Marie Gilstrap
John Kleyn
Eugene W. Nester

MICROBIOLOGY EXPERIMENTS

a health science perspective

Marie Gilstrap
John Kleyn
Eugene W. Nester

illustrations by
Annetje Claringbould

MICROBIOLOGY EXPERIMENTS

a health science perspective

accompanies The Microbial Perspective by Nester, Pearsall, Roberts and Roberts.

SAUNDERS COLLEGE PUBLISHING
Philadelphia New York Chicago
San Francisco Montreal Toronto
London Sydney Tokyo Mexico City
Rio de Janeiro Madrid

Address orders to
383 Madison Avenue
New York, NY 10017

Address editorial correspondence to:
West Washington Square
Philadelphia, PA 19105

This book was set in Century Schoolbook by Caledonia Composition.
The editors were Michael Brown, Amy Satran, Patrice L. Smith.
The art & design director was Richard L. Moore.
The text design was done by Larry Didona.
The cover design was done by Richard L. Moore.
The artwork was drawn by Annetje Claringbould.
The production manager was Tom O'Connor.
This book was printed by Connecticut Printers.

MICROBIOLOGY EXPERIMENTS:
A HEALTH SCIENCE PERSPECTIVE ISBN 0-03-057008-5

Library of Congress catalog card number 81-53093.

234 111 987654321

CBS COLLEGE PUBLISHING
Saunders College Publishing
Holt, Rinehart and Winston
The Dryden Press

PREFACE

This laboratory manual is designed for health science students in both two- and four-year programs who wish to learn not only the basic principles of laboratory microbiology but also how to apply these principles to their profession. For example, nurses need to understand how to collect specimens, how to interpret reports from the clinical microbiology laboratory, and how to prevent the spread of infection. Medical technologists need to understand how to handle the specimen and to process it for actual identification. Other paraprofessionals will be involved in other ways with microbiology. But all health science students require a good introduction to the basic principles and their clinical applications. The laboratory exercises include excellent illustrations and are organized into three sections according to the following outline:

Part I emphasizes *fundamental principles and techniques* in microbiology. Topics covered include the use of the microscope; pure culture and aseptic technique; bacterial staining methods, including commonly used structural stains; growth and metabolism of microorganisms; control of microbial growth by chemical, physical, and antibiotic means in experiments emphasizing disinfection and sterilization. How bacteria may develop resistance to antibiotics is illustrated by the gene transfer experiment.

Part II emphasizes *clinical and diagnostic procedures* in microbiology. Collection and microbiological examination of two clinical specimens, urine and throat, introduce students to the clinical aspect of microbiology. They acquaint students with normal bacterial flora and the potential pathogens from different parts of the body. The procedures most useful in the isolation and identification of these common organisms follow each clinical specimen. A clinical unknown specimen dramatically demonstrates the way in which these techniques are actually used in a clinical microbiology laboratory, and they provide the practical experience necessary for the interpretation of laboratory findings. Other exercises relate to the culture and identification of viruses and to protozoan and helminthic parasites. The exercises on fungi, although emphasizing the fundamental principles of mycology, also deal with medically important fungi, such as *Candida albicans*, frequently isolated from clinical specimens. One final experiment includes methods used in diagnostic immunology.

Part III emphasizes the *preventive aspects* of microbiology: epidemiological and public health procedures. Exercises on finding *Staphylococcus* carriers and on evaluating the safety of milk and water demonstrate surveillance techniques used by hospitals and public health laboratories for infectious disease prevention. The nonlaboratory exercises further help students increase their understanding of infectious diseases and their prevention, especially in hospitals.

Special practical features of this manual include information on ordering stock cultures and prepared media; preparation of media, stains and reagents; notes to the instructor about the preparation and use of each exercise; and an index.

Note to the Student

Most of you are taking this microbiology laboratory course because you plan to enter a health science career that requires a

background in microbiology. Therefore we hope that as you use this manual in the laboratory you will acquire a better understanding of the basic principles of microbiology and the ability to apply the skills and knowledge in your prospective profession. Your success and sense of satisfaction from the laboratory work will be enhanced if you follow these few simple rules:

1. Read the scheduled laboratory exercise *before* coming to the laboratory session. This prior preparation will enable you to plan your work with your partner, to proceed with the experiments efficiently, and to ask the instructor questions about anything you don't understand.
2. Study the Getting Started and Objectives sections so that you will understand *why* you are doing the experiment, *what* you should learn from it, and *how* it relates to the current topic of study.
3. Record your experimental results and observations in the Results section while you are making them; do not take notes on another piece of paper and recopy them onto the Results page later. You can tear the report sheet out of the manual, so that you can read the instructions in the manual and record the results at the same time. This procedure greatly increases the accuracy of your observations and reduces the chance for error.
4. Answer the questions at the end of the Results section. These questions are intended to stimulate your thinking about the *significance* of the experiment and its *relation* to the *basic principles* and *clinical applications* of microbiology. Reference to the Nester text (chapter reference given for the experiment) and especially the Getting Started section of the exercise will help you. Since many of the questions grow directly out of each experiment, answers may not always be found in the text. You may need to put together information from the text, the Getting Started section, and especially your own observations to arrive at an appropriate answer. There may be no one *right* answer!
5. The Clinical Unknown exercise (Ex. 22) provides the opportunity for you to apply the laboratory skills and knowledge you have acquired in the course to a specific clinical culture. This personal involvement in problem solving will not only give you an additional learning experience but will also greatly increase your sense of satisfaction and confidence in your own achievements.
6. The microbiology laboratory poses risk to the beginning student because you are dealing with living organisms, some of which are capable under certain conditions of causing infection; and because you are just learning the techniques, errors may occur in the process. Thus it is *absolutely essential* that you know the procedures used to promote laboratory safety, including those to reduce risk wherever the occasional accidents occur.

For this reason the following section on Laboratory Safety must be studied before laboratory work begins.

We hope you enjoy and profit from these experiments as much as we have enjoyed working in microbiology laboratories and teaching our students over the years. We have appreciated their enthusiasm and constructive comments about microbiology experiments; these have given us inspiration and practical help in writing the manual.

LABORATORY SAFETY

To be read by students *before* beginning any laboratory work.

The microorganisms used in this laboratory manual are of little or no risk to healthy people working with them provided that standard microbiological practices of containment are followed. The few organisms that pose even a slight risk are species normally inhabiting the human body.

Students with special health problems that increase their risk of infection should notify the instructor, discuss the course with their physicians, and be especially diligent in following these precautions. Examples of such conditions are diabetes, mold allergy or tendency to allergies, disorders of the immune system, and immunosuppressant (or immunosuppressive?) therapy.

To maintain safe conditions in the microbiology laboratory, students, instructors, and laboratory assistants should observe the following.

1. Do not eat, drink, smoke, or store food in the laboratory.
2. Bring only necessary equipment and the laboratory manual to the work bench. Leave coats and other textbooks in another part of the room, away from the work area.
3. Wear a protective laboratory coat or apron when you are working with cultures, and avoid wearing long, full sleeves if possible.
4. Tie long hair back or put it up. If hair hangs loose, it becomes a contamination hazard and also risks catching fire in the bunsen burner flame.
5. Wear safety glasses when they are recommended in a laboratory procedure.
6. Carry and store cultures of microorganisms in racks or baskets. Do not leave cultures on the table or in unmarked areas when the laboratory session is completed.
7. Place cultures to be discarded in racks or trays designated for contaminated material; these racks should be clearly labeled. All such materials should be autoclaved before further handling, discarding, or washing.
8. Decontaminate work surfaces after spills and at the end of each laboratory period with the disinfectant provided in the laboratory.
9. In case of spills notify the instructor or laboratory assistant. Cover small spills with paper towels, soak the towels well with disinfectant. Let the towels stand for half an hour. Carefully pick them up with a broom and dustpan or with clean towels. Place these materials immediately in a container with disinfectant or in a plastic bag which can be sealed. Autoclave container before discarding.
10. In the case of a large culture spill, especially one with the possibility of aerosol production, students should be asked to leave the area. Apply the disinfectant to the area of the spill, and then leave the room closed for 1 hr. Clean the area.
11. Gently flame inoculating loops after use and flame glassware after inoculation to avoid breaking or splattering.
12. Mix liquid cultures gently to avoid foaming and splashing, which may produce an aerosol of bacterial culture.
13. Never pipette cultures by mouth.
14. Instructors should demonstrate proper laboratory tech-

niques to students before they carry out the procedures themselves. Work should be planned to avoid haste and shortcuts.

15. Wash hands carefully with the soap provided after any possible contamination and before leaving the laboratory.
16. Keep the laboratory doors closed to avoid unnecessary drafts.
17. Report any laboratory accident to the instructor so that proper procedures may be followed. Cuts should be treated promptly. Open cuts should be protected when you are working in the laboratory.
18. Individuals who have special health problems should be especially careful in following these procedures and any special precautions recommended by their own physicians.
19. The laboratory should be properly labeled as a "Microbiology Laboratory" so that all persons who enter or use the area are aware of the slight potential biological hazard. If other classes are scheduled in this area, cultures should be discarded or stored and labeled, work areas decontaminated, and contaminated materials removed.

CONTENTS

FUNDAMENTAL TECHNIQUES AND PRINCIPLES

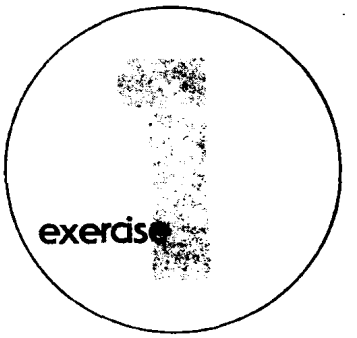
Fundamental techniques and principles of microbiology are included as Part I of this manual since they provide the foundation necessary for proper understanding and appreciation of the clinical and diagnostic procedures in Part II and public health procedures in Part III. Gene transfer is included as a fundamental experiment since such phenomena often affect the resistance of bacteria to antibiotics.

In an initial experiment, students are made aware of the numbers and variety of bacteria in the environment including the human body. This experiment also provides an understanding of normal body flora. You are then made aware of how to prepare culture media and, once prepared, how to isolate microorganisms in pure culture.

Following this introductory material is an exercise on the importance and use of the microscope. A related exercise showing how to measure the size of microorganisms with the aid of the microscope follows. The next two experiments teach you how to help identify microorganisms isolated by the use of the Gram stain and certain other special staining techniques. The use of special media to isolate and differentiate bacteria is included as a related experiment.

The nutritional requirements of bacteria for growth and metabolism are considered next, in Exercises 9 and 10, as well as in Part II (Exercises 17, 18, 20, and 21). Knowledge of metabolic reactions is important for species isolation and identification; e.g., two bacteria which are similar in appearance when cultured and examined microscopically may be quite different when examined metabolically. It is the sum of these microscopic and metabolic tests which help identify species.

The section finally contains a group of experiments illustrating how man may selectively control the growth of bacteria. Methods of control discussed include ultraviolet light, heat, chemicals (antiseptics and disinfectants), and antibiotics. As an example, one of the fundamental concepts taught experimentally is the relationship of cell wall structure and protein synthesis to narrow and broad-spectrum antibiotic susceptibility, respectively.



Natural Habitats and Communities of Bacteria

GETTING STARTED

Even though you may be starting a career in a health-related field, you may be unaware of the number of bacteria and the variety of types of bacteria found everywhere in the environment, including the human body. In this exercise, you and other class members will demonstrate the presence of bacteria by culturing samples taken from different places on the body, from the laboratory, and from the natural environment. Samples from these sources are then grown in a culture medium which contains the nutrients bacteria require, such as sugars, amino acids, vitamins, and minerals. Agar is then added to solidify the nutritious solution; this provides a solid surface for bacterial growth. Material from one source is streaked onto this surface and then allowed to grow (incubate) at an appropriate temperature. Within one or two days each bacterium will reproduce by many cell divisions resulting in a single visible colony which contains millions of cells. Each bacterial species has its own characteristic type of colonial growth, which will vary from that of other bacteria in size, shape, color, and consistency. By observing the *total number* of colonies, the number of different *types of colonies,* the different *growth responses,* different *incubation temperatures,* and results of culturing the *same source* from different individuals, you will find answers to the following questions:

1. Which sources have many bacteria and which sources have few?
2. How many different species of bacteria, as revealed by the different colony types, are found from any given source?
3. Does each human source, even from different individuals, have its own typical community with characteristic types of colonies? Does each source differ from the other human source, even though the cultures are taken from the same individual?
4. Do environmental factors (for example, the temperature) affect the number and types of colonies found in the culture?

Your experience with this exercise is very important because you will learn to carry out new laboratory techniques and make first-hand observations which relate directly to the basic practices and concepts of microbiology studied in this course, and to your future health career. In cultures taken from various body locations you will see groups of bacterial species that normally reside there. These constitute the "normal flora" for that source and may differ considerably from the bacterial community found in a different body location. Health professionals need this knowledge in order to distinguish the normal flora from a possible infectious agent when interpreting microbiology reports. They also need to understand how these normal flora can occasionally serve as a source of infection when they get into a different area of the body, or when the patient's resistance is lowered.

The cultures studied in this exercise were all taken from areas exposed to the outside environment and thus always contain a community of normal flora. However, cultures from parts of the body not so exposed, such as blood, cerebrospinal fluid, internal organs, and bladder urine, are normally sterile.

Thus, any bacteria isolated from these internal areas usually indicate the presence of infection rather than normal flora.

This exercise should also dramatically demonstrate the way in which the human body can become infected or a laboratory culture can become contaminated from the external environment. In non-human environmental cultures, you will observe that all sources are populated with bacterial species. From this you can infer that special precautions must be taken to prevent contamination of your cultures from the outside, and that the environment contains bacterial species that might cause human infection and thus constitute a *reservoir* or source of disease agents.

Finally, in this very first exercise you are being introduced to *aseptic technique,* the procedure followed by microbiologists and health workers to prevent contamination of cultures from outside sources and to prevent the introduction of potential disease agents such as bacteria or viruses into the human body. The precise methods for handling sterile materials, for taking samples, for making cultures, and for disposing of contaminated materials after use are all designed to prevent the spread of bacteria from one area to another. Close attention to the details in the written procedure and in the instructor's demonstrations will prevent contamination and infection, and will be of practical value throughout the course.

OBJECTIVES

1. To demonstrate the presence, total number, and variety of bacteria in environmental and human habitats.
2. To compare different habitats with respect to the total number and to the types of bacteria found.
3. To demonstrate the effect that temperature has on the growth of bacteria.
4. To give you direct experience with normal flora.
5. To give you direct experience with the possible environmental sources of infectious bacteria and laboratory contaminants.

REFERENCES

Nester, Eugene W., Nancy N. Pearsall, Jean B. Roberts, and C. Evans Roberts, *The Microbial Perspective.* Philadelphia: Saunders College Publishing, 1982.

Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. 9th ed. Detroit: Difco Laboratories, 1971.

Other manufacturers' manuals for culture media.

MATERIALS (per team of two students)

1. Four sterile cotton swabs, one per tube
2. Four trypticase soy agar (TSA) Petri dish plates
3. One tube sterile water

Note: An inoculating loop and one glass-marking pencil for each student, 1 Bunsen burner per team, and 1 beaker with chemical disinfectant per table for the discard of swabs, are all part of the supplies provided to the student throughout the course, and will not be listed again.

PROCEDURE

NOTE: Be sure to read this entire procedure before *beginning the actual work.*

This exercise is divided into two parts. In the **First Session** you will *prepare the cultures* from different environmental and human sources, and incubate them at two different temperatures. In the **Second Session** you will *make final observations* of bacterial growth on the culture medium and record the results on the special report sheet. After completing these observations, you will *analyze the significance* of these results and answer the interpretive questions following the report. Most of the laboratory exercises in this manual will follow this two-step procedure, so that on any given day, you will usually be completing an exercise set up during the previous session, and will be setting up a new exercise to be completed at the next session.

FIRST SESSION

Since this is your first day in the laboratory, wait for the instructor to demonstrate the procedures described below and to make the specific assignment for each team. Use this list and additional ones as suggestions for specific culture sources and the method for obtaining the inoculum.

HUMAN BODY

Throat (swab)
Nose (swab)
Skin (swab)
Hair (special method)
Scalp (swab)
Outer ear (swab)
Fingernails (swab)
Fingers, before and after washing (special method)
Cough (special method)
Teeth or gums (swab)

ENVIRONMENT

Laboratory or bench top (swab)
Sink (swab)
Refrigerator (swab)
Floor (swab)
Air (special method)
Soil (special method)
Clothing (special method)
Metal door knobs (swab)
Coin (swab)
Newsprint (swab)

In this exercise, as in most microbiology experiments, you are dealing with *living bacteria,* so it is important to follow the procedures exactly to avoid contamination or infection. Read the section on Laboratory Safety *before* doing this exercise. The following precautions are especially important:

a. Whenever you have materials from the human body, such as the swabs from nose, throat, etc., or cultures of any kind, discard them in the appropriate designated places, after the completion of your work with them. Do not place them on the table top, or touch anyone with them. Put the cultures into the special container for contaminated material. They will then be steam-sterilized (autoclaved) before further handling.

b. When making cultures from a specific source, the materials used (swab, loop, culture medium, and so forth), except for the inoculum itself, must be sterile and remain sterile in order to avoid contamination. Therefore, the loop must be heated until red hot to kill bacteria and must not be allowed to touch any area but the inoculum until you are ready to inoculate. The lid is then held in one hand over the surface while you are inoculating the surface; the lid is immediately replaced (see Fig. 1-2).

c. The cultures you will observe in the **Second Session** have a very high concentration of bacteria because of the 2-day growth period. Even though they are "normal" inhabitants in the environment or on the body, they can cause an infection if they get into an open cut or are transmitted to the mouth or eyes from your hands because of the large number of

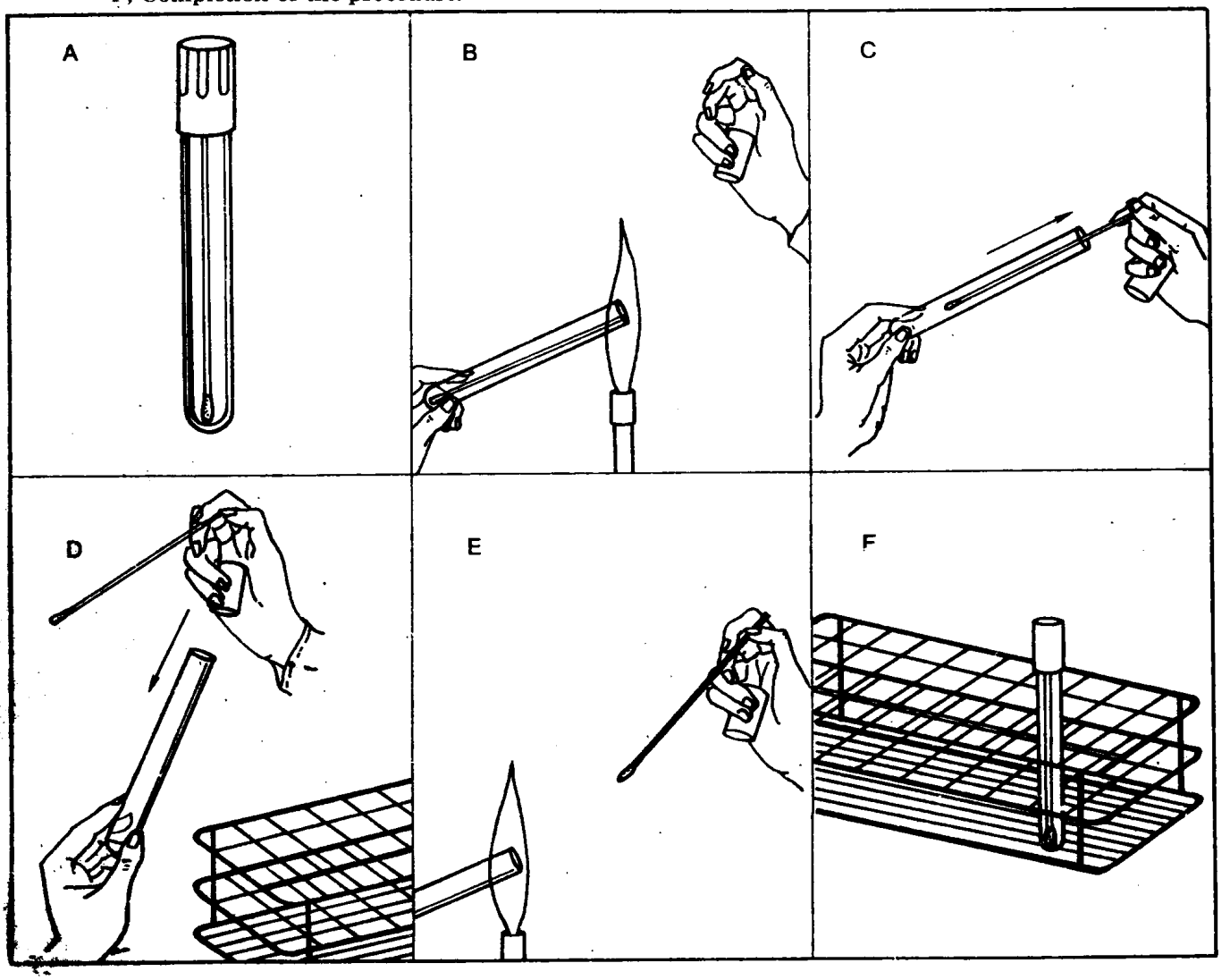
bacteria present. Thus, it is extremely critical that the Petri dishes be examined only when the covers are in place. They should never be handled or handed to someone else with the lids removed. If a spill occurs, notify the instructor immediately and decontaminate the area right away. The inoculating loop must be flame-sterilized, not only before use, but immediately after use as well.

1. After you have received your assignment, take two plates for each source. Using a glass-marking pencil, label both plates on the Petri dish lid with the *date* of inoculation, the *source* of the inoculum, and the type of *medium* used, in this case TSA. One plate should indicate incubation at 20°C (room temperature) and the other incubation at 37°C (body temperature in the incubator).

   Note: Labeling the lid instead of the bottom of the Petri dish is recommended in this manual to assure ease in identifying the culture source when shared with teammates and the instructor. However, removal of the labeled lid would lead to mislabeling of the cultures so you need to be very careful never to separate the lid of the Petri dish from its culture.

2. Turn the plates over and mark the bottom of the Petri dish in two equal parts, and one of the halves into two equal quarters with the glass-marking pencil, as shown in illustration (Fig. 1-2).
3. The cultures, except for the ones requiring special methods, require removal of a sterile swab from the tube, moistening with sterile water, inoculating the culture, and then returning the swab to the tube. Follow the procedure outlined below and illustrated in Figure 1-1. This will probably be demonstrated by your instructor as well. (Special

FIGURE 1-1 Removal of a sterile swab and its return to the tube again. *A*, Sterile tube with sterile swab. *B*, Removal of cap and sterilization of the mouth of the tube. *C*, Removal of the swab from the tube so that the mouth of tube, cap and swab will not be contaminated. *D*, Replacement of the cap, keeping the swab sterile. *E*, Flaming of the mouth of the tube just before replacing the swab after inoculation. *F*, Completion of the procedure.



methods are required for hair, cough, fingers before and after washing, clothing, air, and soil. These procedures will be explained below in Section 5.)

4. Follow the procedure given below for preparing the moist swab:
   a. Holding the tube containing the swab in the left hand (unless you are left-handed), remove the cotton plug or cap with the two small fingers of your right hand by a twisting motion, in such a way that the inside portion of the cotton plug is turned to the outside rather than toward the palm of your hand.
   b. Flame the mouth of the tube by passing it quickly through the Bunsen burner flame, tip the tube slightly to let the end of the swab come out of the tube in such a way that it can be removed without touching the mouth of the sterile tube. Replace the plug or cap on the tube, and return the empty tube to the rack. Take care to avoid touching the swab to any surface but the culture source.
   c. Take the tube of sterile water in the left hand, remove the cap as in Step a, flame as in Step b, carefully insert the swab into the water, press against the walls of the tube to remove the excess water, carefully remove from the tube without touching the tube, flame the mouth again, replace the cap or plug, and place the empty tube in the test tube rack.
5. Inoculate the swab according to the specific instructions given below for each source, and return it to the sterile dry tube. Be sure to follow the same aseptic technique described in Step 3 and illustrated in Figure 1-1 in this inoculation procedure.

   SPECIMEN FROM DRY SURFACE. Rub the moistened swab from Step 4 over about one square inch of surface. Return the swab to the sterile tube.

   SPECIMEN FROM OUTER EAR, UNDER FINGERNAILS, INSIDE NOSTRILS. Gently twist the swab in the specific location and return it to the sterile tube.

   THROAT SPECIMEN. Have the subject open mouth wide, lower the tongue, and gently touch far back in the tonsil area on both sides. Return the swab to the sterile tube.

   SOIL SAMPLE. Pick up a sample of earth with a moistened swab, put in a test tube with about 1 ml of sterile water, and agitate to cause the soil particles to break up and become suspended in the water. Let the large particles settle out. Moisten a fresh swab in the supernatant fluid above the settled-out soil particles.

   AIR SAMPLE. Remove the lids from the two agar plates and let the agar medium stand uncovered on the bench top for one hour, then replace the lid. Invert the plates and incubate at specified temperatures.

   HAIR AND CLOTHING. Remove the lids from two agar plates and shake hair or clothing vigorously over the surface of the uncovered agar medium without actually touching the surface. Bacteria will fall onto the surface. Replace lids after a minute or two. Invert the plates and incubate at the specified temperature.

   COUGH PLATE. Holding the uncovered agar plate 3 to 4 inches from the mouth, cough as deeply and vigorously as possible onto the surface. Replace the lid. Repeat with a second plate. Invert the two plates and incubate at the specified temperatures.

   FINGER TIPS BEFORE AND AFTER WASHING

   a. Remove the lids from two Petri dishes, labeled with two temperatures (20°C and 37°C) and "hand before washing." Place the fingers and thumb of the hand on the surface of the agar and gently rotate *in place*, being careful not to gouge or break the agar surface. Repeat, using the other hand on the second Petri dish.
   b. Wash hands vigorously with soap (or detergent) and a brush or paper towel, rinse well, dry, and inoculate the other two plates in the same manner as described in Section a. Label one plate "hand after washing" at 20°C; the other "hand after washing" at 37°C.
   c. Invert the plates and incubate at the specified temperatures.
6. Complete the inoculation of those samples for which you have obtained a swab in the following way:

a. Remove the swab from the tube using procedure outlined in Step 4 (sections a and b), and roll it over a small area of the agar surface (as shown in Figure 1-2) at the top of the undivided half. Repeat for the second agar plate. Discard swab immediately into the receptacle with disinfectant, located on each table.
b. Flame the inoculating loop by placing it in the flame at an angle so that the hub is

FIGURE 1-2 Preparation of a streak plate.