

Preface

Microbiology is a dynamic science. It is constantly evolving as more information is added to the continuum of knowledge, and as microbiological techniques are rapidly modified and refined. The eleventh edition of *Microbiology: A Laboratory Manual* continues to provide a blend of traditional methodologies with more contemporary procedures to meet the pedagogical needs of all students studying microbiology. As in previous editions, this laboratory manual provides a wide variety of critically selected and tested experiments suitable for undergraduate students in allied health programs, as well as elementary and advanced general microbiology courses.

Our Approach

This laboratory manual is designed to guide students in the development of manipulative skills and techniques essential for understanding the biochemical structure and function of a single cell. Its main goal is to encourage students to apply these laboratory skills in the vocational field of applied microbiology and allied health or to further pursue the study of life at the molecular level.

In this manual, comprehensive introductory material is given at the beginning of each major area of study, and specific explanations and detailed directions precede each experiment. This approach augments, enhances, and reinforces course lectures, enabling students to comprehend more readily the concepts and purposes of each experiment. This also provides a review aid if the laboratory and lecture sections are not taught concurrently. The manual should also reduce the time required for explanations at the beginning of each laboratory session and thus make more time available for performing the experiments. Finally, care has been taken to design experimental procedures so that the supplies, equipment, and instrumentation commonly found in undergraduate institutions will suffice for their successful execution.

Organization

This manual consists of 72 experiments arranged into 16 parts. The experiments progress from those that are basic and introductory, requiring minimal manipulations, to those that are more complex, requiring more sophisticated skills. The format of each experiment is intended to facilitate presentation of the material by the instructor and to maximize the learning experience. To this end, each experiment is designed with the following components.

Learning Objectives

This introductory section defines the specific principles and/or techniques to be mastered.

Principle

This is an in-depth discussion of the microbiological concept or technique and the specific experimental procedure.

Clinical Application

Clinical or medical applications that appear within each experiment help students connect what they are learning in lecture with what they are doing in the lab. For students who intend to have careers as nurses or in other allied health fields, Clinical Applications explain the relevance of each lab technique to their career plans.

At the Bench

This section signals the beginning of the experiment, and includes the materials, notes of caution, and procedural instructions—all of the things students will need to know at the bench, during the course of the experiment.

Materials

This comprehensive list helps students and instructors prepare for each laboratory session. Materials appear under one of the following headings:

Cultures These are the selected test organisms that have been chosen to demonstrate effectively the experimental principle or technique under study. The choice is also based on their ease of cultivation and maintenance in stock culture. A complete listing of the experimental cultures and prepared slides is presented in Appendix 6.

Media These are the specific media and their quantities per designated student group. Appendix 3 lists the composition and method of preparation of all the media used in this manual.

Reagents These include biological stains as well as test reagents. The chemical composition and preparation of the reagents are presented in Appendices 4 and 5.

Equipment Listed under this heading are the supplies and instrumentation that are needed during the laboratory session. The suggested equipment was selected to minimize expense while reflecting current laboratory technique.

Procedure

This section provides explicit instructions, augmented by diagrams, that aid in the execution and interpretation of the experiment.

 A caution icon has been placed in experiments that may use potentially pathogenic materials. The instructor may wish to perform some of these experiments as demonstrations.

Lab Report

These sheets, located at the end of each experiment, facilitate interpretation of data and subsequent review by the instructor. The Observations and Results portion of the report provides tables for recording observations and results, and helps the students draw conclusions from and interpret their data. The Review Questions aid the instructor in determining the student's ability to understand the experimental concepts and techniques. Questions that



call for more critical thinking are indicated by the brain icon.

New to the Eleventh Edition

For this eleventh edition, the primary aim was to build upon and enrich the student experience. The changes described below are intended to impart the relevance of microbiological lab techniques to published standard protocols, and to enhance student understanding in the validity of each of the microbiological procedures as they apply laboratories in both the educational and industrial setting.

New Tips for Success

The eleventh edition includes new Tips for Success that will help students avoid common mistakes while they learn laboratory techniques. These tips alert students to potential issues or mistakes that other students have encountered while doing the same experiments. Warning students about potential issues before they begin experiments will reduce the number of procedural mistakes and maximize the number of successful experiments.

New Experiment 39: Propagation of Isolated Bacteriophage Cultures

This experiment builds on previous experiments by utilizing procedures for the cultivation and enumeration of coliphages isolated from individual plaques produced in Experiment 38. These techniques when combined should allow students to isolate, cultivate, and further propagate bacteriophages from commercial or environmental sources.

New Experiment 46: Microbial Fermentation

The previous version of this experiment examined alcohol fermentation by yeast cells. The current experiment has been expanded to include lactic acid fermentation. Students will produce yogurt in an experimental setting, examining changes in culture pH and liquid consistency over time as a means to study bacterial lactic acid production during anaerobic metabolism.

Information Concerning Governing Bodies

Where appropriate, information concerning governing bodies, such as the Environmental Protection Agency (EPA), the Clinical and

Laboratory Standards Institute (CLSI), and AOAC International, has been included in the introductory material for some experiments. By drawing attention to governing bodies beyond the American Society for Microbiology (ASM) that have published laboratory standards, students will be introduced to the various industry standards that regulate microbiology laboratories.

Updates and Revisions

Throughout the manual, updates and revisions have been made to background information, terminology, equipment, and procedural techniques, including the following:

- Added a new procedure for a streak plate method in Experiment 2.
- Updated protocols in many experiments, including Experiment 20, to utilize micro-volume procedures.
- Added an alternate protocol in Experiment 12 that uses the McFarland Standards to culture preparations for each lab.
- Protocols for the utilization of plate readers in measuring turbidity and bacterial growth have been added to Experiment 12 and other experiments.
- Results tables have been updated for many experiments (e.g., Experiments 20 and 21) to allow instructors to modify or customize the lab to include organisms of interest not listed in the protocol.
- Added biosafety level (BSL) markers throughout the text. Organisms are clearly labeled with biosafety level 2 if they require additional precautions (**BSL-2**).

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Chad Welsh

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Contributor

Kaiser I Sheikh, *The University of Sheffield*

Reviewers

Sumitra Datta, *Amity University Kolkata*
Rajeev Kaul, *Delhi University*
Smriti Srivastava