qualifications of microbiologists must be established and maintained. The management of the laboratory must be of high caliber and, if qualified personnel are to be recruited and retained, must permit professional development. Opportunity must be available for scientific study of problems arising from the work of the laboratory.

While this manual is written primarily for the microbiologist, it is of first importance that other health personnel become familiar with the work of the laboratory. Only when the laboratory is used

† The author is indebted to the authors of Chapter 1 of *Diagnostic Procedures and Reagents* (4th ed.), 1963, for much of the material presented in this chapter.

with intelligence and understanding can its work yield the most rewarding information. Otherwise, the efforts that should be spent on important tests will be dissipated on needless and costly activities. Laboratories are expensive to operate. Reasonable restraint in utilizing laboratory services is therefore necessary. Waste can be avoided through prompt communication with appropriate laboratory personnel at a time when the laboratory scientist can offer information about the methods available and the laboratory approaches best used to obtain the information desired. Often, the objective to be achieved will determine the kind of laboratory work needed. The methods available may also determine whether the objectives can be realized.

Health department personnel and clinicians should appreciate the limitations of the laboratory from the technical and logistics point of view. Tests which are available for diagnostic assistance cannot always be applied to large numbers of specimens because of the limitations of time and facilities. On the other hand, the resourceful laboratory scientist can often devise modified procedures that will provide the needed information.

The solution to these problems lies in the use of an established mechanism for communication and planning which will involve laboratory personnel at the earliest possible time. Informal consultations in the laboratory, in the field or clinic and over the telephone, are of great value and should be encouraged despite their time-consuming nature. Of particular worth are scheduled conferences in which the clinician or health department worker can exchange his experience and special knowledge with the information and special skills of the laboratory scientist. Tests are performed more effectively and with greater understanding when laboratory personnel are informed about the case or situation under consideration and the surrounding circumstances. Specimens are selected and taken more intelligently when it is known what the laboratory can do and how the results may be used. If the laboratory is to perform its function, laboratory staff should be regarded as full and equal members of the health team and should be encouraged to contribute of their special knowledge and skills toward the planning and conduct of department or clinic activities.

Laboratory Standards and Methodology

Quality control of work in the laboratory is based on several factors. Of greatest importance are adequate education and experience for the work to be done. Most microbiological work requires staff who have at least a bachelor's degree with a major in microbiology, since much of the quality control in microbiology is dependent on professional judgment. Of next importance is the application of intralaboratory quality control procedures. While these procedures are limited in their application to microbiology, many things can be done to insure high quality. One of these is strict adherence to the procedures called for, whether they concern test methods or the preparation of supplies and reagents. While variations are to be expected in the technics of different laboratory workers, these can be minimized by the exercise of precision and care in following the procedures that are approved in a given laboratory. This must be accepted as a guiding principle by all laboratory personnel. Research for improved methods and the trial of new methods described in the literature are definite responsibilities of the public health laboratory and are to be encouraged and fostered at every opportunity. Changes in procedure, however, are to be made only with the approval of the director and after comparative studies have shown that the method is of adequate sensitivity, specificity and reproducibility. Laboratory methods should be available in writing and supplied to every worker in the laboratory.

Evaluations of laboratory performance should be undertaken periodically. Every laboratory should participate in some proficiency testing program and every person in the laboratory should participate in some intralaboratory performance evaluation activity. These programs are designed to provide information on the performance of one laboratory as compared with that of other laboratories. The information so obtained can be useful in spotting areas where training is needed, methods which may be inferior to others currently available, and weaknesses in the intralaboratory quality controls. Such evaluations should be part of the total program for professional development and training of laboratory personnel.

Selection and Use of Supplies and Equipment

The public health laboratory will necessarily have a large amount of expensive equipment. The personnel should be instructed in the reasonable care and maintenance of this equipment. There should be one worker in every laboratory, preferably a competent mechanic, who will be responsible for the maintenance and periodic checking of all equipment. He will do well to maintain spare parts for some of the elements most commonly needing replacement. Sources of information regarding equipment specifications are readily available from the manufacturers. Careful consideration should be given to such specifications in selecting the kind of equipment needed. Since each laboratory will have different needs, the choice of equipment should be geared to the basic requirements. Specialized equipment should not be purchased when less expensive equipment will suffice; and expensive research should not be authorized when alternative ways of accomplishing the work can be found that will not increase the amount of work involved or adversely affect the quality of the test results. On the other hand, the most expensive item in laboratory operation is the salaries of personnel. Accordingly, every effort should be made to use effective labor-saving devices.

LABORATORY SAFETY

The director of the laboratory is responsible for the safety of the laboratory personnel. Accidents *can* be prevented. If a safety program is conducted in the laboratory, accidents can be reduced to a minimum. Accidents or illness may result from physical, chemical or microbiological hazards present in the laboratory. A recognition of these hazards, coupled with the knowledge of what can be done to minimize them, should be one objective of the training of all laboratory personnel. The director of a laboratory should establish a safety committee representing all levels of workers in the laboratory. This committee should be charged with a review of the facilities, equipment and procedures of the laboratory to uncover hazards and to make recommendations for correction. The committee should review all accidents that have occurred in the laboratory and recommend action for prevention of future accidents from the same cause. The committee should also assist the director in implementing and maintaining a safety program, including a regularly scheduled personnel training program.

Hazards due to the physical environment and to chemicals in the laboratory are so numerous that only a few suggestions can be made here. Rather, the reader is referred to a volume entitled *Handbook of Laboratory Safety* published by the Chemical Rubber Company in 1967.[1] This or a publication of comparable content should be a working manual of every laboratory and should be made available especially to the laboratory safety committee.

Electrical shock and fire rank high among physical hazards. Such dangers are well known and in general are not peculiar to the laboratory, but a few cardinal principles ought to be emphasized. First, the danger of electric shock is greatly minimized when all machines are properly grounded; second, the danger is further reduced if laboratory workers learn never to handle the electric machines or their switches and controls with wet hands. For the prevention of fire, strict rules should be enforced against smoking around flammable materials, and persistent vigilance exercised by every laboratory worker using a Bunsen burner or gas oven. Fire-resistant blankets should be located at strategic points to wrap at once about any victim whose clothes catch fire. There should be an adequate number of properly placed fire extinguishers, which must be serviced regularly at 6-month intervals. Fire drills, with emphasis on the turning off of gas burners and the closing of doors, should be held about twice a year to familiarize each worker with his proper duties in the event of fire. The fire department should be called at once for even small fires. Some of the worst fires on record have been devastating largely because they were fought locally too long before the fire department was called.

The laboratory worker is daily confronted with the potential hazards of toxic chemicals and poisons. Unlike the delayed response to accidental infections of microbial origin, most of the toxic reactions present their effects almost immediately after the accident has occurred. Such accidents occur when a worker inhales toxic vapors, ingests chemicals by mouth in the act of pipetting, unexpectedly introduces the agent into the eye or spills it on the skin. In particular, the pipetting of chemicals must be done with the greatest caution. The laboratory technician, with his basic training in chemistry and related sciences, should have at least fundamental information concerning the hazards that attend improper handling and disposal of poisonous, corrosive, flammable or explosive chemicals.

Some general directions in the handling and storage of chemicals may be useful:

1. Store volatile, flammable and explosive materials in a metal cabinet or closed room. The storage place should be cool and well ventilated.
2. Keep volatile, flammable, explosive and corrosive materials in their original containers, in glass, or in resistant plastic containers.
3. Use pumps in transferring large volumes of dangerous liquids. Transfer small volumes with a bulb attached to a suitable pipet.
4. Transfer toxic or irritating materials in a well-ventilated area, preferably under a hood.
5. Avoid storing incompatible chemicals together.
6. Label all laboratory reagents. Attach poison stickers where indicated.
7. Have available in the immediate area suitable neutralizers for

acids and alkalies (sodium bicarbonate vs mineral acids, dilute acetic acid vs alkalies).

8. Make available in the immediate area of volatile, flammable and explosive materials suitable fire-fighting equipment (fire extinguishers, fire blanket, respirators).
9. Make periodic inventory of dangerous chemicals to avoid stockpiling of hazardous materials.
10. Familiarize other laboratory personnel with the precautions necessary in handling new hazardous materials.

Following are specific suggestions for the more common hazardous chemicals:

a. Acetone, ether—Highly flammable. Keep in a cool place (spark free refrigerator) and remote from sparking apparatus and open flames, including pilot flames. Keep containers tightly closed. Use with adequate ventilation. Avoid prolonged or repeated contact with the skin.

b. Acids—Avoid breathing vapor; avoid contact with the skin or eyes. Add concentrated acid to water in making dilutions. *Never add water to concentrated acids.*

c. Alkalies, caustics—Avoid contact with the skin, eyes and clothing. Wear goggles or shield when handling. Add crystals slowly to water to avoid spattering.

d. Bichloride of mercury—Avoid contact with skin and eyes.

e. Chloroform—Nonflammable, but keep vapors away from open flame, electric hot plates, or any hot metals to guard against formation of the toxic gas phosgene. Use only with adequate ventilation. Avoid breathing vapor. Avoid prolonged or repeated contact with the skin.

f. Alcohols (methyl alcohol, ethyl, etc.)—Use only with adequate ventilation. Avoid breathing vapor.

g. Formaldehyde—Use with adequate ventilation. Avoid breathing vapor. Avoid prolonged contact with the skin.

h. Hypochlorites—Store powders in cool dry place. Dust can be damaging to the skin and injurious internally if inhaled. The calcium salt of hypochlorous acid is explosive when heated suddenly to above 100 C; when mixed with combustible substances, deflagration occurs. Wear chemical safety glasses and a respirator.

i. Iodine—Work with crystals only in areas adequately ventilated; transfer under hood. Avoid contact with the skin and eyes.

j. Phenol—Rapidly absorbed through the skin. Causes severe burns. Avoid contact with the skin, eyes and clothing. Avoid breathing vapor.

k. Potassium dichromate—Dust harmful. May cause rash or external ulcers. Avoid breathing dust or solution spray. Avoid contact with the skin or eyes.

l. Xylene—Rapidly absorbed through the skin. Flammable. Keep in a cool place and use with adequate ventilation. Avoid breathing vapor. Avoid contact with the skin and eyes.

The preceding list covers most of the hazardous chemicals commonly used in the public health laboratory. It is not exhaustive, however, and full caution must be exercised when any other chemicals are used in the laboratory.

Following are instructions for the treatment of accidental poisoning from hazardous chemicals. *For immediate advice call the nearest poison information center.*

1. **Volatile substances and gases**—Whether the victim is overcome by ether, chloroform, carbon monoxide or any other volatile substance or gas, the principles of immediate treatment are the same: *First*—At once remove the patient well out of the area of the toxic agent into air as fresh as possible. *Second*—If the patient is not breathing, see that the air passages are open and then apply artificial respiration by the mouth-to-mouth method, using oxygen inhalator if available. Keep the artificial respiration going until a physician sees the patient. *Third*—Seek medical help, but institute the first two measures immediately.

2. **Substances dangerous internally**—Whatever the toxic agent ingested, with the important exception noted below, the cardinal principles are immediate dilution of the agent and prompt emptying of the stomach. Both dilution and emptying can be effectively accomplished by having the patient drink at once huge quantities of lukewarm water until vomiting takes place. Repeat the process again and again until thorough stomach lavage has taken place. If a stomach tube is available, *use it*.

The special emetics and antidotes that are recommended in texts are rarely at hand for quick use when needed, nor are they always effective. Instead, if the drinking of copious amounts of water has not induced the expected vomiting, simply tickle the back of the patient's throat with your finger or try soapsuds in warm water or two tablespoons of table salt in a half pint of warm water as simple