FUNDAMENTAL EXPERIMENTS in MICROBIOLOGY

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The Cover: A scanning electron micrograph showing a typical bacterial population in the digestive system of the rat. (Courtesy of S. E. Erlandsen and G. Wendelschafer.)

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PREFACE

Fundamental Experiments in Microbiology is designed for students taking their first course in microbiology. The main objective is to acquaint the student with some of the principles and techniques which are unique to the field of microbiology. The manual should prove useful for bacteriology majors and for students majoring in natural science, agriculture, sanitary engineering, nursing, and paramedical fields who require a fairly broad introductory course in microbiology. Many of the experiments incorporated in this manual originated in W. B. Sarles, J. B. Wilson, S. C. Knight, W. C. Frazier, and E. H. Marth: "Experiments in General Bacteriology," 4th ed., 1957—a manual used for many years on the Madison campus of the University of Wisconsin.

Most experiments include a somewhat lengthy introduction to aid the student in understanding the objectives of the experiment and a discussion section at the end to determine his depth of comprehension. This approach, while increasing the size of the manual somewhat, shortens the time required for introductory remarks by the instructor and will be found particularly useful where less experienced teaching assistants must assume much of the teaching responsibility. Although the outline appears to contain a great number of experiments for a semester course, we have found that all of them can be completed with judicious planning. Instructors should, of course, feel free to substitute or delete experiments or to treat some or all of the projects in Sections V and VI as optional, to be performed as time and interests dictate.

The first few experiments included in this outline are staining technique, effective use of the microscope in observing bacterial cells, and handling of pure cultures of bacteria, viruses, and fungi. As the manual progresses, physiological and biochemical activities of the bacteria, effects of physical and chemical agents on the growth of microorganisms, and bacterial genetics are presented. The later experiments include aspects of applied microbiology, covering soil, water, and foods, with emphasis on ecology and the sanitary aspects of microbiology.

To gain interest in the applied aspects of microbiology and to learn first hand of problems facing microbiologists and of employment possibilities, students should be encouraged individually or as a group to visit various local industries, public health laboratories, and sewage treatment plants. Students have also reacted very favorably to the last experiment involving the isolation

and identification of an unknown, particularly when they have been allowed to bring a sample which is of interest to them. For safety's sake, however, the instructor may wish to exclude potential pathogens by prohibiting samples from dead animals, infected pets, and the like.

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CONTENTS

Laboratory Suggestions and Rules

SUGGESTIONS

1. It is expected that you will have read and thoroughly acquainted yourself with the subject matter before coming to class. Frequently you will be given a five to ten minute quiz on concepts given in the introduction to the experiment. The examinations may cover objectives, procedures, and results of the experiments and discussion.

2. Laboratory notes should be recorded as you complete the experiment. Accuracy and neatness are considered above artistry. Periodically, your instructor may call for the submission of your results. Be ready to hand them in at any time.

RULES

1. Cleanliness is next to virtue in bacteriology. You are expected to wipe clean your own bench space *before* and *after* the laboratory period with the bacteriocide (Roccal) provided. If you spill stains and reagents on your bench, be sure to remove them immediately.

2. Accidents, such as spilled strong acids and bases, cuts, burns, and swallowed or spilled cultures, should be reported immediately to your instructor.

3. Many of the cultures with which you will be working (including your own isolate) are potentially pathogenic; therefore, you must always practice aseptic techniques in handling and transferring them.

4. Properly label all of the materials to be used in the experiment; unlabeled test tubes and Petri dishes will be discarded by your instructor. All dirty and used glassware should be returned promptly to the receptacle provided. Do not accumulate unnecessary glassware in either your drawer or incubators. All used pipettes should be discarded into the tray or receptacle.

5. Microscopes must always be returned to their proper storage place, generally a locked cabinet, when not in use. Your instructor will examine your

microscope for cleanliness and position of objectives during storage (read Experiment 2 for proper maintenance of microscope). Any damage or defect in the microscope should be reported immediately to your instructor.

6. The common equipment needed for the laboratory will be found in your desk drawer, basket, or equivalent. Be sure to inventory the equipment assigned to you on the first and last day of laboratory. It is your responsibility to replace any lost articles.

SECTION

I

BASIC TECHNIQUES, MICROSCOPY AND CYTOLOGY

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BASIC TECHNIQUES, MICROSCOPY AND CYTOLOGY

Staining of Bacteria: The Simple Stains

Unstained bacterial cells are nearly transparent when observed by the conventional light microscope and hence are very difficult to see. Most bacteria, however, readily react with simple stains because the protoplasm of bacteria is basophilic. These simple stains allow one to distinguish bacterial cells of various morphological types (cocci, bacilli, vibrios, spirilla, and so forth) from extraneous material present in the stained smear. In addition, stained cells are firmly fixed on the slide, in contrast to living specimens. Stained slides may be retained as a record for a long period of time. Simple stains such as methylene blue, crystal violet, safranin, and acid and basic fuchsin are very widely used.

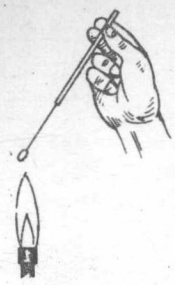
MATERIALS

1. Löffler's methylene blue stain.
2. Hucker's crystal violet stain.
3. Ziehl's carbol-fuchsin stain.
4. Pure cultures of *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Spirillum serpens.*

PROCEDURE

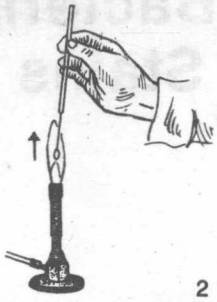
1. Preparation of Slides. A clean, grease-free glass slide is essential for obtaining good stained preparations and the slide should be cleaned as follows: Wet the tip of your index finger and rub it on a cake of Bon Ami; spread the paste thus formed over both surfaces of the slide; allow the paste to dry and then wipe it off with a clean paper towel. Cleaned, grease-free slides should be handled with care. Always hold the slide at the edges. Remember, grease from your fingers causes water to collect in tiny droplets and interferes with even spreading of bacterial specimens over the slide. Aseptic techniques for preparing bacterial smears are presented in Figure 1-1.

ASEPTIC TECHNIQUES IN PREPARING BACTERIAL SMEARS



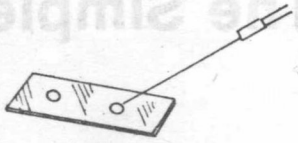
1

Place the loop above visible flame to prevent sputtering of cells.



2

Bring the loop to just above the cone; heat it until red-hot.



3

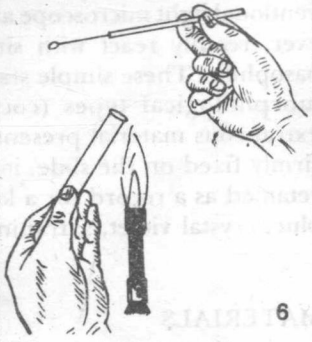
Place two drops of water on a cleaned glass slide and flame the loop again.

4

Remove the cotton plug and quickly flame the neck of the tube.

5

Remove a loopful of the culture with the cooled loop.

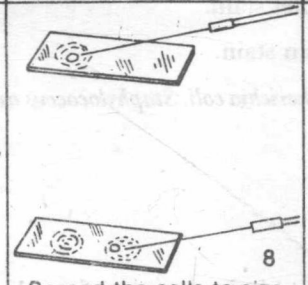


6

Flame the tube again.

7

Return the cotton plug to the test tube.



8

Spread the cells to size of a dime; allow the smear to air dry. Flame the loop, but avoid spatter.

9

Heat fix the cells by quickly passing the slide over the flame twice.

FIGURE 1-1

2. Following Figure 1-1, sterilize the wire inoculating loop by flaming it until it is red hot. Cool for a few seconds. Do not touch it to anything.

3. Using the sterile loop, place two small drops of tap water on the slide approximately 1.5 cm apart.

4. Resterilize the inoculating loop in the flame as shown in Figure 1-1; cool for a few seconds. With the tip of this sterile loop, remove a very small amount of growth from the surface of the *E. coli* agar slant culture. Do not dig into the agar medium. Only a very small amount of the culture is needed to make a smear. (Your instructor will demonstrate the detailed aseptic techniques involved in the handling of pure cultures.)

5. Mix the culture on the tip of the inoculating loop with one of the drops of water on the slide. With a circular motion, spread the drop out to the size of a dime. A well-mixed smear has a *pale*, milky appearance. Flame the loop again as soon as the smear is made by holding the loop above the flame until the inoculum is dry. Only when the inoculum is dry should the loop be plunged into the flame and heated until red hot. If heated correctly, inoculum will not be spattered on your bench top or clothing.

6. Repeat Steps 4 and 5 with the second culture, *S. aureus*, on the other droplet on the same slide. Remember, each time the inoculating loop is used it is essential to flame sterilize it in order to avoid contamination of the succeeding pure culture.

7. On a second grease-free slide, follow the same procedure for cultures of *B. cereus* and *S. serpens*.

8. Allow the smears to air dry, then "heat fix" them by quickly passing the slides through the flame (right side up) two or three times (see Fig. 1-1). Properly "heat-fixed" slides can be handled easily; if they are too hot to handle, you have heated them too much.

9. Place the air-dried, heat-fixed slides on a staining rack over the sink or staining tray; make sure the slides are level.

10. Stain *E. coli* and *S. aureus* smears with crystal violet and *B. cereus* and *S. serpens* smears with Löffler's methylene blue for one to two minutes. Do not allow to dry. (You may consult with a classmate on the selection of the simple stains; your classmate or partner may use carbol-fuchsin in place of crystal violet.)

11. After the smears have been exposed to the stains for 1 to 2 minutes, pour off the excess stain into the sink or staining tray. Wash the slides by placing them under a gentle stream of water or by dipping them in a tumbler of water until the water draining from the slides becomes colorless.

12. Remove all excess water from the slides by touching one corner of each slide to a clean, absorbent paper pad and gently blotting dry, as shown in Figure 1-2. Label the slides with a marking pencil.

The dried slides are now ready for microscopic examination. Keep them in your slide box until you have learned the parts and proper use of the microscope.