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LABORATORY MANUAL AND WORKBOOK IN Microbiology APPLICATIONS TO PATIENT CARE

Second Edition

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Applications to Patient Care

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The photograph on the cover is an electron micrograph showing numerous gonococci attached to the surface of epithelial cells from the urethra of a man with symptoms of gonorrhea for 24 hr. Gonococci are between adjoining cells. (26,500 X.) From Michael E. Ward and Peter J. Watt: Adherence of *Neisseria gonorrhoeae* to Urethral Mucosal Cells: An Electron-Microscopic Study of Human Gonorrhea, *J. Inf. Dis.*, **126**:601-604, 1972.

All of the photographs illustrating laboratory techniques or equipment (Figures 2-1, 2-2, 2-3, 2-4, 8-1, 9-1, 10-1, 19.5-2, and 27-1) were taken by, and supplied through the courtesy of, Mr. Gordon Bowie, photographer for the Department of Pathology, University of Chicago, Chicago, Ill.

Figure 31-1 and Tables 13-1, 28-1, 29-1, and 29-2 are reproduced from *Microbiology in Patient Care,* by Marion E. Wilson, Helen Eckel Mizer, and Josephine A. Morello, 3rd ed. (New York: Macmillan Publishing Co., Inc., 1979). Table 30-1 is adapted from the same source.

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Laboratory Manual and Workbook in Microbiology

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Preface

This laboratory manual and workbook, now in its second edition, maintains its original emphasis on the basic principles of diagnostic microbiology for students preparing to enter the allied health professions. It remains oriented primarily toward meeting the interests and needs of those who will be directly involved in patient care and who wish to learn how microbiologic principles should be applied in the practice of their professions. These include nursing students, dental hygienists, dietitians, hospital sanitarians, inhalation therapists, operating room or cardiopulmonary technicians, optometric technicians, physical therapists, and physicians' assistants. For such students the clinical and epidemiologic applications of microbiology often seem more relevant than its technical details. Thus, the challenge for authors of textbooks and laboratory manuals, and for instructors, is to project microbiology into the clinical setting, and to relate its principles to patient care.

To this end, the authors of this manual (now four in number) have emphasized the purposes and functions of the clinical microbiology laboratory in the diagnosis of infectious diseases. The exercises illustrate as simply as possible the nature of laboratory procedures used for isolation and identification of infectious agents, as well as the principles of asepsis, disinfection, and sterilization. The role of the paramedical professional is projected through repeated stress on the importance of the clinical specimen submitted to the laboratory – its proper selection, timing, collection, and handling. Equal attention is given to the applications of aseptic and disinfectant techniques as they relate to practical situations in the care of patients. The manual seeks to provide practical insight and experience rather than to detail the microbial physiology a professional microbiologist must learn. We have approached its revision with a view toward updating basic procedures and reference sources, adding some new and pertinent material, strengthening its organization, and heightening the value of its illustrative material, while retaining the tenor of the first edition. Every exercise has been carefully reviewed and revised, if necessary, to conform to changing practices in clinical laboratories.

The material has been reorganized into five parts of increasing complexity designed to give students first a sense of familiarity with the nature of microorganisms, then practice in aseptic cultural methods in clinical settings. Part One introduces basic techniques of microbiology. It includes general laboratory directions, precautions for handling microorganisms, the use of the microscope, microscopic morphology of microorganisms in wet and stained preparations, pure culture techniques, and an exercise in environmental microbiology. In the section pertaining to stains, modifications in the Gram stain technique have been made (Exercise 5); and Exercise 7, formerly the spore stain, has been completely rewritten to include capsule and flagella staining.

Part Two provides instruction and some experience in methods for the destruction of microorganisms, so that students may understand the principles of disinfection and sterilization before proceeding to the study of pathogenic microorganisms. There is an exercise on antibiotics (chemical antimicrobial agents) that includes antibiotic susceptibility testing using the Bauer-Kirby technique, and a new experiment on bacterial resistance to antibiotics.

The principles thus learned are then applied to diagnostic microbiology in Part Three. Techniques for collection of clinical specimens (microbiology at the bedside), and precautions for handling them,

are reviewed. The normal flora of various parts of the body is discussed. The five sections of this part now cover the principles of diagnostic bacteriology; the microbiology of the respiratory, intestinal, urinary, and genital tracts; and the special techniques required for the recognition of anaerobes, mycobacteria, fungi, protozoa, animal parasites, and the smallest microorganisms (mycoplasmas, rickettsiae, chlamydiae, viruses). Sections VIII and IX, dealing respectively with the microbiology of the respiratory and intestinal tracts, have been reorganized first to present exercises on the common pathogens and normal flora of these areas, then exercises dealing with methods for culturing appropriate clinical specimens. New experiments have been added for performing antibiotic susceptibility testing on relevant isolates from such specimens. The exercise on streptococci and staphylococci (Section VIII) includes the CAMP test for Group B streptococci. Some of the new rapid methods for identification of the Enterobacteriaceae have been presented in the section on the microbiology of the intestinal tract (Section IX). The discussion of anaerobic bacteria (Exercise 27 in Section XI) has been broadened to include many genera of opportunistic anaerobes whose isolation from serious infections has increased with improved laboratory techniques for achieving anaerobiasis and for identifying isolates.

Part Four reviews the principles of serologic procedures for identification of microorganisms and detection of serum antibodies. Part Five presents some simple microbiologic methods for examination of water and milk.

The sequence of the exercises throughout the manual, but particularly in Part Three, is intended to reflect the approach of the diagnostic laboratory to clinical specimens. In each exercise the student is led to relate the practical world of patient care and clinical diagnosis to the operation of the microbiology laboratory. To learn the normal flora of the body, and to appreciate the problem of recognizing clinically significant organisms in a specimen containing mixed flora, students collect and culture their own specimens. Simulated clinical specimens are also used to teach the microbiology of infection. The concept of transmissible infectious disease becomes a reality, rather than a theory, for the student who can see the myriad of microorganisms present on hands, throat, and in feces, urine, clothes, hair or environmental objects. Similarly, in learning how antibiotic susceptibility testing is done, the student acquires insight into the basis for specific drug therapy of infection and the importance of accurate laboratory information.

In acquiring aseptic laboratory technique and a knowledge of the principles of disinfection and sterilization, the student is better prepared for subsequent encounters with pathogenic, transmissible microorganisms in professional practice. It is the authors' belief that one of the most valuable contributions a microbiology laboratory course can make to patient care is to give the student repeated opportunity to understand and develop aseptic technique through the handling of cultures. Mere demonstrations have little value in this respect. Although the use of pathogenic microorganisms is avoided in these exercises, students are taught to handle all specimens and cultures with respect, since any microorganism may have potential pathogenicity. To illustrate the nature of infectious microorganisms, material to be handled by students includes related "nonpathogenic" species of similar morphologic and cultural appearance, and demonstration material presents pathogenic species. Occasional exceptions are made in the case of organisms such as pneumococci, staphylococci, or clostridia that are often encountered, in any case, in the flora of specimens from healthy persons. If the instructor so desires, however, substitutions can be made for these as well.

Teaching flexibility has been sought throughout the manual. There are 35 exercises, many of which contain several experiments. These may be tailored selectively (and sequentially) to meet the needs of any prescribed course period, the weekly laboratory hours available, or the interests and capabilities of individual students. The order and emphasis of the material were planned so that the manual could be used in conjunction with the textbook entitled *Microbiology in Patient Care* (Wilson, Mizer, and Morello, 3rd ed., Macmillan Publishing Co., Inc., New York, 1979). However, it can be adapted to follow any textbook on basic microbiology appropriate for students entering the allied health field. Chapters in the latest editions of some of these have been cross-referenced with each

exercise in this manual. These reference tables appear on the inner side of the front and back covers. For the instructor's use, a more complete listing of current literature and other source material is provided in Appendix V.

Each exercise begins with a discussion of the material to be covered, the rationale of methods to be used, and a review of the nature of microorganisms to be studied. In Part Three, tables are frequently inserted to summarize laboratory and/or clinical information concerning the major groups of pathogenic microorganisms. Several new tables have been added to Section XI to cover organisms that require special techniques. The revised questions that follow each exercise are designed to test the ability of students to relate laboratory information to patient-care situations and to stimulate them to read more widely on each subject presented. *Bergey's Manual of Determinative Bacteriology* (R. E. Buchanan and N. E. Gibbons, editors, 8th ed., Williams & Wilkins Co., Baltimore, 1974) has been followed in all nomenclature. Current metric terminology is used throughout.

To facilitate advance preparation by instructors or students, the "Purpose" and "Materials" listings for each exercise and experiment are highlighted by a gray tint so that they can be readily located. Highlighting is also applied to tables and information of particular importance. Many of the line drawings that appeared in the first edition have been replaced with photographs that more effectively illustrate simple laboratory techniques.

Five appendixes have been included to provide instructors with information and assistance in presenting the laboratory course. Appendix I contains a series of notes for instructors. These provide suggestions on how to obtain and prepare the material required for many of the exercises, and have been thoroughly revised to suit the needs of this edition. Appendix II gives methods for preparing the stains and chemical reagents needed for all exercises. Appendix III contains information on the preparation and storage of culture media and lists current sources of media and laboratory supplies. Sources from which reliable stock cultures can be obtained are listed in Appendix IV, and details of practical methods for maintaining stocks are provided. A complete list of all bacterial strains needed for the entire group of exercises can be found in this appendix. Finally, Appendix V lists sources of audiovisual materials, titles and sources of a large number of pertinent films, sources of projection and/or microscope slides for demonstrations, and reference literature on medical microbiology, including several method manuals on diagnostic microbiology. All lists have been revised and updated.

Special thanks are due those professional colleagues who generously gave their time and experienced opinion to our effort to construct a pertinent and practical manual. The continuous guidance of Dr. Yvonne C. Faur, Senior Research Scientist, Public Health Laboratory Services, New York City Department of Health, has been invaluable, as always. Dr. Nancy Morello, Associate Professor of Microbiology, Lasell Junior College, Newton, Mass., also made constructive suggestions that were most helpful in our preparation of the revised manuscript.

We are particularly grateful to Mr. Gordon Bowie, photographer for the Department of Pathology, University of Chicago, Chicago, Illinois, whose excellent photographs appear in this edition. These were taken specifically for our use and were supplied through his courtesy. Others who played a supportive role in the details of manuscript preparation include Ms. Belle Barzman, Ms. Lorraine Toaldo, and Ms. Shafia Hyder, to whom we offer special thanks.

Finally, the many courtesies of Macmillan Publishing Co., Inc., are acknowledged again with pleasure and gratitude. As in the past, the guidance of Ms. Joan C. Zulch, medical editor, and the professional skill of Ms. Pat Larson, production supervisor, have been pivotal in the presentation of a finished product.

M.E.W.
M.H.W.
H.E.M.
J.A.M.

During the preparation of the manuscript for the second edition we were deeply saddened by the sudden and untimely death of Martin H. Weisburd, our coauthor and friend of many years. "Marty's" ideas and skills as a teacher were an integral part of the manual from its inception. The depth of his knowledge as a clinical microbiologist was coupled with a perspective on the particular needs of the beginning student – a combination of qualities that brought pleasure and a sense of excitement to his laboratory as well as his classroom. It is our hope that his presence will continue to be felt by all who work with this manual, whether teaching or first learning about the compelling role of microbiology in the clinical world.

M.E.W.
H.E.M.
J.A.M.

Contents

PART One

Basic Techniques of Microbiology

In beginning the study of medical microbiology, one must learn the basic laboratory techniques used to visualize and to cultivate microorganisms. With knowledge of the microscope and staining techniques, one can study their morphology (features and structures). With an understanding of culture media and how they are used, one can cultivate, and study the behavior of, these minute organisms that are of so much importance to us, in health or disease.

Section I
Orientation to the Microbiology Laboratory

Safety Procedures and Precautions

The microbiology laboratory, whether a classroom or a working diagnostic laboratory, is a place for handling and examining cultures of microorganisms. This type of work must be conducted with good aseptic technique in a scrupulously clean, well-ordered environment. Even if the microorganisms being studied are not considered to be pathogenic, *any* culture of *any* organism should be handled with respect for its potential pathogenicity.

Each student must quickly learn and continuously practice aseptic laboratory technique. It is important to avoid any risk of contaminating oneself (hands, hair, clothing) or one's neighbors with culture material. It is also necessary to prevent contamination of the work itself with microorganisms from the environment. The importance of asepsis and proper disinfection is stressed throughout this manual and demonstrated by experiment. Once learned in the laboratory, these techniques are applicable to virtually every phase of patient care, especially to the collection and handling of specimens ordered for laboratory diagnosis of infectious disease. Such specimens should be handled as meticulously as cultures, for the same reasons, so that they do not become sources of infection of others. People who are sick, whatever the reason, are often targets for infection by easily transmitted microorganisms. Well-trained professionals caring for the sick should never be responsible for transmitting infection between them.

In general, all safety procedures and precautions followed in the microbiology laboratory are designed to:

1. *Restrict microorganisms present in specimens or cultures* to the vessels in which they are contained or studied.
2. *Prevent environmental microorganisms* (normally present on hands, hair, clothing, laboratory benches, or in the air) from entering specimens or cultures and interfering with results of studies.

Hands and bench tops are kept clean with disinfectants, protective clothing is worn, hair is controlled, working areas are kept clear of all extraneous items, glass- or plasticware used for specimen collection or culture material is presterilized and protected from entry by unsterile air, sterile tools are used for transferring specimens or cultures. *Nothing* unsterile (or not disinfected) is placed in the mouth.