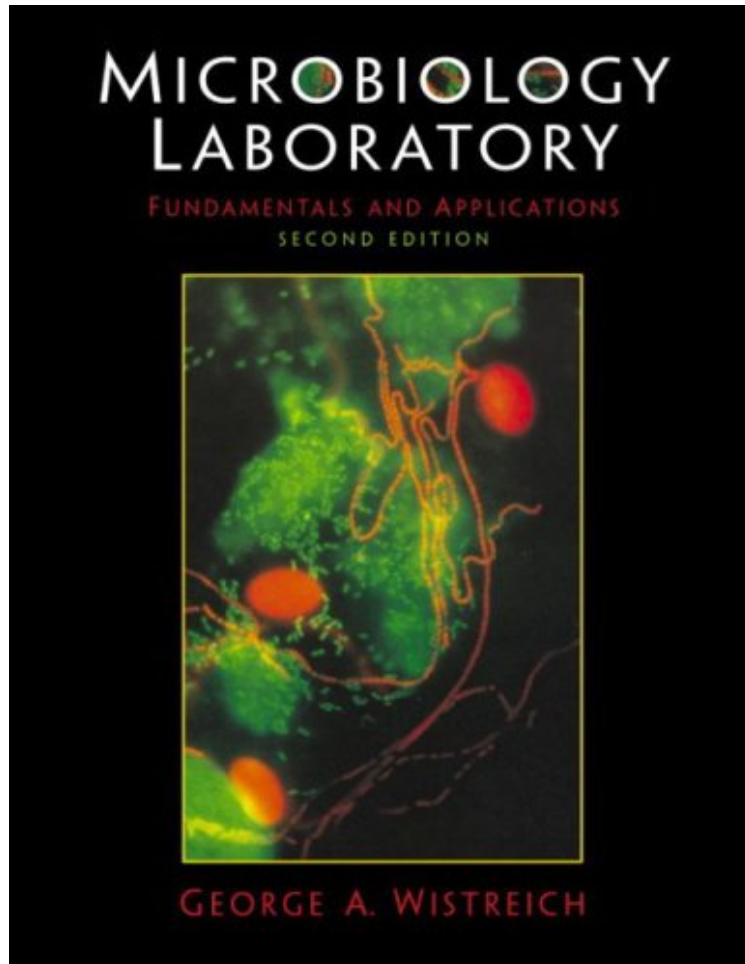


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Microbiology Laboratory Fundamentals and Applications (2nd Edition)

George A. Wistreich

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This comprehensive laboratory manual provides state-of-the-art techniques, concepts, and applications of

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All rights reserved. Microbiology Laboratory Fundamentals and Applications provides students beginning their study with functional and effective experiences that will enable them to work with, understand, and appreciate the importance of microorganisms to basic life processes. The exercises have been chosen, designed, and arranged to help students learn to observe and to recognize fundamental similarities and differences among microorganisms. The second edition of Microbiology Laboratory Fundamentals and Applications contains a number of new illustrations, exercise improvements, and new up-to-date procedures. The elements of critical thinking have also been interwoven within the various exercises and experiments to build and to add to the scientific perspective and abilities of students. Learning Aids To make this manual an effective tool for students, we have developed and incorporated a number of learning aids. These include: Pronunciation Guide to Organisms. The scientific names of each organism studied are presented in a way to aid the student in acquiring a greater ease with which to discuss, describe, and use microorganisms. A phonetic pronunciation guide is listed on the front and back inside covers of this manual. These lists contain all organisms discussed and/or used. In addition, the phonetic pronunciation of an organism's genus and species is given the first time the organism appears in the manual. Section Overviews. Each major section of the laboratory manual is preceded by a description of the range of topics and experiments to be covered. Learning Objectives. Each exercise begins with a list of learning objectives to emphasize the purpose of the procedures and what the student is to accomplish. Illustrations. More than 500 photographs and diagrams have been incorporated into the manual to provide a clear and functional understanding of techniques, properties of microorganisms, and specific test results. Procedure Diagrams. Exercises that introduce basic techniques contain step-by-step illustrations of the procedures to show the proper handling of equipment and cultures. An alphabetical listing of Procedure Diagrams can be found on pages xix-xx. Referral Boxes. In exercises requiring the use of specific procedures and/or techniques described in other parts of the laboratory manual, special Referral Boxes have been inserted to help students to quickly locate needed procedures. Laboratory and Critical Thinking Corner. Laboratory s accompany all exercises to help students determine whether they understood the major features of the exercise and related topics. Several exercises also contain Critical Thinking Corners. Here the student is expected to answer questions, quite frequently accompanied by illustrations, pertaining to specific results or concepts of the exercise. Answers to these sections can be found in Appendix 1. Key Terms. Each exercise contains a specific section of definitions together with the phonetic pronunciations of selected terms. Experimental Exercises. A separate section containing a number of specially developed exercises, techniques, and applications can be found at the end of the manual. These exercises lend themselves to experimentation and additional applications of the principles of microbiology and molecular biology. Index. An index has been included in this manual because of the large number of tests and procedures it contains. Students should have no difficulty in locating a particular test or procedure. Organization of the Manual The 66 instructional exercises, 4 experimental exercises, and related items in this manual are arranged into 15 sections, as follows: Microbiology Laboratory Fundamentals and Applications begins with a short visual survey of equipment generally used in a microbiology laboratory. Familiarity with these and the other equipment items presented in specific exercises will contribute to a smoother-operating laboratory. Section I teaches certain fundamental procedures used in microscopy and in the study of microorganisms. These procedures include oil-immersion objective usage, smear preparations, simple staining, and hanging-drop and temporary wet-mount techniques. Section II stresses methods employed in the isolation and study of both aerobic and anaerobic microbial cultures. Dilution techniques and the use of pipettes together with an application of the pour technique to determine colony-forming units of cultures also are included. Section III compares the representative microbial groups, which include bacteria, algae, protozoa, and fungi, as to their structure, morphological arrangement, and selected activities. Exercises in the section also deal with the distribution of microorganisms in the environment, and spontaneous generation. Microscopic measurements and an examination of Leeuwenhoek's peppercorn water also are important aspects of this section. Section IV demonstrates specific staining techniques, such as the Gram stain and acid-fast

procedures, which are used in the identification and classification of bacteria. Both the hot and cold forms of acid-fast staining techniques are presented. Section V familiarizes students with the structure and function of bacterial cells. Techniques used to observe flagella and bacterial motility by means of motility agar are included. Section VI demonstrates the extracellular and intracellular metabolic activities of bacteria. The section begins with a comparison of various selective and differential plating media and then proceeds to the use of a number of tube and plate media to show the enzymatic activities of various bacterial species. The use of rapid and miniaturized testing procedures to identify microorganisms is also covered. The section ends by introducing the use of a biochemical flowchart (key) in the identification of an unknown enteric bacterial species. Section VII stresses the principles of sterilization and disinfection and the effectiveness of chemical and physical agents in the control of microorganisms. Particular attention is given to the thermal resistance of microorganisms, the use of ultraviolet light in sterilization and as a mutagenic agent, and the inhibitory actions of dyes, different pH levels, and heavy metals. Antibiotic sensitivity testing and the detection of antibiotic resistance also are demonstrated. Section VIII emphasizes certain properties of viruses. Both bacterial and animal viruses are studied and techniques for their detection and cultivation are included. Section IX includes exercises involving gene transfer processes found in bacteria. Experiments contain safe procedures designed to present the basic features of bacterial transformation, conjugation, and transduction. Rapid colony transformation with plasmid DNA and the application of the Ames test for the detection of mutations and/or cancer-causing chemicals also are contained in this section. Section X demonstrates some of the industrial applications of microorganisms. Exercises covering the distribution of microbes in food and their involvement in the production of sauerkraut, cheese, yogurt, and wine are included. Section XI contains exercises designed to increase students' understanding of basic immunological principles and their application. Modifications of standard procedures and concepts and a survey of commercially available tests are presented. Such tests include a survey of commercially available diagnostic devices. The section begins with microbial agglutination reactions and concludes with an enzyme immunoassay. Section XII provides a number of exercises dealing with epidemiology. Particular topics covered include a demonstration of Koch's postulates, the effectiveness of universal precautions, and the proper handling of specimens and their transport. Consideration also is given to aerosols and their importance to laboratory and hospital sepsis. Section XIII includes exercises relating to several aspects of basic medical microbiology. Experiments dealing with the normal flora (microbiota) of the body demonstrate that the presence of microbes on or in the body is not necessarily indicative of disease. Procedures for handling and identifying various unknown laboratory specimens are considered. In addition, representative disease-producing microorganisms and procedures used for their identification and study are stressed in specific exercises. Section XIV provides an introduction to the fundamental areas of medical helminthology. The distinguishing features of helminths and arthropods associated with specific infectious diseases are considered. Section XV presents a number of experimental exercises that reinforce critical thinking and the scientific method. Several of the topics considered lend themselves to class demonstrations and to special student projects. The exercises include topics such as determining the effects of temperature on the bacterial growth curve, immunoelectrophoresis, the polymerase chain reaction (PCR), and DNA restriction analyses. Instructor's Manual An updated Instructor's Manual to accompany the Laboratory Manual is available and contains specific directions for the preparation of laboratory materials, alternate procedures, the use of supplementary aids, suggested sources for audiovisual aids, laboratory equipment and supplies, questions for laboratory examinations and quizzes, and answers to exercise Laboratory questions. George A. Wistreich