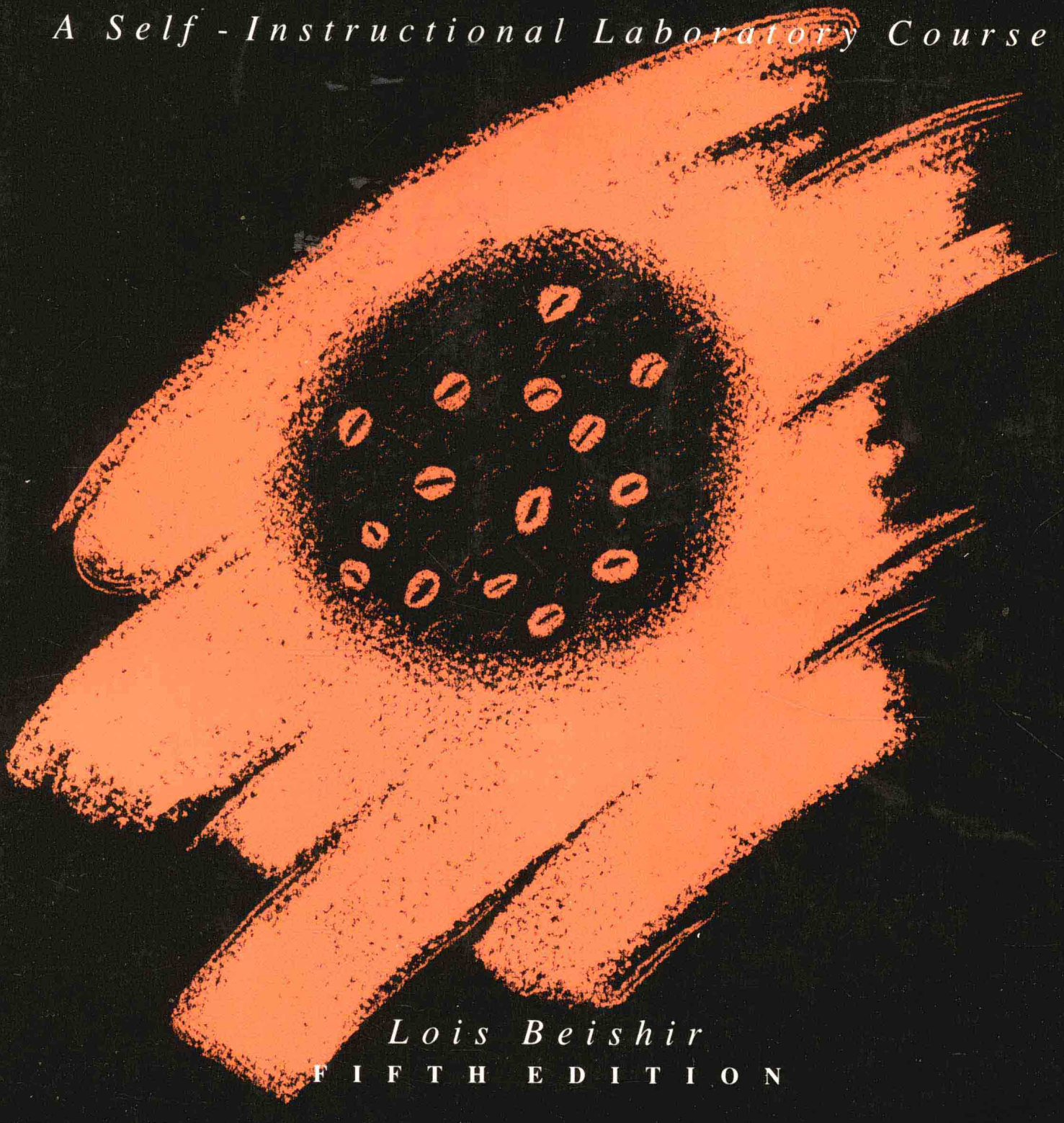
MICROBIOLOGY IN PRACTICE

A Self-Instructional Laboratory Course



Lois Beishir

FIFTH EDITION

MICROBIOLOGY IN PRACTICE

A Self-Instructional Laboratory Course

Fifth Edition

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Microbiology in Practice: *A Self-Instructional Laboratory Course,* Fifth Edition

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Introduction to the Student

The step-by-step procedure found in the activities of this edition will allow you to use your laboratory time more efficiently and makes the course more self-instructional. The more self-reliant you become, the greater your interest in microbiology will be. This new approach to learning depends on your being honest in evaluating yourself, since your instructor will only infrequently measure your mastery of the material. Therefore, your honest self-evaluation of your mastery of each module, by means of the posttest and worksheet, is vital to your success. Do not be satisfied with partial understanding. Nobody knows better than you do whether you are "getting it."

The format of each self-instructional package (module) is as follows:

1. *Prerequisite skills* are taught as the course proceeds. Skills and techniques learned in early modules must be understood before later materials can be grasped.
2. *Materials* needed for the activities are listed at the beginning of each module.
3. An *overall objective* for each module states clearly the general principle or procedure that you are expected to learn.
4. *Specific objectives* set down explicit terms to be defined, particular concepts to be grasped, and descriptions to be made.
5. *Discussion* provides explanation of theories and procedures, describes specific aspects of each module's topic, explains each objective, and prepares you for performing the activities.
6. *Activities* are techniques or experiments that support the discussion and the objectives. These are the core of the module. If you can explain why you were asked to perform each activity, i.e., what you were expected to learn from it, then you should understand most of the specific objectives.
7. *Related experiences* provide optional activities that will add to your understanding and improve your skills.
8. *Phonetic pronunciations* are found at the end of each module before the post test. You are strongly encouraged to learn these pronunciations as you work with each of the organisms. The phonetic pronunciations are repeated in an alphabetized appendix in the back of your manual for convenient future reference.
9. The self-evaluating *post test* allows you to determine whether you have completed the module successfully and thereby fulfilled the objectives. The post test also helps determine which parts of the module you have not learned and should therefore review or repeat.
10. *Figures* are used throughout the text to give visual explanations of complex techniques, schemes, and expected results. Illustrations are especially helpful in learning techniques. You must study them and imitate them exactly. *Tables* show you how to collect data and record them.

11. *Worksheets* include drawings, charts, thought questions, conclusions, and the like. Worksheets for each module requiring them are placed immediately after the module.

These components support and reinforce the objectives and make this presentation a self-taught course. This method gives you the choice of working at a slower or faster pace, without feeling pressured. Speed of performance is not a measure of solid learning.

Each module contains essentially a single technique or concept that is explained by the discussion, activities, and post test. In modules that deal with difficult techniques, the activities may seem at first to be overdone, but they will save you time in the long run. For example, the aseptic tube transfer of bacteria is a technique that is difficult to learn, but it is the single most important technique to learn in microbiology since it protects you, your neighbor, and your pure cultures from contamination. If you do the practice activities thoroughly, you can learn such difficult-to-grasp techniques in one lab period.

The phonetic pronunciations will be particularly useful to you. As a future health professional it will be important for you to pronounce these scientific names correctly. For example, as you discuss the disease process with colleagues on the ward, your mispronunciation of these terms would be a negative reflection on your professionalism.

As you perform the activities in the modules, you will be asked to place descriptions, drawings, some tables, and scientific conclusions on your worksheets. From this collection of data your instructor can then determine if you have made the correct observations and learned the procedures that the activities were designed to teach you. Your worksheets must be complete at the end of the semester for you to be successful in the course. Your instructor may also make an unannounced spot check of the worksheets intermittently.

Periodically in the course, a MODS (module objective discussion session) may be scheduled. This is an informal discussion period, conducted by a student leader or the instructor, during which you explain the specific objectives to the other students and discuss them together. If you prepare for the MODS by reviewing your completed modules and worksheets, these specific objective discussions are a very useful learning aid.

Helpful Hints

1. The biggest mistake you can make with materials that you submit to your workbook is merely to copy the figures in each module. Many of these figures are included to help you determine whether you are seeing the correct organisms and/or structures through your microscope. It is to your advantage to make your drawings as you see them so that you will be able to recognize and identify them when you see them through the microscope again. In summary, don't dry-lab. It is very easy for your instructor to determine whether you are giving some thought and effort to your drawings and descriptions.
2. It is advisable to supplement the information in each module with reference reading. At the end of this book, you will find a list of reference books that complement this lab course. This supplementary reading will help you attain a better overall view of microbiology.
3. If you do not score 100% on your post test, review or repeat the parts of the module that will enable you to achieve a perfect score. As another aid to learning, go back to the post test a week after you have evaluated yourself, and see if you can still pass it with 100%. If you can do this, you have indeed learned the information.
4. In modules without a related experience (designed to expand your learning), try to invent one yourself. Discuss this related experience with your instructor before

you proceed.

5. Materials and cultures for each lab session will be made available to you in a designated area. Less frequently used materials and equipment will be kept in specified storage areas.
6. Always read the label of the various dehydrated media. Learn the reason each medium is designed differently.

Laboratory Rules

Certain rules should be followed while you are working in the laboratory. Some are listed here. Your instructor may add other appropriate rules or suggest more safety procedures to remember.

1. Never eat or drink in the laboratory, and avoid putting objects in your mouth. Remember that you are working with living microorganisms, most of which are harmless, but others, if ingested, can cause you physical discomfort.
2. Disinfect your working area and wash your hands thoroughly at the beginning and end of each laboratory period. Wash your hands before you leave the lab for any reason, even a coffee break.
3. If you spill living microorganisms, cover the spilled material with paper towels, and pour your laboratory disinfectant over the towels and the contaminated area. Wait 15 minutes before you clean it up.
4. If you are injured (burned or cut), notify your instructor immediately.

A drawer, tray, cabinet, or other storage area will be assigned to you for the equipment that you will be using almost daily. You will also be assigned a microscope. It is your responsibility to keep it clean and to report any malfunctions to your instructor. Care of your equipment and microscope is most important because you may be sharing them with students in other lab sections. Suggested materials to supply for yourself:

1. Protective garment such as a lab coat or apron
2. Glass marking pencil
3. 1 lb coffee can for storage and incubation of culture tubes
4. Hot pads or pot holders
5. Colored pencils (red, blue, and green)

You are expected to read the assigned modules before attending each laboratory period. This will allow you to use your time efficiently, which is important since you will be performing different stages of two or three different modules during the same lab period. That is, you may be preparing the media and reagents for one module, inoculating for another module, and collecting the results for a third module.

These self-instructional modules are presented in such a manner that you should be able to proceed with the activities (if you have preread the module) without a lengthy discussion and explanation from your instructor. Upon your arrival at the lab, you should be ready to go to work. Students who preread and preorganize their laboratory time will inevitably finish their labs early while learning more.

Lois Beishir

Contents

PART ONE

Use of Equipment

MODULE 1

Triple Beam Balance

PREREQUISITE SKILL

Working knowledge of the metric system of length, mass, and volume as commonly used in the science laboratory.

MATERIALS

triple beam balance
glazed weighing paper
150 ml beaker or weigh boat
spatula or weighing spoon
clean sand

OVERALL OBJECTIVE

Demonstrate your ability to weigh a granular substance accurately using a triple beam balance.

Specific Objectives

1. Demonstrate your understanding of the relationship between the three beams of the triple beam balance.
2. Describe how to correct for the weight of the paper or container used to hold the substance being weighed.
3. Demonstrate your ability to weigh a large amount and a small amount of a granular substance.
4. Define the term *tare*.

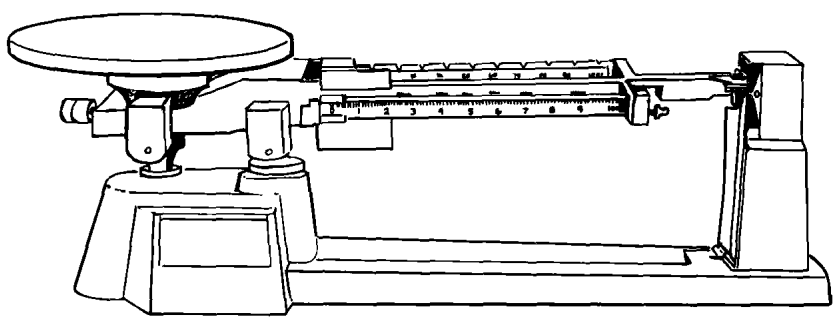
DISCUSSION

Weighing powders and granular substances accurately is essential to the preparation of microbiological media. When you reconstitute dehydrated media, you add a

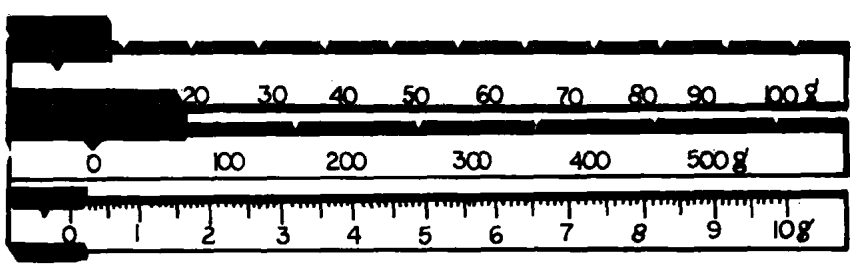
specific number of grams of the powdered medium to distilled water and heat this to dissolve the powder completely. If you have not weighed the powder and measured the water accurately, you will not have the proper proportion of nutrients in the finished medium. In the case of solid media, if you do not weigh accurately, you run the risk of having your medium turn out semisolid or too dry.

Although not as accurate as an analytical balance, the triple beam balance can weigh quantities larger than 1 g with sufficient accuracy for your purposes if used carefully. Familiarize yourself with the balance shown in Figure 1-1. Notice the three beams, each with a separate scale. These are shown in more detail in part (b) of the figure. Each beam has a weight that can be moved along the scale. The first scale, the one closest to you, is graduated in grams from 0 to 10. Each major division equals 1 g, and the smaller lines mark off 0.1 g. The second scale has no subdivisions but is graduated from 0 to 500 g in 100 g increments. There are notches in this beam to mark the position of these 100 g increments. The third beam also has notches to mark the increments, but on a scale from 0 to 100 g. Be sure the sliding weight rests *in* the desired notch. When you move one of the weights to a given number, it counterbalances the platform with that amount of weight. For example, if you move the weight on the third beam to the first notch (10 g), you could add material to the platform until the pointer swings to 0, which would mean that you have placed 10 g of the material you are weighing on the platform. This concept is difficult to grasp just from a description, so you should go over to the balances and look at the beams and their weights. Move the weights back and forth, and weigh your pencil, keys, or some other object until you feel that you understand the scales and their relationship to each other.

When you weigh powders or granular materials, you must never put them directly on the weighing platform of the balance. If you did, every substance you weighed would be contaminated with the last powder weighed on the same balance. Even traces of certain chemicals could affect the medium enough to interfere with the



(a)



(b)

FIGURE 1-1
(a) Triple beam balance. (b) Detail of beam scales and weights.

growth of microbes. So for amounts up to 10 to 15 g, you should use glazed weighing paper to hold the material you are weighing, and for amounts larger than this you should use a small beaker or weigh boat.

Of course, you will have to correct for the weight of the paper, beaker, or weigh boat, or you will not have the right amount of the substance you are weighing. This deduction of the weight of the container or weighing paper from the total weight to determine the weight of the contents is called *taring* or finding the *tare*. To tare, you must first weigh the container and *add* this weight to the weight of the powdered medium. For example, if your glazed paper weighs 0.6 g and you want to weigh 8.2 g of dehydrated nutrient agar, you would add the two figures for a total of 8.8 g. Then move the weights on the beams of the balance to the setting that corresponds to this total. Now you can add the powdered medium carefully until the pointer swings to 0.

Many labs today are equipped with top loading balances that tare automatically. These balances are reset to zero after the weighing paper or container is placed on the platform. Then you may dispense only the weight of dehydrated medium needed without adding the weight of the paper or container. If your lab is equipped with automatic tare balances, your instructor will provide instruction specific to the brand you will be using.

ACTIVITIES

Activity 1: Using Weighing Paper

Weigh 5.5 g of clean sand.

1. Place glazed weighing paper on the scale platform.
   - Remember to tare by weighing the paper and adding 5.5 g to the weight of the paper: for example, 0.4 g + 5.5 g = 5.9 g.
2. Using a weighing spoon, add sand to the platform until the pointer rests at 0.
   - Remember to add the sand to the platform slowly so that you do not add a surplus, which you will have to remove.
3. Return the sand to the container from which you obtained it.

Activity 2: Using a Weighing Container

Weigh 43 g of clean sand.

1. Place a 150 ml beaker or a weigh boat on the scale platform.
   - Tare by weighing the beaker or weigh boat.
   - Add 43 g to the weight of container: for example, 59.7 g (beaker) + 43 g (sand) = 102.7 g.
2. Using a weighing spoon, put sand in the container until you obtain the tared weight.
3. Return the sand to the container from which you obtained it.

Take the post test next.

POST TEST

The post test is a self-evaluation. It is not used for a grade. It is designed only to let you decide if you have successfully completed this module.

True or False

_____ 1. It is best to make it a habit to calculate your tare mentally.

_____ 2. The weight of the glazed weighing paper varies considerably from one sheet to the next.

_____ 3. It is best to weigh amounts less than 15 g on glazed paper and larger amounts in a small beaker or weigh boat.

_____ 4. Each major division of the first scale equals 1 gram and is subdivided into tenths of a gram.

_____ 5. Because the glazed paper is so light, you can disregard its weight as negligible.

_____ 6. To weigh 315 g, you must set the weight on the second beam at 300 g, the third beam weight at 10 g, and the first beam weight at 5 g.

_____ 7. The scales on all three beams have subdivisions between the major divisions.

_____ 8. You should not attempt to weigh amounts smaller than 1 g on a triple beam balance.

_____ 9. The triple beam balance should only be used to weigh powdered or granular materials.

_____ 10. Traces of foreign chemicals would not be likely to affect a large amount of prepared media.

KEY

1-F, 2-F, 3-T, 4-T, 5-F, 6-T, 7-F, 8-T, 9-F, 10-F.

MODULE 2

Preparing and Dispensing Media

PREREQUISITE SKILL

Ability to weigh materials accurately using a triple beam balance.

Suggestion: Do this module and Module 3, "Sterilization of Media and Equipment," in the same lab period.

MATERIALS

500 ml graduated cylinder
hot plate stirrer or Bunsen burner and ring stand
stirring magnet or glass stirring rod
stir bar retriever
triple beam balance
600 ml beaker
dehydrated nutrient agar
250 ml beaker
dehydrated nutrient broth
distilled water
8 oz screw-cap bottle or flask of similar size
Salvarsan burette
culture tubes and closures (27):
10 tubes for slants
3 tubes for deeps
14 tubes for broths
wire or plastic basket
Kimrack

OVERALL OBJECTIVE

Properly reconstitute dehydrated nutrient media and dispense them in standard quantities and containers for various uses.

Specific Objectives

1. Demonstrate your ability to calculate from the grams per liter directions on the medium bottle the amount of powdered medium necessary to make less than a liter of medium.
2. Demonstrate your ability to measure liquids accurately with a graduated cylinder.

3. Use a magnetic stirrer–hot plate combination, or the available equipment, to prepare media that require heat to dissolve completely.
4. Prepare a liquid medium and a solid medium.
5. Demonstrate your ability to use a Salvarsan burette to dispense measured amounts of medium into the appropriate containers.
6. Describe the correct and complete labeling of the various containers of medium according to standard format.
7. Name the type of balance most commonly used to weigh microbiological materials.
8. Name the containers used to weigh less than 15 g and more than 15 g.
9. List the preferred amounts of medium and the containers used to sterilize the medium for each of the following: slant, broth, stab, deep, culture plate, 8 oz bottle.

DISCUSSION

Essential to the preparation of microbiological media is the accurate weighing of powders and granular substances. When dehydrated media are reconstituted, a specific number of grams of the powdered medium is added to distilled water and heated to dissolve the powder completely. If you have not weighed the powder and measured the water accurately, you will not have the proper proportion of nutrients in the finished medium. If you do not weigh solid media accurately, you run the risk of having the medium turn out semisolid or too dry.

A triple beam balance is generally used to weigh media since large amounts are involved. When you weigh powders or granular materials, you must never put them directly on the weighing platform of the balance. For amounts up to 15 g, you should use glazed weighing paper to hold the powdered media you are weighing, and for amounts larger than this you should use a small beaker or weigh boat.

The directions on the labels of most commercially prepared media bottles give you the amount of powdered medium to be rehydrated in 1000 ml (1 liter) of water. The amount of powdered medium per liter of water varies for each medium. Therefore, be sure to *read the label* for each type of medium. You rarely need to make 1000 ml of medium, so you need to calculate for smaller amounts.

Obviously, if you need 500 ml of medium (½ liter), you simply divide both water and powdered medium by 2. If you need 250 ml (¼ liter), you divide both liquid and powder by 4. The easiest way to determine amounts necessary for various other quantities of medium is to find the amount needed for 100 ml by simply moving the decimal point one place to the left, which is actually dividing by 10 ($1000 \div 10 = 100$). Then multiply the number of grams necessary to make 100 ml by the number of hundreds ($400 = 4 \times 100$) of milliliters you wish to make. For example, if the label on the bottle directs you to add 15 g of medium to 1000 ml of distilled water, how many grams of powdered medium should you weigh to make 200 ml?

$$(\text{g}/1000\ \text{ml}) \div 10 = \text{g}/100\ \text{ml}$$

Moving the decimal point one place to the left would give the amount needed for 100 ml:

$$15\ \text{g}/1000\ \text{ml} = 1.5\ \text{g}/100\ \text{ml}$$

Now multiply by the number of 100 ml you wish to make:

$$(1.5\ \text{g}/100\ \text{ml}) \times 2 = 3.0\ \text{g}/200\ \text{ml}$$

So you need 1.5 g/100 ml or 3.0 g/200 ml.

Another method for figuring how much powdered medium you need is by using a direct proportion. In this method you must set up a ratio between the grams/liter directions on the label and the amount of medium you wish to make. Using the earlier example, your proportion is

$$\frac{15 \text{ g}}{1000 \text{ ml}} = \frac{x \text{ g}}{200 \text{ ml}}$$

To solve for x:

$$1000x = (15)(200)$$

$$x = \frac{3000}{1000}$$

$$x = 3 \text{ g}$$

You may use either method of calculation. Use the one that you understand better. Most important is that you are confident that your calculations are accurate.

The graduated cylinder is the basic measuring device for liquids. If you need to measure 10 ml or less, it is important to use a pipette of appropriate size. The various cylinders are graduated differently, depending on their sizes and total volumes. For example, a 10 ml cylinder is graduated in 1 ml quantities with subdivisions of 0.1 ml, but a 500 ml cylinder has major graduations of 50 ml with 10 ml subdivisions. Whenever you use a graduated cylinder, you should first inspect the graduations to determine exactly what you can measure accurately with it. For instance, if you want to measure 142 ml, you need to use a 250 ml cylinder that has 2 ml subdivisions, rather than a 500 ml cylinder that has 10 ml subdivisions.

Agar media must be heated to boiling for several minutes before they will dissolve completely. This can be accomplished by setting up a ring stand or tripod with a wire gauze square over a Bunsen burner, as shown in Figure 2-1. You must stir the media almost constantly with a glass stirring rod to prevent burning or boiling over.

If you are fortunate enough to have one, the hot plate stirrer is an extremely useful aid in making media. Any medium that requires heating to dissolve can be made on the hot plate stirrer, which can also be used simply to stir without heat.

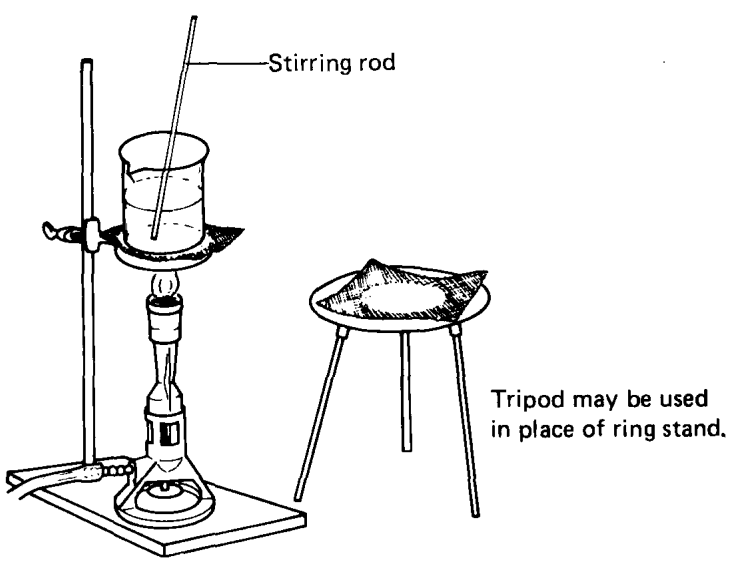


FIGURE 2-1
A ring stand or tripod is set up over a Bunsen burner to boil agar media.