LABORATORY EXERCISES IN

MICROBIOLOGY

PELCZAR AND CHAN FOURTH EDITION

LABORATORY EXERCISES IN MICROBIOLOGY

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FOURTH EDITION

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This book was set in Primer by Black Dot, Inc. The editors were William J. Willey, Thomas A. P. Adams, and Michael LaBarbera; the designer was Hermann Strohbach; the production supervisor was Joe Campanella. The drawings were done by E. H. Technical Services.

PREFACE

The experiments in this manual are designed for an introductory course in microbiology for college or university students, regardless of their background or field of specialization. Satisfactory completion of the exercises should acquaint the student with the basic techniques of microbiology and the fundamental characteristics of microorganisms.

It is not an exaggeration to state that microbial cells are prototypes, as well as models, of biological structure and processes such as cellular anatomy, biological energetics, biosynthesis, molecular genetics, and cellular physiology. Studies of microorganisms also make their contribution to the broad areas of ecology and evolution. Microbes not only provide the tools for an understanding of living processes at a molecular level but also the specific systems used in solving the reactions, enzymatic, genetic, or synthetic that form the basis of life as a whole. Indeed we find in microbes the simplest ideal systems for the study of life in all its manifestations. We can see then that microbiology, as an independent discipline, has much to offer in unifying and integrating biological science as advances in modern biology crumble the barriers between its distinctive (and traditional) branches. It is inconceivable that today's student could learn genetics, biochemistry, or even evolution without first getting a clear understanding of the life of microorganisms. As a discipline microbiology has helped in no small measure to change the nature of biology from a largely descriptive science to a quantitative, precise science sharing many of the characteristics of physics and chemistry.

One must also not forget the extensive applications of microbial knowledge in the human environment. These applications range from improvements in fermentation, agriculture, and medicine to modern extensive industries based upon the activities of selected microorganisms. Acids and alcohols, vitamins and enzymes, flavoring substances and antibiotics, hormone derivatives, and a host of other products are now produced by microbes.

From what has been said about microbiology, students may gather that they can do their "thing" in microbiology, whatever their interests are in the broad spectrum of biological activities. It is hoped that this manual will serve as the first turn of the key to the kingdom of microorganisms.

The exercises are intended to be brief and to include only the steps required to accomplish the particular experiment. Details such as composition and preparation of staining solutions, media, and other reagents are given in the Appendixes. Appendix material includes information to the instructor for each exercise where deemed necessary, e.g., helpful demonstrations and mechanisms of reactions of certain tests. However, the laboratory instructor will generally supplement the brief introductory remarks to each exercise with further explanation of the principles or techniques deemed helpful to the student. It is essential that the student understand the what, the how, and the why of each exercise before performing it.

The student is encouraged to supplement interpretation of results, explanations of the various biological phenomena, and so on, by reading the corresponding chapters of the text *Microbiology*, by Pelczar, Reid, and Chan, cited near the beginning of each exercise. Those using other textbooks can easily find the appropriate chapters covering the same material. In this edition, audiovisual material has been keyed to the exercises where appropriate. A list of references on selected laboratory subjects is also included in Appendix E. The number and choice of experiments in this edition of the manual can be modified by the instructor to accommodate laboratory classes which meet either two days per week for one semester or once a week for the academic year. In the latter case, the availability and use of refrigerated incubators (inoculated cultures are refrigerated until 24 to 48 h before the next class, when the incubators will be brought up to incubation temperature) make this schedule quite convenient.

We thank Phyllis Vineberg for her assistance in this revision and Danuta Iwanicka for modeling for some of the illustrations.

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CONTENTS

INTRODUCTION

MICROBIOLOGY AND ITS TECHNIQUES

Microbiology is the study of the biology of microbes. But microbes are a mixed lot. Bacteria are microbes, but so are the single-celled protozoa, the noncellular viruses, and the many-celled fungi and algae (as well as some parasitic metazoan animals). However, they all share the common properties of diminutiveness, rapid multiplication, and ubiquitous geographical distribution. This unique assemblage of properties has compelled microbiologists to develop unique methods for studying microorganisms and in so doing has defined their discipline. One might even say that microbiology is a science defined more by the techniques it uses than the subjects it covers!

Even the advancement of the discipline had to be tied to the development of techniques. For example, development of sterilization methods and pure-culture techniques were of paramount importance because of the ubiquity of microorganisms and their natural occurrence in mixed populations. It is only from the pure culture consisting of one kind of microbe in a sterile medium that the microbiologist can distinguish the particular behavior of a microbe. This requirement at once points out the difficulties in the study of mixed cultures or natural populations. The techniques of microbiology, then, are very important to the science. We hope that this manual introduces you to the proper use of basic techniques and methods in microbiology.

THE ORGANISMS IN MICROBIOLOGY

Among living organisms, there are two fundamental and different cell organizational patterns: the eucaryotic and the procaryotic (protocaryotic) types. They may be compared as tabulated below.

On the basis of this division and the mode of nutrition (photosyn-

	PROCARYOTIC CELLS	EUCARYOTIC CELLS
Groups where found as unit of structure	Bacteria, blue-green algae	Most algae, fungi, protozoa, higher plants and animals
Nucleus:		
Nuclear membrane	Absent	Present
Mitotic division	Absent	Present
Chromosome number	1	More than 1
Movement:		
Cytoplasmic streaming	None	May occur
Locomotor organelles	Simple	Complex
Ameboid movement	None	May occur
Functional structure:		
Chloroplasts	None	May be present
Mitochondria	None	Present

thesis, absorption, or ingestion), the world of living organisms can be classified into five kingdoms: Monera, Protista, Fungi, Plantae, and Animalia. Of interest to microbiologists are the first three kingdoms (usually lumped together into the kingdom Protista of Haeckel):

Kingdom Monera, the procaryotic cells
Kingdom Protista, the unicellular eucaryotic organisms
Kingdom Fungi, the multinucleate eucaryotic higher fungi

Viruses, a very important group of microorganisms studied by microbiologists, are not included in the scheme of living things above because they are not regarded as cellular entities.

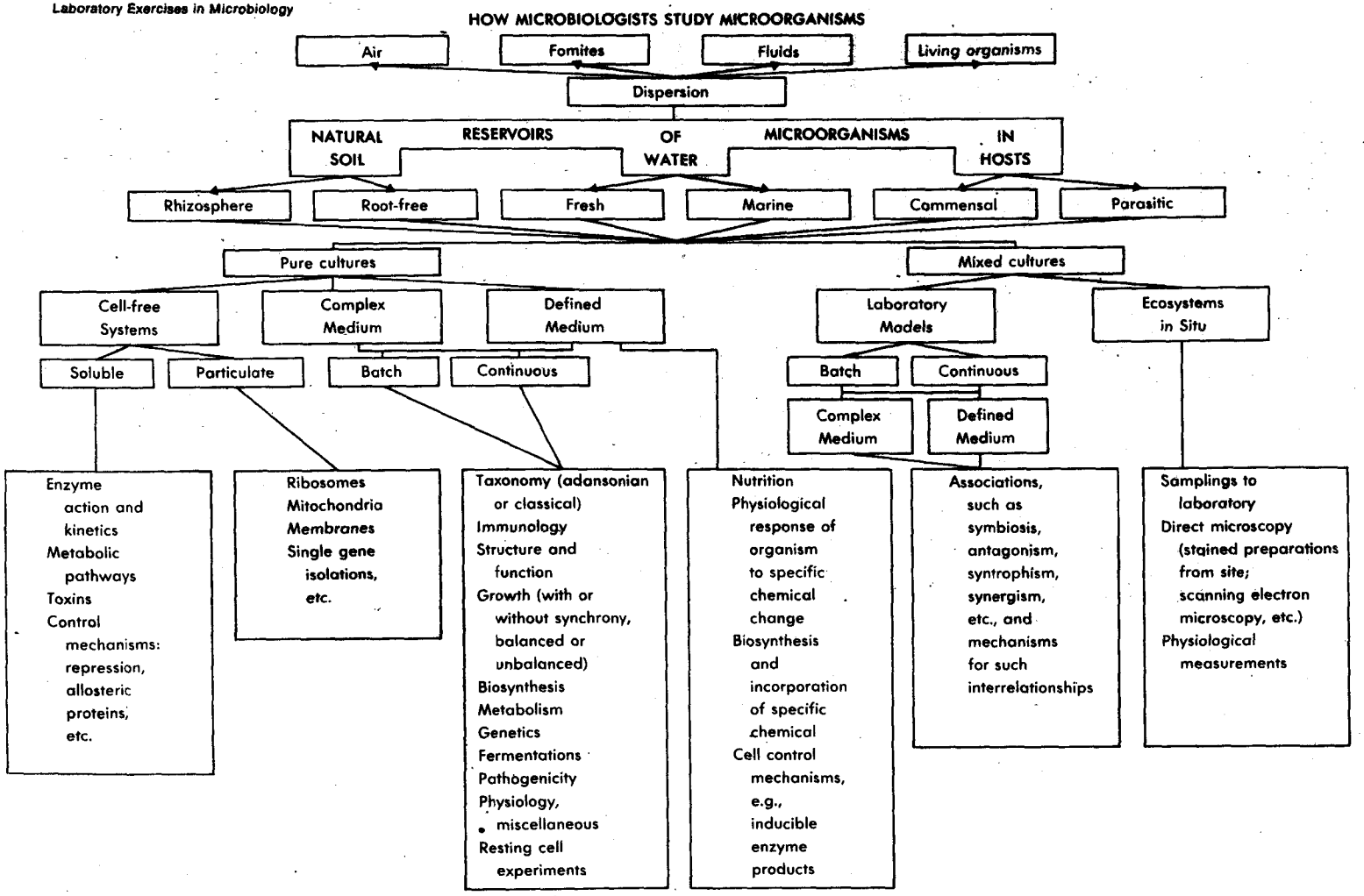
We can see then that the microbes encountered by microbiologists include all the microscopic forms of life. They can be autotrophic and heterotrophic; chlorophyll-free and chlorophyll-containing; aerobic and anaerobic; acellular, unicellular, and multicellular; depending for their nitrogen on fixed compounds or on gaseous nitrogen; saprophytic and parasitic; capable of living within the cells of higher forms of life or of living outside of cells; and ranging from viruses and bacteria to algae, fungi, protozoa, and even certain worms. This kaleidoscopic array of microbial forms is the concern of the student of microbiology.

HOW MICROORGANISMS ARE STUDIED

Before beginning work on specific experiments, it is instructive for a student to grasp an overall idea of how microorganisms are studied. The schematic diagram (on page 3) summarizes various ways of studying microorganisms. It also gives a general impression of the broad scope of microbiology. With this in mind, the individual experiments that follow become more meaningful when placed in the context of the diagram.

GENERAL LABORATORY INSTRUCTIONS

1 You must familiarize yourself *in advance* with the exercise(s) to be performed.
2 Preliminary instructions and demonstrations will be given at the beginning of each exercise by the laboratory instructor. You should be prepared to ask any questions concerning the procedure to be followed and should thoroughly understand the purpose of the exercise. Do not attempt to start work before receiving instructions.
3 Accurate and detailed results are to be recorded at the completion of each exercise. Use the tables and other spaces provided for recording results and answering questions. Drawings of microscopic findings should be enclosed within the circular outlines. In making your drawing of a specimen, do not attempt to draw everything in the microscopic field. Simply select a few representative specimens, i.e., cells, their arrangements, or structures.
4 The writing of a laboratory exercise is to be completed within 1 week from the time the exercise was performed unless directed otherwise by the instructor.
5 A permanent slide collection is helpful. A representative stained slide (or slides) from each exercise that calls for stained preparations should be properly labeled and placed in a slide box after appropriate examinations have been made. This collection will prove useful in reviewing your work.


HOW MICROBIOLOGISTS STUDY MICROORGANISMS
Air
Fomites
Fluids
Living organisms
Dispersion
NATURAL RESERVOIRS OF MICROORGANISMS IN
SOIL
WATER
HOSTS
Rhizosphere
Root-free
Fresh
Marine
Commensal
Parasitic
Pure cultures
Mixed cultures
Cell-free Systems
Complex Medium
Defined Medium
Laboratory Models
Ecosystems in Situ
Soluble
Particulate
Batch
Continuous
Batch
Continuous
Complex Medium
Defined Medium
Enzyme action and kinetics
Metabolic pathways
Toxins
Control mechanisms: repression, allosteric proteins, etc.
Ribosomes
Mitochondria
Membranes
Single gene isolations, etc.
Taxonomy (adansonian or classical)
Immunology
Structure and function
Growth (with or without synchrony, balanced or unbalanced)
Biosynthesis
Metabolism
Genetics
Fermentations
Pathogenicity
Physiology, miscellaneous
Resting cell experiments
Nutrition
Physiological response of organism to specific chemical change
Biosynthesis and incorporation of specific chemical
Cell control mechanisms, e.g., inducible enzyme products
Associations, such as symbiosis, antagonism, syntrophism, synergism, etc., and mechanisms for such interrelationships
Samplings to laboratory
Direct microscopy (stained preparations from site; scanning electron microscopy, etc.)
Physiological measurements

LABORATORY RULES

The following rules must be observed for the safety and convenience of everyone working in the laboratory:

1 Wearing apparel not worn should be left outside the laboratory on the clothes racks provided.
2 Pencils, labels, or any other materials should never be placed in your mouth.
3 Transfer needles and loops are sterilized by heating the entire length of the wire to redness *before* and *after* using. Spattering is avoided by first holding the needle above the flame.
4 If a culture is spilled, cover the area with disinfectant (5% Lysol solution) and notify the instructor.
5 Cultures are never to be taken from the laboratory.
6 Inoculated media placed in the incubator must be *properly labeled*, i.e., with your name, date, and the nature of the specimen, and put on the assigned shelf.
7 Gas burners must be turned down or off when not in use during the laboratory period. Be sure gas burners are turned off at the end of the laboratory period.
8 Eyepiece, lenses, and objectives, as well as the microscope stage, should be cleaned before and after use. Lenses of the microscope must be wiped off with lens paper only.
9 All reagents and equipment must be returned to their proper place at the end of each laboratory period.
10 All used tubes, petri dishes, pipettes, etc., must be placed in designated receptacles at the end of the laboratory period.
11 All scraps of paper, cotton, etc., must be placed in wastebaskets and not left on desk tops or on the floor.
12 The laboratory desk top should be cleaned with a disinfectant (5% Lysol) at the beginning and end of each laboratory period.
13 Personal accidents, such as cuts and burns, must be reported immediately to the instructor.
14 Every student should wash his hands—if necessary, with a disinfectant—before leaving the laboratory.

EQUIPMENT AND SUPPLIES NEEDED BY EACH STUDENT

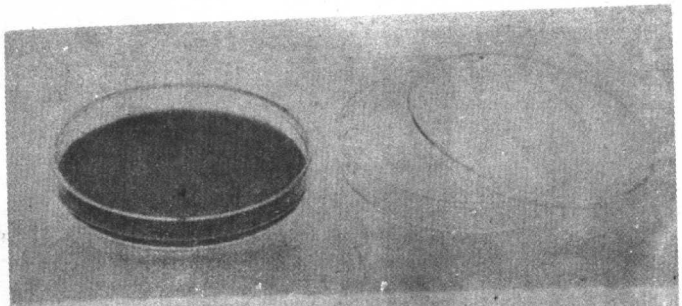
SUPPLIES CUSTOMARILY FURNISHED BY THE STUDENT

Drawing pencil (4H)
Wax glass-marking pencil (red) or Venus Sanford's "Sharpie" marking pen
Celluloid metric rule (6-in)
Microscope slides (3 by 1 in), one box
Microscope slide box (50 capacity)
Microscope slide labels (box or booklet)
Culture microscope slide (one depression)
Lens and bibulous paper (booklet)
Cover slips ($^7/_8$ in square), $^1/_2$ oz
Safety matches
Cheesecloth, 1 yd

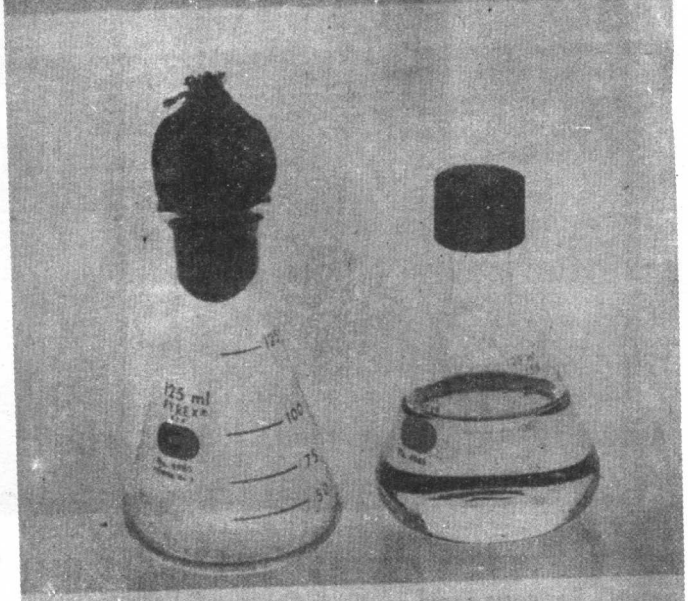
PERMANENT EQUIPMENT GENERALLY FURNISHED

Microscope
Microscope lamp
Loop transfer needle
Straight transfer needle
Forceps
Staining tray or trough
Bunsen burner
Staining block complete with stains

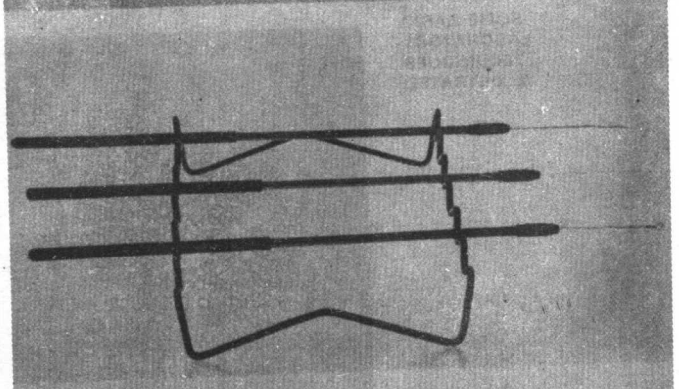
SOME EQUIPMENT OF THE MICROBIOLOGY LABORATORY



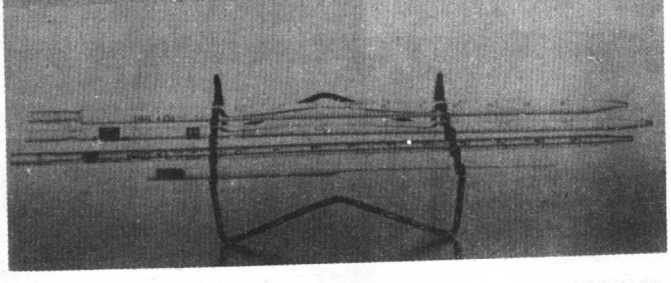
Petri dishes. Left one has medium.



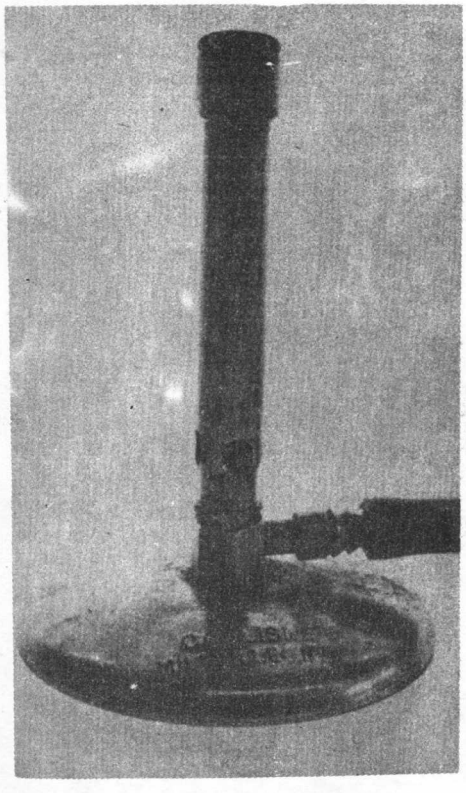
Erlenmeyer flasks (may be used with cotton plugs or screw caps).



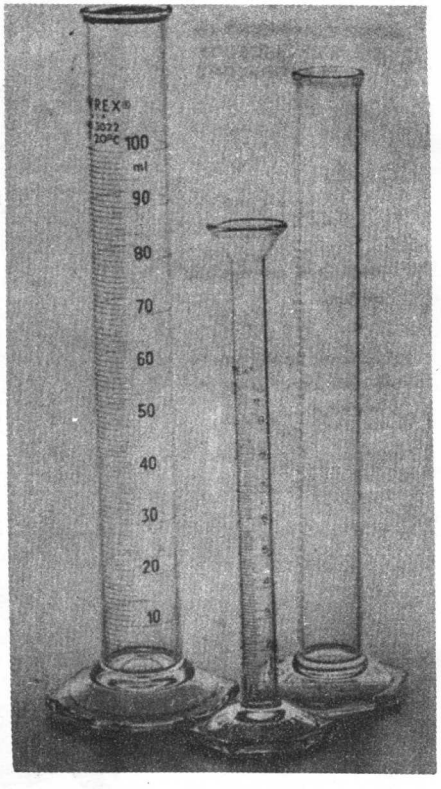
Inoculating needles (top to bottom): straight transfer needle (thick) for molds; straight transfer needle (thin) for bacteria; loop transfer needle.



Serological pipettes and a Pasteur pipette (bottom). (The Pasteur pipette is generally used with a rubber bulb.)

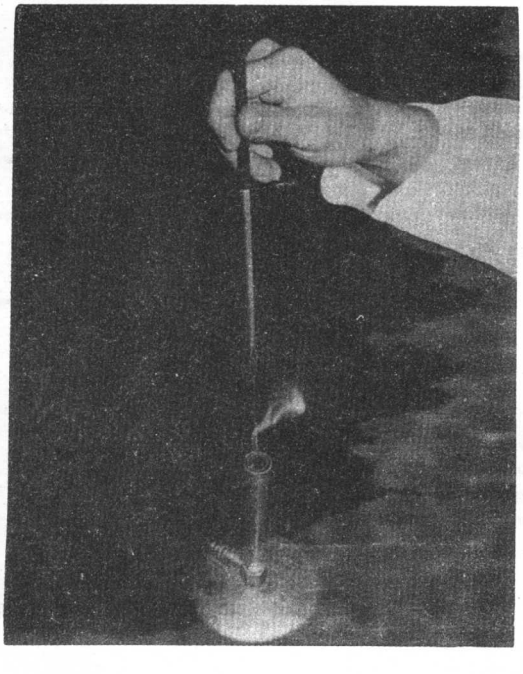


A bunsen burner.



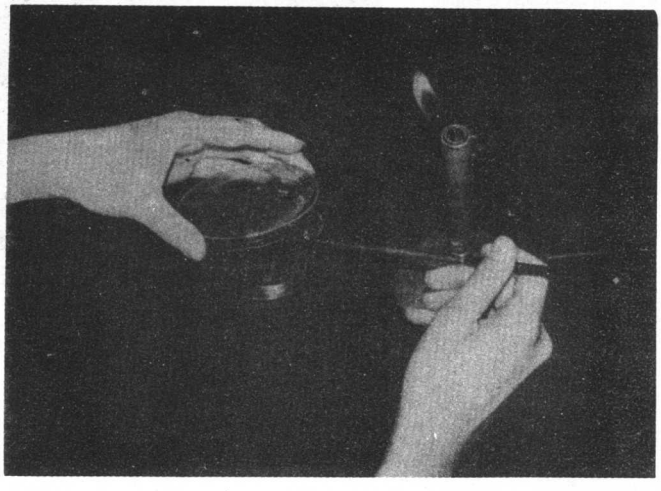
Graduated cylinders.

SOME BASIC LABORATORY TECHNIQUES ILLUSTRATED

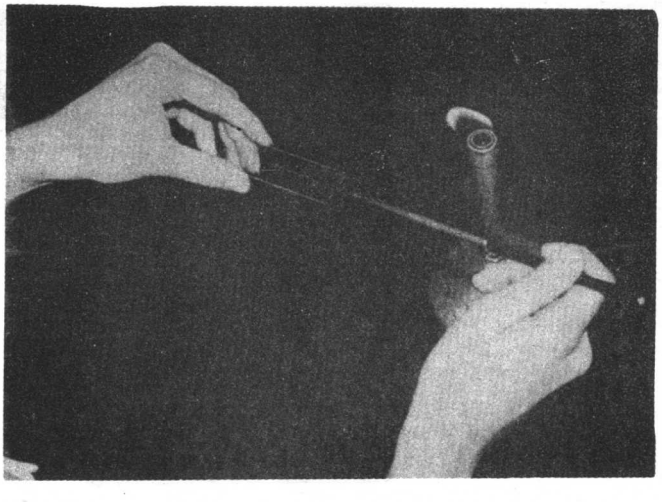


PREPARATION OF STAINED SMEAR. Transfer needle is flamed (sterilized) before and after removal of microorganisms.

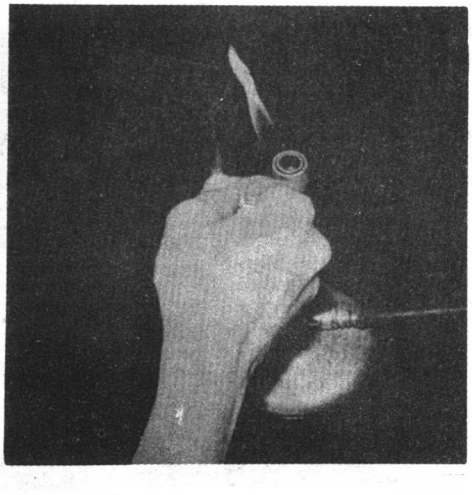
Specimen is removed with transfer loop from colony.



Specimen is smeared (spread) on a clean slide about the size of a dime. Smear is allowed to air-dry.

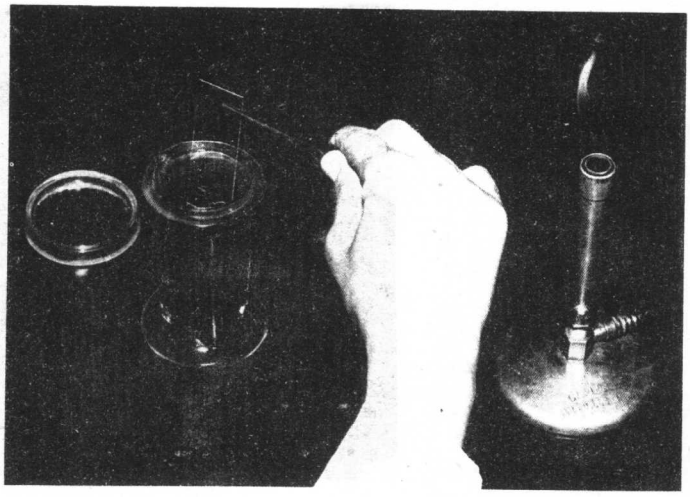


Smear is fixed by passing slide (smear side up) over the flame of a bunsen burner several times.



Staining solution(s) is applied and then washed off. Smear is air-dried and examined under the microscope.

The smear can also be stained by immersion in staining solution contained in a Coplin jar.



INOCULATION OR TRANSFER OF A CULTURE. Transfer needle is first sterilized as shown before. Caps are removed from the tubes by grasping them between the fingers of the hand holding the needle.