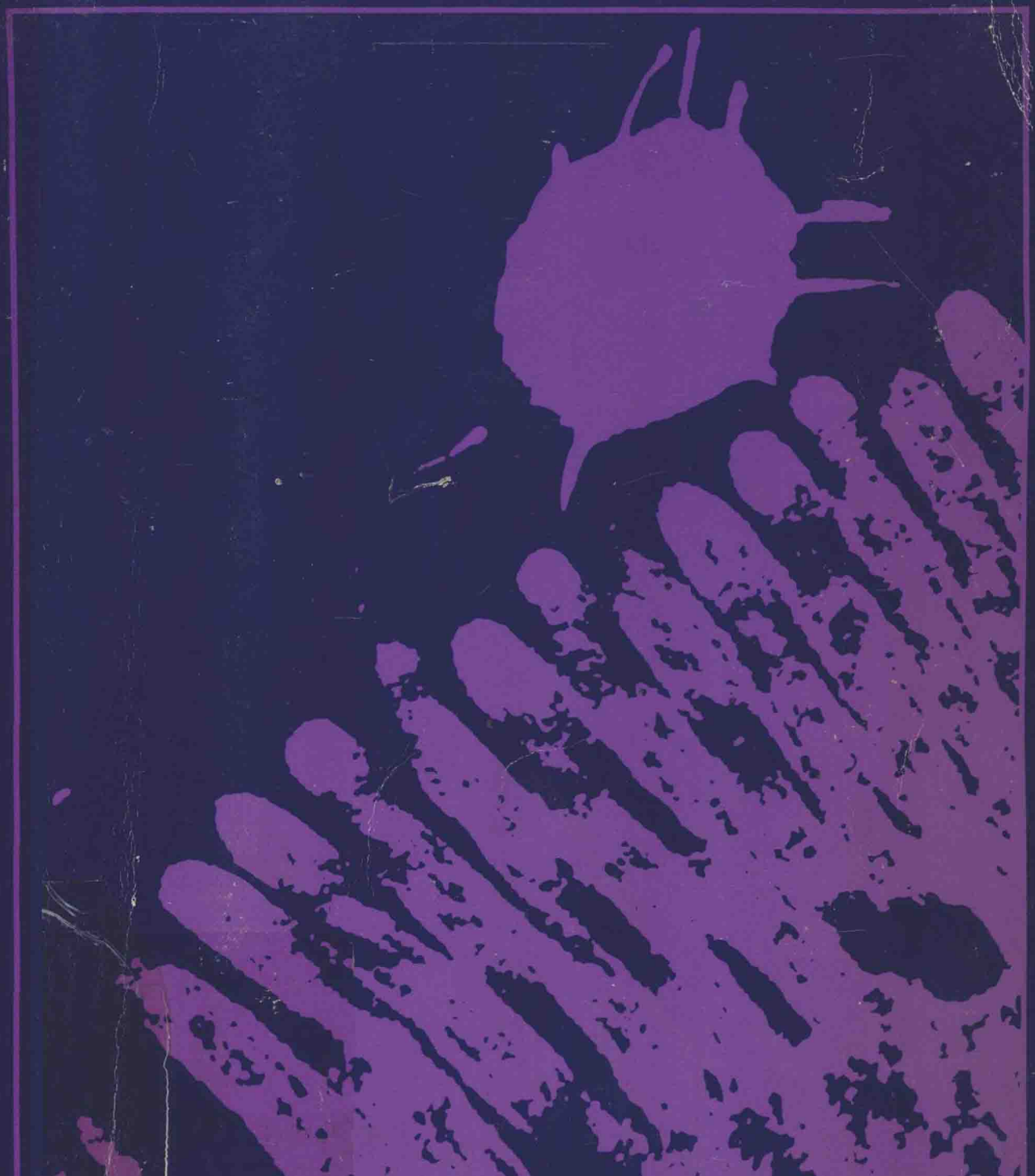
LABORATORY MANUAL TO ACCOMPANY BASIC MEDICAL MICROBIOLOGY

SECOND EDITION

ROBERT F. BOYD, Ph.D., AND BRYAN G. HOERL, Ph.D.



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PREFACE

This laboratory manual was written for students enrolled in a medically oriented microbiology laboratory course and as a supplement to our text (Boyd, R. F., and Hoerl, B. G., *Basic Medical Microbiology*, Second Edition, Boston, Little, Brown, 1981). It is designed to acquaint the student with the basic techniques currently being utilized in the clinical microbiology laboratory. It will be helpful not only to the future medical technologist but to any microbiology student interested in the strategies used for isolating and identifying microorganisms pathogenic to humans. Exercises and discussions are presented in a manner that allows the student to follow the development of methods and techniques that are required for the isolation and identification of infectious agents. We feel strongly that a laboratory manual should teach as well as offer procedural methods and techniques. Nearly all of the exercises are preceded by introductory discussions. These brief introductions may include the background and theory of the procedures or reactions that are part of the experiment, the purpose of the experiment, and when appropriate a description of the strategies employed in the isolation of the infectious agent. Within the text we have asked many questions and also have answered them. Someone might object that this type of approach closes off student inquisitiveness. As previously stated, we believe that a medical microbiology manual should be a teaching tool. We hope that the questions that have been answered will stimulate the student to ask other questions.

The manual is divided into seven parts. The first three are devoted to general techniques and procedures that must be learned by any student of microbiology no matter what the applied area. If the techniques in these parts are mastered, the student will be ready to apply them to actual clinical procedures. Part IV presents individual biochemical tests that are routinely used in identification procedures. They also demonstrate the scope of the problems associated with the identification of an infectious agent by any or several of these tests. Part V deals with the control of microorganisms. With a greater understanding of antibiotic sensitivity, for example, the student will realize the importance of the clinical laboratory in terms of patient treatment. Part VI is concerned with methods for the isolation and identification of microorganisms from pure cultures as well as specimens obtained from patients. It also contains tables describing the various tests used to differentiate species within a particular genus or group. Part VII presents exercises that demonstrate the more common types of antigen-antibody reactions, i.e., agglutination and precipitation. The appendixes comprise a correlation of the exercises with discussion in the textbook (1), instructions for the instructor's preparations (2), a brief treatment of the dilution technique (3), a description of some mini-tests currently used in the identifica-

tion of the Enterobacteriaceae (4), and an alphabetical list of reagents, stains, and media (5).

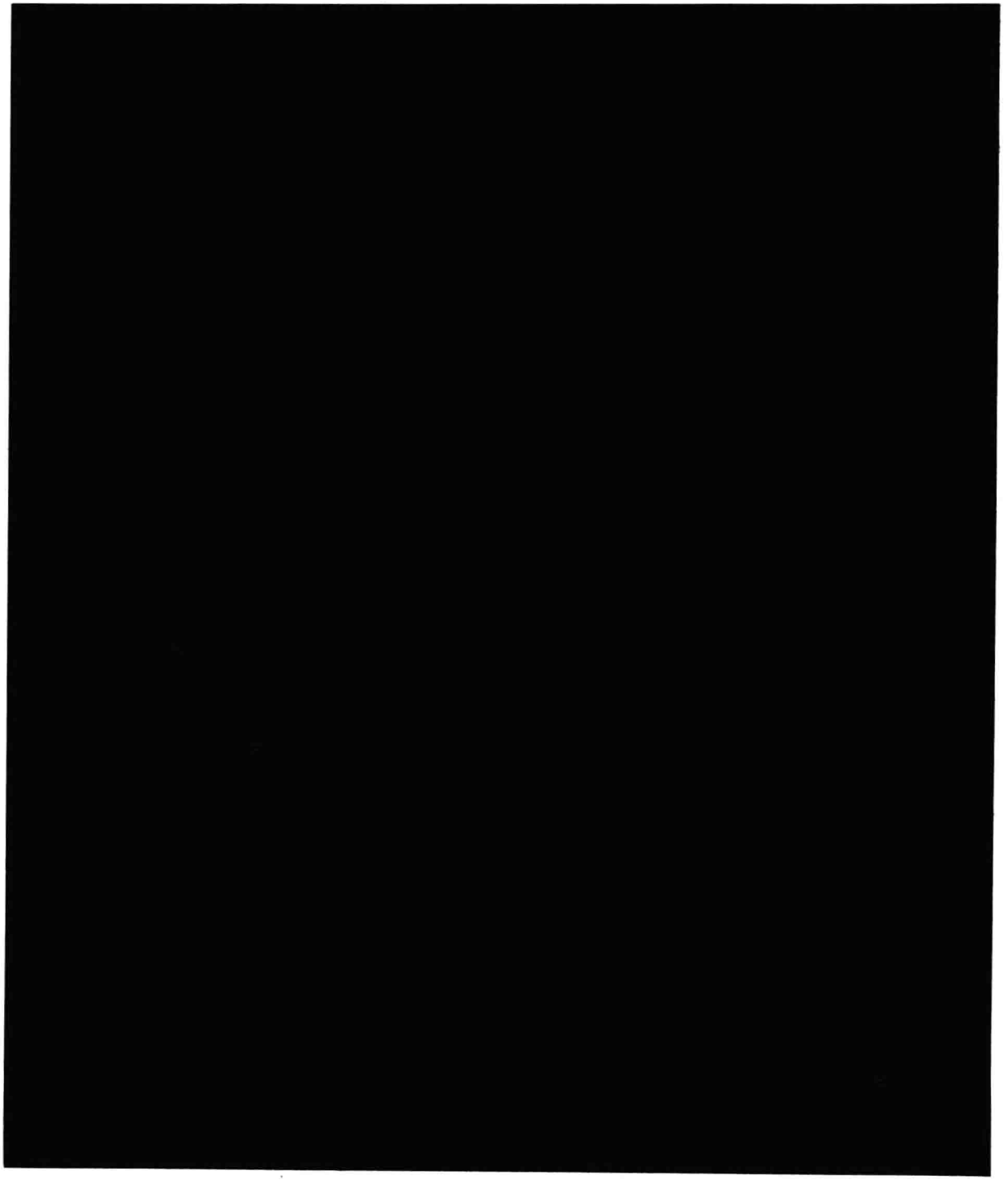
A special thanks to Beverly Boyd, who typed the major portion of this manuscript, and to Mary Ann Komorowski, our illustrator.

R. F. B.
B. G. H.

CONTENTS

APPENDIXES

PART I GENERAL LABORATORY TECHNIQUES AND PROCEDURES



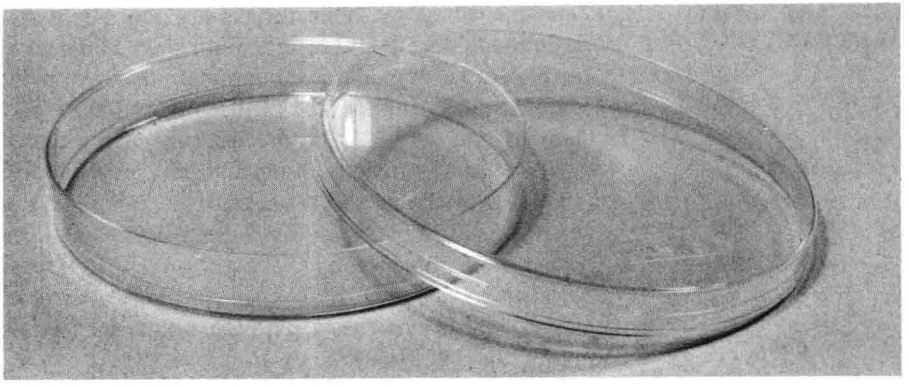
RULES AND SAFETY MEASURES

The microbiology laboratory is a place where microorganisms are cultivated, identified, stored, and tested. With such a high concentration of microorganisms in one area, accidental contamination of yourself and your neighbor through carelessness or ignorance of the proper safety measures can be a serious problem. If you take specific precautions each time you enter the laboratory, they will eventually become a normal part of your routine and you will not have to think twice about safety measures. Some microorganisms to be used for the exercises described in this laboratory manual are pathogenic species while others are considered "nonpathogens." It is best to remember that any microorganism under the proper circumstances is a potential pathogen, and the same precautions used for pathogenic species should also be applied to the nonpathogen.

When aseptic technique is maintained in the laboratory, few contamination problems will arise. Aseptic technique, which will be described in the manual and by your instructor, is designed to prevent the contamination of clinical specimens as well as pure cultures of microorganisms to be tested. In addition, aseptic technique prevents contamination of the environment, including yourself and your neighbor, from a clinical specimen or from the cultures issued for study. Other precautionary steps that will help to avert contamination are as follows:

1. Wear a lab coat or apron at all times in the laboratory. The lab coat should be cleaned after every laboratory experiment.
2. Treat any cuts or abrasions before entering the laboratory. Report any cuts acquired in the laboratory to your instructor for treatment.
3. Before entering the laboratory and when leaving the laboratory wash your hands with soap and water or a disinfecting solution. Those of you entering hospital work will find that handwashing is the greatest deterrent to accidental contamination of patients. The hospitalized patient is most susceptible to infection even from so-called nonpathogenic species.
4. Keep fingers, pencils, and pens away from your mouth. Do not lick labels (use tap water).
5. Do not bring food or drink into the laboratory.
6. Keep your bench area free of extraneous materials such as papers, books, and any unnecessary equipment or glassware.
7. Disinfect your bench area when you come into the laboratory and after you have finished your experiment. Your laboratory bench area may be used by others and you would not want to accidentally contaminate them.
8. Do not throw any refuse into the sink. Containers for disposal of materials will be provided by your laboratory instructor. Containers for the disposal of slides and pipettes will also be provided.
9. If a culture is spilled on the bench top or on the floor, ask a classmate to stand guard over the area and then notify your instructor or laboratory assistant immediately. The area of contamination should be covered with paper towels that have been soaked in disinfectants such as 2% phenolics (O'Syl, Staphene, Vesphene) or buffered glutaraldehyde (Cidex).

Figure 1. Petri dish.



MATERIALS AND EQUIPMENT

THE PETRI DISH AND ITS CONTENTS

The Petri dish (Fig. 1), made of glass or a disposable plastic, holds a quantity of an agar medium and is used to isolate microorganisms in the form of colonies. Disposable plastic Petri dishes obtained commercially are presterilized and have for the most part supplanted glass dishes, which must be sterilized in the laboratory. The standard Petri dish used in the laboratory is 15 × 100 mm. Approximately 20 ml of a molten agar medium is the standard amount used in the dish. Once the molten agar has been poured, the top of the dish is replaced and the agar is allowed to harden (this takes about 15 to 20 minutes at room temperature). If bubbles appear on the surface of the molten agar, the flame from a Bunsen burner passed rapidly over the bubble will burst it. The agar plate should be inverted to prevent the condensation that accumulates on the lid from falling on the agar surface. Inverting of plates also prevents the agar from drying out rapidly. Agar plates can be left out at room temperature for 24 hours or placed in a 37°C incubator for 3 to 4 hours to speed the drying of the agar surface. Any agar surface should be relatively free of moisture before any attempt is made to streak it with microorganisms. Most agar plates can be stored in the refrigerator for weeks and still support bacterial growth.

CULTURE TUBES

A culture tube is basically a test tube (varying size) that can hold either a broth solution or an agar medium. In the tube biochemical as well as physical characteristics of microorganisms may be determined, depending on the medium used. The mouth of the tube is closed by a cotton plug, a metal cap, or a plastic screw cap. Tubes containing a fermentation broth may also contain a small inverted glass vial called a *Durham tube* (Fig. 2). The purpose of the Durham tube is to trap gases produced by microorganisms during metabolism. For preserving microorganisms in the laboratory an agar slant prepared in the test tube is often used for short-term storage (weeks to months, depending on the microorganism). Tubes containing a culture streaked onto an agar slant are sometimes used for short-term storage of the culture. In upright tubes in which the agar remains horizontal, the motility of microorganisms can be determined by

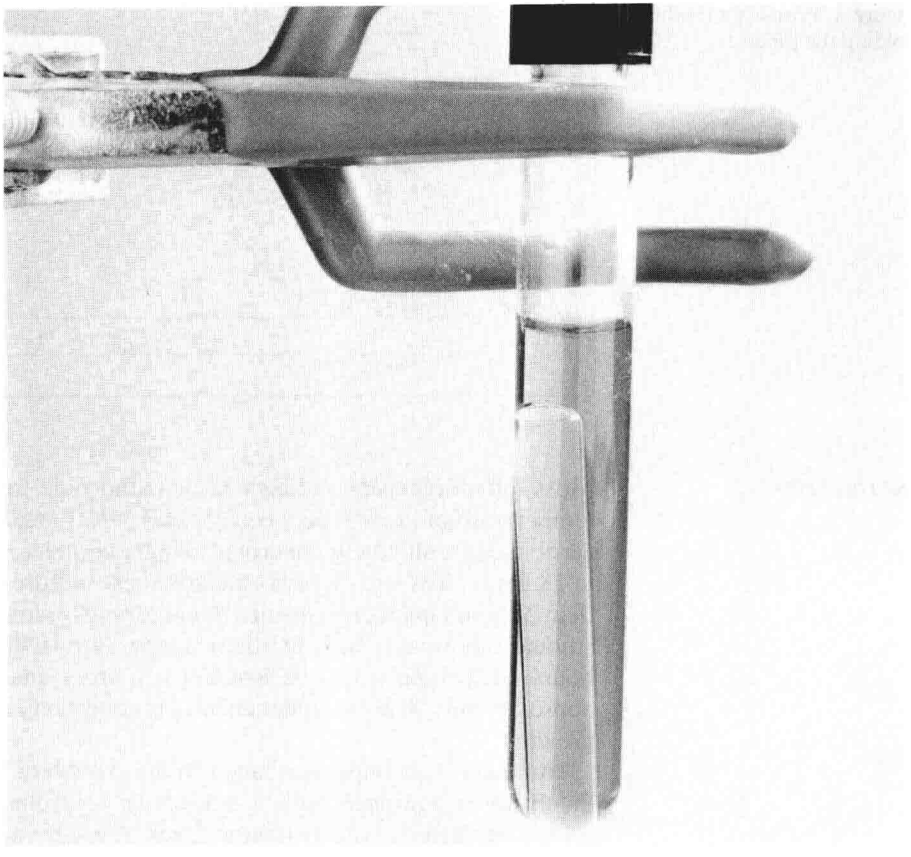


Figure 2. Fermentation broth tube containing the smaller Durham tube for trapping gases.

stabbing the agar with an inoculating needle (this technique is discussed later in the manual). Because of the unavailability of oxygen in the depths of the agar, stabbing also permits the growth of microorganisms that can grow anaerobically. Cultures on a slanted surface stored in the refrigerator should not be relied on to remain viable for more than a week. To ensure greater viability over a longer period the agar slant can be covered with sterile mineral oil.

MICROSCOPE SLIDES

Microorganisms and clinical material are applied to microscope slides and usually stained in preparation for microscopic examination. The glass slide may also be used to ascertain serological reactions such as the agglutination of bacteria. Slides should be free of any fatty or oily residues that often result from handling. If the slides are cleaned with detergent or solvents, the material to be stained spreads more uniformly over the surface of the glass and adheres better to the slide. Some slides with a hollowed-out center are used for motility determinations. These are discussed in Exercise 8.

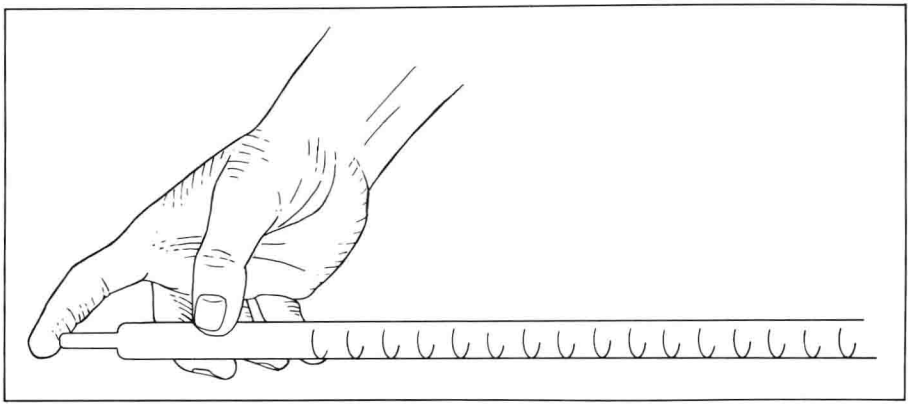


Figure 3. Proper method for holding the pipette.

INCUBATOR

Most microorganisms indigenous or pathogenic to humans have an optimum temperature of growth between 35° and 37°C. At 37°C microorganisms that are aerobic or facultatively anaerobic usually complete a cycle of growth within 12 to 18 hours. Tests to be performed on these cultures should be initiated no later than 24 hours unless the species is a notoriously slow grower. Cultures left in the student laboratory desk at room temperature will grow in approximately 48 hours. If the species to be isolated is a strict anaerobe, the culture will take approximately 48 hours under anaerobic conditions at 37°C to complete a cycle of growth.

Humidity is an important factor in the growth of microorganisms. Unless the incubator is equipped with a device for controlling humidity, pans of water should be placed in the incubator. Lack of water vapor causes the agar plates to dry out more rapidly than they would otherwise.

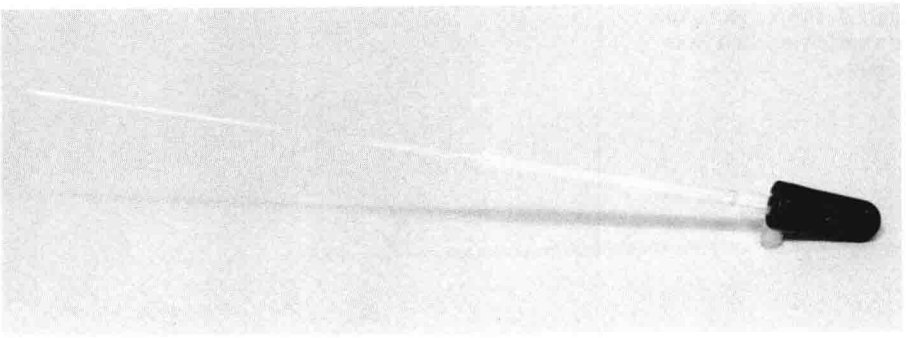
PIPETTES

Pipettes used for transfer of specified quantities of broth cultures or of solutions may be plastic and disposable or reusable glass. Single-use plastic pipettes are presterilized; the glass type must be cleaned and sterilized after each use. Glass pipettes are usually sterilized in metal canisters. The canister should be left flat on the tabletop when a sterile pipette is being removed. As soon as the pipette is taken out, the lid of the canister should be carefully replaced so as not to touch the mouths of the remaining pipettes with the outer part of the lid. It is particularly important to remember that a sterile pipette should not be removed from the canister until one is ready to use it. Placing it on a nonsterile surface or handling the lower end breaks the sterility. When fluid is being transferred, the pipette should be held in such a way that the index finger and not the thumb controls the flow of fluid (Fig. 3). Once the fluid or culture has been transferred, contaminated pipettes should be placed in a special receptacle containing a disinfectant or in a container that can be sterilized.

The Pasteur Pipette

Pasteur pipettes are uncalibrated fluid-delivering devices that are drawn out to capillary size for about half their length. With a rubber bulb providing suction,

Figure 4. Pasteur pipette.



they are used for mixing solutions and suspensions and for transferring unmeasured amounts of fluids (Fig. 4).

ASEPTIC TECHNIQUE

In any laboratory, whether a research, clinical, or teaching laboratory, it is extremely important that the microbial material being examined or tested be free of unwanted or contaminating microorganisms. A procedure that prevents the introduction of unwanted microorganisms or destroys contaminating agents already present is called *aseptic technique*. As students in the microbiology laboratory you will be given laboratory media, pipettes, flasks of broth, etc., that have been previously sterilized by laboratory assistants. In most laboratory manipulations unwanted microorganisms must be prevented from contaminating these solutions or glassware. Since microorganisms are present in the air on dust particles, they can also be found on clothing, skin, and fomites that have been exposed to the air. Certain special precautions must be taken in the laboratory to prevent contamination. Two important procedures require aseptic technique.

PIPETTE TRANSFER OF FLUID

It is sometimes necessary to transfer aliquots of a broth culture to another flask or tube of broth—for example, when preparing dilutions of a culture. Remove a sterile pipette from a canister and hold it between the first and third fingers. Remove the lid or cap of the flask or tube containing the culture with the same hand using the fourth and fifth fingers (Fig. 5). Flame the top of the culture tube and insert the pipette to remove the appropriate aliquot of fluid. Replace the lid on the culture tube. Remove the cap or lid of the tube to receive the aliquot and flame the lip of the tube. Insert the pipette and allow the fluid to flow down the side of the tube above the surface of the broth. Do not plunge the pipette into the fluid (Fig. 5).

INOCULATING LOOP TRANSFER OF MICROORGANISMS

The inoculating loop must always be free of debris or contaminating microorganisms before it can be used in culture transfer. Just before use, sterilize the inoculating loop by inserting the major portion of the loop first into the coolest part of the flame (yellow) of a Bunsen burner, then into the hot blue cone. After the entire wire becomes red hot, remove it and allow it to cool for 6