microbiology series volume 11

Laboratory Manual for Medical Microbiology

edited by
The Faculty of the Department of Microbiology
Schools of Medicine and Dentistry
State University of New York at Buffalo

Laboratory Manual for Medical Microbiology

edited by
THE FACULTY OF THE DEPARTMENT OF MICROBIOLOGY
Schools of Medicine and Dentistry
State University of New York at Buffalo
Buffalo, New York

MARCEL DEKKER, INC. NEW YORK AND BASEL

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

Preface

This laboratory manual is a product of the work of many faculty members of this department who, over a period of 40 years, have described various exercises for students of medical microbiology, and who have introduced constant changes and corrections. The manual was initiated by the late Dr. Ernest Witebsky who was chairman of this department from 1941 to 1967. The faculty members who have contributed to this manual are Drs. Erwin Neter, James F. Mohn, Noel R. Rose, Sidney Shulman, Reginald M. Lambert, Almen L. Barron, Ernst H. Beutner, Felix Milgrom, Joseph H. Kite, Jr., William R. Bartholomew, Joseph Puleo, Konrad Wicher, C. John Abeyounis, Thomas D. Flanagan, Kyoichi Kano, Carel J. van Oss, Eugene A. Gorzynski, Murray W. Stinson, Russell J. Nisengard, Joseph M. Merrick, Arlene R. Collins, Richard T. Evans, Roger K. Cunningham, Harshad R. Thacore, Diane M. Jacobs, Marek Zaleski, Philip T. LoVerde and several others. A great contribution to the creation of this manual has been made by Miss Anne M. Heide who, for 40 years, has been responsible for arranging and preparing the laboratory exercises in this department.

Our many years of teaching experience have permitted the creation of this manual which should be a valuable learning aid for students of medical microbiology. The main emphasis is placed on microorganisms pathogenic for humans: bacteria, fungi, viruses, and protozoa. The exercises demonstrate the most important features of these organisms and present many important diagnostic procedures used in hospital laboratories for identification of causative agents of infectious diseases. Several exercises are devoted to immunological procedures with emphasis on immunodiagnosis of infectious diseases. The manual

is primarily designed for laboratories on pathogenic microorganisms and as such should serve medical students. Additional laboratory exercises dealing with microorganisms of the oral cavity will be edited in the near future to serve the special needs of dental students. Many parts of this manual can serve as exercises for graduate students in microbiology as well as for students of pharmacy, nursing, and medical technology.

Felix Milgrom

Chairman, Department of Microbiology
Schools of Medicine and Dentistry
State University of New York at Buffalo
Buffalo, New York

Equipment and Supplies

The following should be purchased and brought to class for the first laboratory:

1 box of 72 microscope slides, select grade, 3 x 1 inches, frosted ends
1 china marking (wax) pencil, red
1 laboratory coat, white, knee-length
1 ruler, graduated in millimeters
1 small magnifying glass
1 book of matches

Laboratory Rules

The laboratory facilities should fulfill the criteria for biosafety level 2 practices as established by the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia.

It is essential that laboratory personnel have specific training in handling pathogenic agents and are supervised by competent scientists.

The following standard and special practices apply to agents assigned to biosafety level 2.

STANDARD MICROBIOLOGICAL PRACTICES

1. Laboratory doors are kept closed when experiments are in progress.
2. A knee-length, cloth laboratory coat must be worn in the laboratory. Laboratory clothing must not be worn in nonlaboratory areas. This is necessary to avoid carrying infectious agents out of the laboratory. Outside wraps are not to be brought into the laboratory at any time.
3. Do not eat, drink, store food, or apply cosmetics in the laboratory. Do not moisten labels with the tongue. Do not rub eyes or put fingers in the mouth or nose while handling cultures. The mucous membranes of the eyes, nose, and mouth are excellent portals of entry for infectious agents.
4. Smoking is absolutely prohibited in the laboratory.
5. Laboratory desks should be decontaminated by washing with "roccal" solution after each laboratory period and after each spill of viable material.
6. Before leaving the laboratory immerse and wash hands in roccal or

"zephiran" disinfectant solution and then wash thoroughly with soap and water. Use this procedure to disinfect hands during the laboratory in case of an accident.

7. Cultures are never to be taken out of the laboratory.
8. Mechanical pipetting devices are to be used; mouth pipetting is prohibited. All procedures are conducted carefully to minimize the creation of aerosols.
9. The gas burner is a very dangerous device and should be treated with care. All burners must be turned off or to pilot when not in use.
10. All contaminated materials including cultures should be placed in proper receptacles for decontamination. Culture tubes are to be discarded in baskets and petri dishes in pails which are supplied in each laboratory. No cultures should ever be kept or stored in the drawer or on the desk.
11. Discard pipettes and microscope slides in proper disinfectant receptacles. Under no circumstances should an attempt be made to recover microscopic slides once they have been discarded.
12. Stock cultures of microorganisms must be equitably shared by the students in a manner satisfactory to all concerned.
13. Students should have no fear of being reprimanded for accidentally spilling infectious materials. All accidents while handling such materials *MUST BE REPORTED IMMEDIATELY* to the instructor so that proper steps may be taken safely to remove the material. A written record must be prepared and maintained.
14. Put all supplies and equipment in their proper places and turn off all utilities at the end of each laboratory period.

SPECIAL PRACTICES

1. Access to the laboratory must be limited by the instructor when experiments are being conducted. In general, persons who are at increased risk of acquiring infections, such as children, and individuals who are immunodeficient or immunosuppressed, should not be allowed in the laboratory.
2. When infectious materials or infected animals are present in the laboratory, a hazard warning sign incorporating the universal biohazard symbol must be posted on all laboratory doors.

3. An insect and rodent control program should be in effect in each laboratory.
4. Hypodermic needles and syringes must be disposed of properly.
5. A safety or operations manual which identifies known and potential hazards and which specifies practices and procedures to minimize or eliminate such risks should be prepared or adopted. Personnel should be advised of special hazards and required to read and follow standard practices and procedures.

Basic Bacteriologic Procedures

1. Always sterilize inoculating loops in the flame of the burner *BEFORE* and *AFTER* use.

After the loop has been used in picking up infected material, dry the contaminated end in the coldest cone of the burner flame. Flame first the loop end and then the entire wire in the hottest cone, allowing the wire to become cherry red in color. After this flaming process pass the chuck and the distal one-third of the holder through the flame several times. If the material on the loop is not dried before the bacteria are killed, masses of live bacteria may be scattered about the room by the sudden steaming.

Always allow the heated wire to cool before using; otherwise the bacteria will be killed and no growth will be obtained.

2. Always flame the open mouth of a culture tube *AFTER* the closure has been removed and *BEFORE* it is replaced.

A variety of tube closures are used in bacteriology. Cotton plugs are used in culture tubes because they permit atmospheric oxygen to reach the culture and yet act as a filter by trapping dustborne bacteria from the air. Since the cotton contains contaminating bacteria, the area of the tube in which it is placed is considered not sterile. Consequently, before the loop is inserted into the culture tube this area of the glass must be heated in the flame of the burner. In order to destroy any bacteria from the air which may have fallen onto the lip of the tube while it was open, the mouth is again flamed before the cotton is returned. Culture tubes are frequently covered with metal or plastic closures. These fit a tube so as to provide an air passage. They do not fit tightly; therefore, be careful not to

allow liquid medium to spill by tipping the tube. *Caution*: metallic closures become very hot when placed on the flamed culture tube.

3. The flame of the gas burner must be used by one student at a time.

It is not possible to sterilize adequately an inoculating loop or to flame an open tube if more than one person is using the flame of the burner. The hottest sterilizing cone of the flame is too small to effect more than one adequate sterilization at a time.

4. Use only 10X or 15X microscope eyepieces for studying bacteria.

Most bacteria pathogenic for humans are quite small, ranging from 0.5 to 3.0 μm in size. To obtain sufficient magnification for morphologic studies it is necessary to utilize the greatest resolving power of the light microscope.

General Considerations for the Culturing of Specimens

Specimens for bacteriologic examination, especially those for culture, should be obtained and handled with sterile technique. They should be taken with sterile instruments and placed into sterile containers. If a body fluid (such as blood, pleural fluid, or a joint fluid) is to be examined for bacteria, it should be taken in a sterile tube containing sterile anticoagulant solution, preferably sodium citrate. Unless this is done, body fluid with high protein content will clot and cannot be examined.

Before the culture is done, a direct film should be prepared and stained with Gram's stain and any other recommended stains necessary to ascertain the number and types of bacteria present. When many bacteria are seen on the film, only a very small amount of the specimen should be taken for culture. On the other hand, in dilute specimens where few bacteria are present, such as spinal fluid or urine, the specimens must be centrifuged and the sediment used for films and cultures. The primary direct film of a specimen often acts as a guide in determining whether or not special culture media should be employed. In addition, this film is of importance in the recognition of bacteria which may fail to grow or which may be overgrown by other organisms also present.

Contents

Laboratory Manual for Medical Microbiology

edited by

THE FACULTY OF THE DEPARTMENT OF MICROBIOLOGY
Schools of Medicine and Dentistry
State University of New York at Buffalo
Buffalo, New York

MARCEL DEKKER, INC. NEW YORK AND BASEL

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

BACTERIOLOGY

1
Bacterial Morphology

I. MICROSCOPIC MORPHOLOGY OF THE THREE BASIC SHAPES OF BACTERIA

Broth cultures of the three basic shapes of bacteria are distributed. These are:

--*Staphylococcus aureus* (coccus)

--*Bacillus subtilis* (bacillus)

--*Rhodospirillum rubrum* (spirillum)

Note: Always flame opening of tube after plug is withdrawn and before it is replaced. Always flame loop before and after transfering culture material. Proceed as follows:

A. Preparation of Films

(1) With a china marking pencil draw three complete rings on a clean microscope slide so that the three types of bacteria may be placed on the slide (Fig. 1.1).

(2) Gently shake culture tube labeled "coccus" to resuspend organisms.

(3) Sterilize bacterial loop by heating in flame of burner. Hold it almost vertically but at sufficient angle to avoid burning fingers until entire wire becomes cherry red and then flame chuck and distal one-third of metal holder.

(4) Allow wire to cool in air for a moment without shaking.

(5) Remove plug by twisting it to right with last two fingers of right hand and at the same time twisting tube to left with left hand.

(6) Flame open mouth of tube in burner.

(7) Taking care not to contaminate the loop or the culture, insert

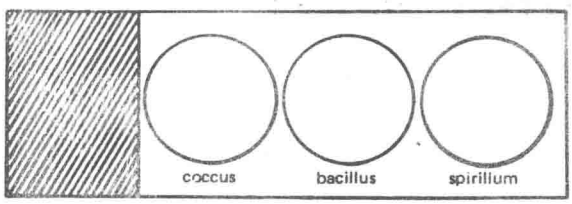

FIGURE 1.1. Microscope slide with marked rings.

loop into culture and pick up a loopful of material. (*Note:* No part of holder should touch broth.)

(8) Flame open mouth of tube in burner.

(9) Replace plug by twisting it to the right with the last two fingers of right hand while twisting the tube to the left with left hand.

(10) Spread material uniformly in first circle of the slide, making a thin, even film.

(11) Sterilize loop thoroughly in flame.

(12) Repeat entire procedure, steps (3) through (11), using culture labeled "bacillus," placing material in the second circle of slide.

(13) Repeat entire procedure, steps (3) through (11), using culture labeled "spirillum," placing material in the third circle of slide.

(14) Allow films to dry in air.

B. Staining of Film

(1) Pass slide, film side upward, slowly through flame of burner three times. (*Note:* This fixes films to slide by coagulating albuminoid material.)

(2) Place slide on staining rack in sink.

(3) Cover each of three film areas completely with methylene blue.

(4) Stain for 2 min.

(5) Wash off stain with tap water.

(6) Dry by blotting.

(7) Examine slide under oil immersion and record your observations.