Name: ______________________________ Block: ___ Date: ________________

Lab: Making Observations, an Introduction

Background:
Man has learned much about his environment by making observations, and as such making observations is the first step of the scientific method. In this lab we will begin mastering the skills of observation. Some are subtle and depend only on our sense of sight and smell (remember it is never safe to directly touch or taste anything in microbiology laboratory), other observations require manipulation of the subject material.

Objectives:
The student will be able to:

- Draw the field of view seen on a microscope slide.
- Use his/her sense of smell to describe an odor.
- Describe the appearance of an organism based on its growth on a blood agar plate.
- Develop the proper vocabulary needed to describe organism growth

Vocaulary:

- **Betahemolysis** – beta (β) h. — complete hemolysis of sheep and ox erythrocytes by bacteria in culture media. Note that β-hemolysin of staphylococci causes incomplete hemolysis.
- **Translucent** – Clear; lucid.
- **Sheen** - Glistening brightness; luster:
- **Opaque** - Not reflecting light; having no luster
- **Swarming** – growth on solid media in which there is progressive surface spreading from the parent colony.
- **Gram positive organism** - bacteria that retain a crystal violet dye during the Gram stain process.
- **Gram negative organism** - bacteria appear red or pink after the gram staining process.
- **Cocci** – organism having a round or spherical shape
- **Bacilli** – organism having a rod or rectangular shape

Materials:
Microscope Gram Stain
Stock organisms:

Ps.aeruginosa	S. marcescens	S.pyogenes
P.mirabilis	S. aureus	M.lutus

Procedure:
Repeat the following procedure for each of the six stations.

1. View the organism on the blood agar plate, observe any coloring of the colony, its shape, is it betahemolytic or have any other prominent characteristics. Record your observations on the data table provided.
2. Waft the "fragrance" of the organism. Record your observations.
3. Observe the gram stain, record your observation.
4. Draw an illustration of the field of view from the microscope on your data sheet in the circle provided.

DATA Table:

Station 1	
Colony growth:	
Odor:	
Gram stain:	Illustration:

Station 2	
Colony growth:	
Odor:	
Gram stain:	Illustration:

Station 3	
Colony growth:	
Odor:	
Gram stain:	Illustration:

Station 4	
Colony growth:	
Odor:	
Gram stain:	Illustration:

Station 5	
Colony growth:	
Odor:	
Gram stain:	**Illustration:**

Station 6	
Colony growth:	
Odor:	
Gram stain:	**Illustration:**

From your observations and the descriptions below identify each of organisms.

Descriptions:

Pseudomonas aeruginosa – gram negative rod, green sheen, swarming, sweet smell

Proteus mirabilis – gram negative rod, swarming, smells like dirty feet

Serretia marcescens – gram negative rod, reddish orange to brick red, opaque colonies, no particular odor

Staphylococcus aureus – gram positive cocci in groups, beta hemolytic, white to golden yellow, opaque colonies, smells like dirt

Streptococcus pyogenes – gram positive cocci in chains, beta hemolytic, translucent to white colonies, no particular odor

Micrococcus lutus – gram positive cocci in groups, bright yellow, often smells like dirt

Determinations:

Station 1: ____________________ **Station 2:** ____________________

Station 3: ____________________ **Station 4:** ____________________

Station 5: ____________________ **Station 6:** ____________________

MODULE 2

Preparing and Dispensing Media

PREREQUISITE SKILL

Ability to weigh materials accurately using a triple beam balance.

Suggestion: Perform Modules 2 and 3 (Sterilization of Media and Equipment) in the same lab period.

MATERIALS

- 500 ml graduated cylinder
- hot plate stirrer
- stirring magnet
- stir bar retriever
- triple beam balance or digital electronic balance
- 600 ml beaker
- dehydrated nutrient agar
- 150 ml beaker
- dehydrated nutrient broth
- distilled water
- 8 oz screw-cap bottle or flask of similar size
- Salvarsan burette or dispensing funnel
- culture tubes (16 mm × 150 mm) and closures (16):
  - 8 tubes for slants
  - 2 tubes for deeps
  - 6 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 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 available substitute, to prepare media that require heat to dissolve completely.
4. Prepare a liquid and a solid medium.

5. Demonstrate your ability to use a Salvarsan burette or dispensing funnel to dispense measured amounts of medium into the appropriate containers.
6. Correctly and completely label the various containers of medium according to standard format.
7. Name the type of balance 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 containers used to sterilize the medium for the following: slant, broth, stab, deep, culture plate, 8 oz bottle.

DISCUSSION

Weighing powders and granular substances accurately is essential to preparing microbiological media. 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, the finished medium will not contain the proper proportion of nutrients. If you have not weighed the solid media accurately, the medium may turn out semisolid or too dry.

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

Directions on the labels of most commercially prepared media bottles state 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, so, be sure to *read the label* for each type of medium. You rarely need to make 1000 ml of medium and will usually calculate for smaller amounts.

If you need 500 ml of medium ($\frac{1}{2}$ liter), simply divide both water and powdered medium by 2. If you need 250 ml ($\frac{1}{4}$ liter), 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 moving the decimal point one place to the left, which means you are 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 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:

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

You need 1.5 g/100 ml or 3.0 g/200 ml.

Another method for figuring how much powdered medium you need is to use a direct proportion. 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:

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

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

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

Use the method of calculation you understand better. It is important you be confident that your calculations are accurate.

The graduated cylinder is the basic device for measuring liquids. If you need to measure less than 10 ml use a pipette of appropriate size. Cylinders are graduated differently depending on size and total volume. For example, a 10 ml cylinder is graduated in 1 ml quantities with subdivisions of 0.1 ml, and a 500 ml cylinder bears graduations of 50 ml with 10 ml subdivisions. When you use a graduated cylinder, you should first inspect the graduations to determine the exact increments you can measure with it. For instance, if you want to measure 142 ml, use a 250 ml cylinder that has 2 ml subdivisions, not a 500 ml cylinder that has 10 ml subdivisions.

Agar media must boil for several minutes to dissolve completely. The hot plate stirrer is extremely useful in making media. Any medium that requires heating to dissolve can be made on the hot plate stirrer, which can also be used to stir without heat.

Study Figure 2-1 to familiarize yourself with the magnetic stirrer. Place a Teflon-coated magnet of appropriate size in the beaker with the measured water and powdered ingredients. When you turn the stirrer on (left-hand switch) a magnetic field is created that causes the magnet in the beaker to spin, stirring the medium. If you are preparing an agar medium that dissolves slowly or if the label directs you to use heat in broth preparations, turn on the right-hand switch, which controls the hot plate. Always bring agar media to a full boil. When the medium has come to a boil and is ready to dispense, it will no longer be turbid.

Examine the Salvarsan burette setup shown in Figure 2-2a. It is a modified graduated cylinder with an inverted scale and a pinch clamp to control the flow of a liquid. A dispensing funnel setup, shown in Figure 2-2b, has a funnel and works just as well as the much more expensive Salvarsan burette.

By using an accurately measured guide tube, you will soon be able to dispense roughly equivalent amounts of agar into each of several tubes or other containers. See Table 2-1 for amounts of medium to be used in various culture preparations. After you fill the first few tubes, watch the liquid in the tube you are filling and close the pinch cock when the level is about the same as in the first tube. To do this, hold a guide tube and an empty tube beside each other in one hand. The guide tube allows you to disregard the graduations on the Salvarsan burette or use an ungraduated dispensing funnel.

Agar to be used for culture plates is dispensed into 8 oz bottles. The bottled agar, after autoclaving, can then be poured into several sterile petri dishes to make

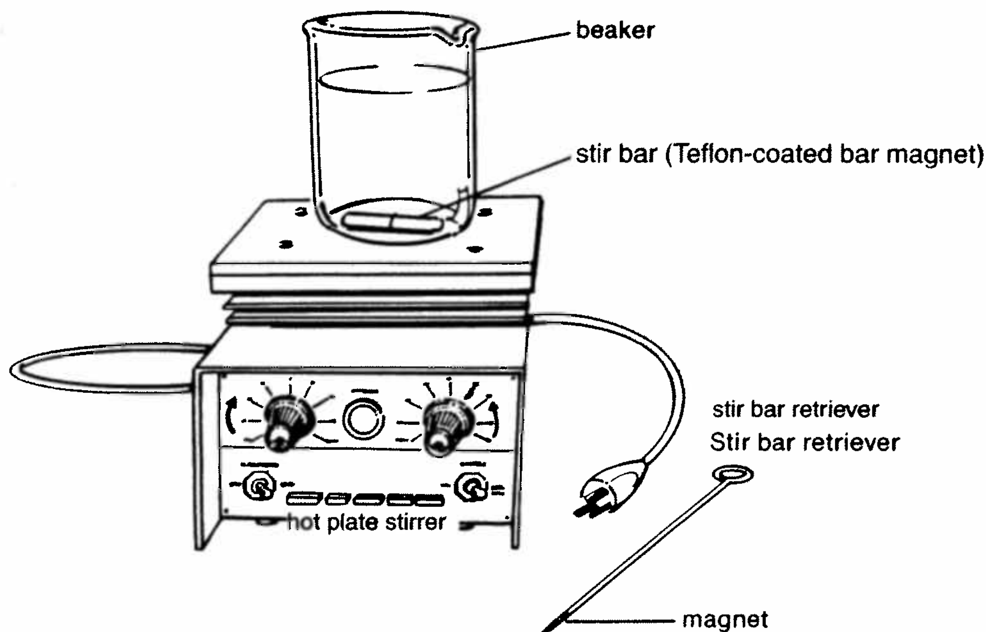
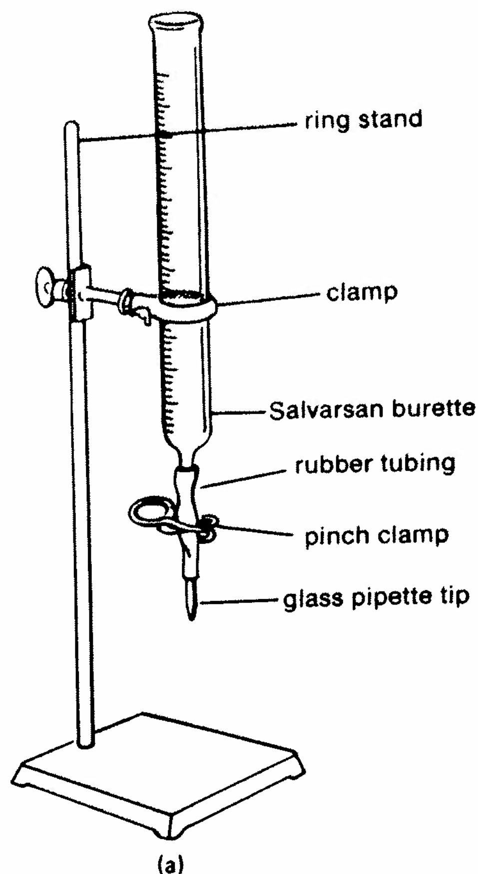


FIGURE 2-1
The hot plate stirrer, used to make media.



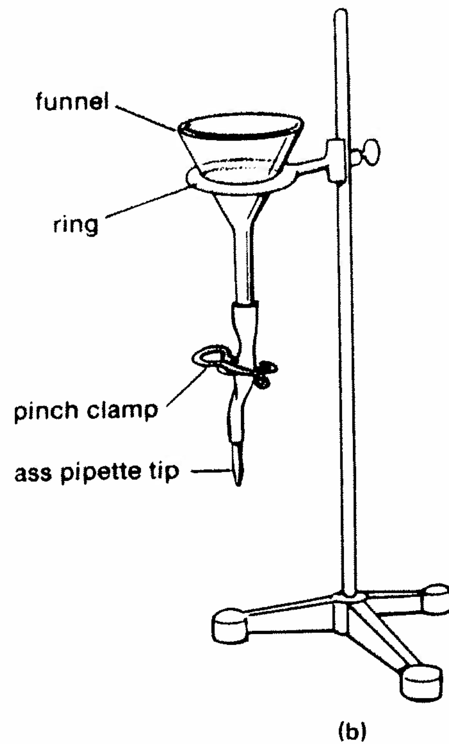


FIGURE 2-2
(a) The Salvarsan burette setup. (b) Alternate dispensing funnel setup.

culture plates. A culture plate contains approximately 20 to 25 ml of sterile agar medium. Media are never sterilized in the autoclave while in the petri dish because the agar would bubble out of the petri dish.

Rinse containers that have held agar with hot water as soon as you empty them. It is crucial to rinse dispensing burettes and tips because the solidifying agar can clog the narrow openings.

When media are sterilized in a steam sterilizer, they are superheated under pressure, that is, the boiling point is raised as the steam pressure rises in the sealed sterilizer. Because of the extreme heat, a tightly stoppered container could explode when the medium expands. To avoid this, leave about 2 inches of air space between the agar level and the neck of the bottle. Even if the cap is loose, expanding agar can boil over the top of the bottle if it is too full. Using cotton-stoppered flasks eliminates the problem because air escapes freely.

TABLE 2-1 Amounts of Medium to Use

Medium	Amount	Autoclaving container
Broth	5–10 ml	Culture tube
Agar slant	10 ml	Culture tube
Agar deep	12 ml	Culture tube
Agar stab	7 ml	Culture tube
Agar plate	200 ml (20–25 ml/petri dish)	8 oz bottle (3/4 full)

ACTIVITIES

Activity 1: Calculating Grams of Media Necessary

Record your calculations of the following problems on the worksheet.

1. The label on the bottle directs you to add 42 g of medium to 1000 ml of distilled water. How many grams should you weigh to prepare 300 ml of medium?
    - Move the decimal one place to the left and multiply by the number of 100 ml you are making.
2. The medium label calls for 25 g/liter, but you need only 400 ml. How many grams of medium do you weigh?
3. You need 12 g of medium per liter of distilled water. You want to rehydrate 350 ml of medium. How many grams do you weigh?
4. You wish to make 600 ml of medium. The label calls for 30 g/liter. How many grams do you weigh?
5. You need 400 ml of nutrient agar, which requires 23 g/liter. How many grams do you need?
6. To make 150 ml of a medium that requires 60 g/liter, how many grams of dry medium do you weigh?

Check your calculations against the answer key and note any discrepancies. If you have responded incorrectly, recalculate and see if you arrive at the right answer. Do several more calculations of this type. Make up your own problems for practice or ask your instructor for help.

Activity 2: Making Nutrient Agar

Prepare 300 ml of nutrient agar.

1. Obtain a 500 ml graduated cylinder, a hot plate stirrer, a Teflon-coated magnet and a 600 ml beaker.
2. With the 500 ml cylinder, measure 300 ml of distilled water.
    - You must always have the bottom of the meniscus (the curved line you see at the top of a liquid) on the graduation for the quantity you wish to measure.
3. Weigh 6.9 g of dehydrated nutrient agar (NA).
4. Pour about two-thirds of the distilled water into a 600 ml beaker and add a Teflon-coated magnet.
    - Select a beaker that will hold about twice as much liquid as you will be working with to allow room for stirring and to prevent the agar from boiling over the top.
5. Place the beaker over the heat source very gently. The beaker can break from the slightest impact.
6. Slowly turn the left-hand control knob (stirrer knob) until the water moves vigorously.
7. Turn the hot plate on (right-hand knob) and set the control at 9 or 9 ½.
8. Add the 6.9 g of dehydrated NA.
9. After you have poured the powdered medium into the beaker of water, hold the glazed weighing paper over the beaker and rinse any remaining powder from the paper into the beaker with the remainder of the water in the graduated cylinder.
10. Stir the medium continuously as it heats to a boil to prevent burning and sticking to the bottom. Remove the medium from the hot plate stirrer when it comes to a rolling boil. Even after you turn the heat switch off, enough heat remains in the hot plate to cause the temperature in the beaker to continue to rise.

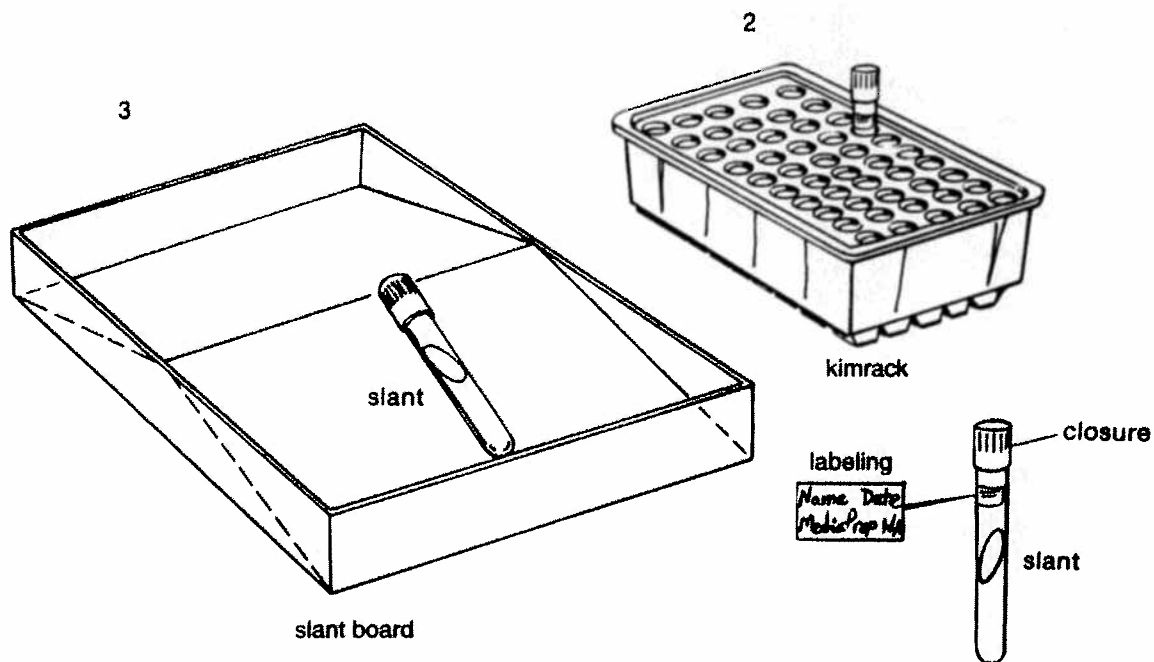


FIGURE 2-3
A tube of agar is labeled, placed on a Kimrack, and then autoclaved. The sterile tube of molten agar is placed on a slant board where it solidifies.

Activity 3: Dispensing Nutrient Agar

1. Obtain a Salvarsan burette setup or a dispensing funnel setup.
2. When the NA has come to a full boil, remove it from the hot plate stirrer and turn off both heat and stir controls. Remove the stirring magnet from the beaker with a stir bar retriever (shown in Figure 2-1). Rinse the magnet and stir bar retriever at once.
3. Carefully pour the hot NA into the burette or dispensing funnel. Make *sure* the pinch clamp is closed before you pour the agar.
4. Using a measured guide tube, dispense 10 ml of NA into each of 8 tubes for slants.
5. Now dispense 12 ml of NA into each of two tubes for deeps.
6. Allow the remaining agar to run into an 8 oz bottle.
   • Dispense all agar before it solidifies in the burette.
7. Remove the burette or funnel from the ring stand and rinse it immediately with hot water. Return it to the ring stand and leave the pinch clamp open so it can drain.
8. Place the screw cap loosely (backed off a turn) on the 8 oz bottle.
9. Place closures on all tubes.
10. Label each tube and your 8 oz bottle using waterproof permanent ink on a piece of tape. Print your name, the date, type of medium, module, and activity. Do not use a felt tip pen or one with erasable ink.
    a. Label each tube with 10 ml of medium "NA slant."
    b. Label deeps "NA deeps."
    c. Label the bottle "NA."
11. Place all NA slant tubes together in a Kimrack and NA deep tubes in a basket.
    • After sterilizing them, all the tubes can be slanted at once by laying the Kimrack on its side on the slant board.
12. Label the basket and Kimrack with a piece of tape containing the information listed in number 10. This agar is now ready to be sterilized.

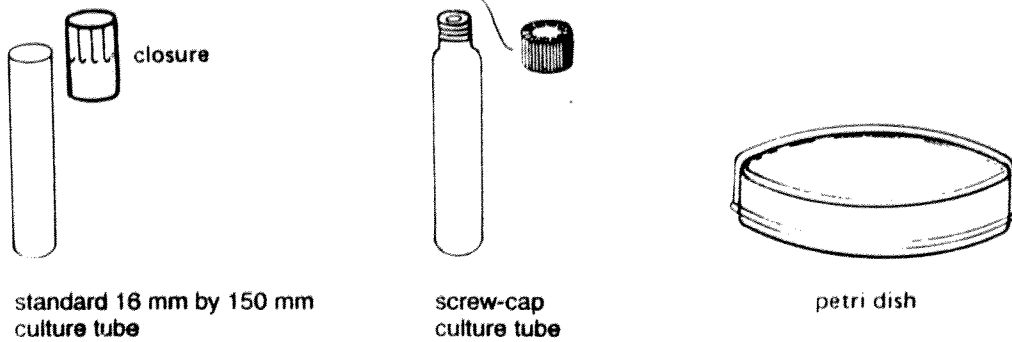


FIGURE 2-4
Various types of culture containers.

13. After the tubes and 8 oz bottle are sterilized and while the medium is still liquid, place the tubes for slants on the slant board (see Figure 2-3) and allow them to solidify. After cooling, refrigerate sterile media until you are ready to use them.

Activity 4: Making Nutrient Broth

Prepare 75 ml of nutrient broth.

1. Obtain a bottle of dehydrated NB and weigh the proper amount of powdered medium you will need to prepare 75 ml of NB.
2. Measure 75 ml of distilled water, and mix the broth in a 150 ml beaker using the same method used in Activity 2. It is rarely necessary to boil broth. In fact, the medium will often dissolve completely with no heat.
3. Gather a basket, six tubes, and a dispensing burette or funnel.
4. Dispense the broth into the tubes in 10 ml amounts. Make an extra tube in case one breaks or you make an error. Discard any extra broth.
5. Rinse the burette and allow it to drain.
6. Label the tubes with your name, the date, type of medium, module, and activity.
7. Place closures on the tubes and place them in a basket. They are now ready to sterilize.

After you sterilize the broth tubes, store them in the refrigerator with the NA until you are ready to use them.

Table 2-1 summarizes the amounts of medium used in various culture preparations. Common containers for media are depicted in Figure 2-4.

Take the post test.

Phonetic Pronunciation

agar = ag´-ur

Name ______________________________

Lab Section ______________________________

MODULE 2: PREPARING AND DISPENSING MEDIA

Activity 1

Calculating Grams of Media Necessary

1. ______________________________

2. ______________________________

3. ______________________________

4. ______________________________

5. ______________________________

6. ______________________________

MODULE 4

Compound Microscope for the Study of Microbes

PREREQUISITE SKILL

MATERIALS

compound light microscope with oil-immersion objective
light source
clear plastic millimeter ruler
microscope slides (2)
concentrated salt solution
prepared slides of stained blood smear
stained smear of yeast, *Bacillus* sp. (any species of the genus *Bacillus*), *Escherichia coli*, and a yeast–*E. coli* mixture
prepared slide of mixed bacteria types

OVERALL OBJECTIVE

Demonstrate your ability to use a compound light microscope at all powers of magnification.

Specific Objectives

1. Draw various bacteria using the 10×, 40×, and 100× objectives of the microscope.
2. Define the terms magnification at eyepoint, object, and parfocal.
3. Name the optical parts of the microscope and their functions.
4. Name the movable parts of the microscope that are not part of the optical system and describe their functions.
5. Name the two parts of a microscope that are most critical in controlling light.
6. Label all the parts of your microscope that are described in the module.
7. Relate the magnification of the various objectives to the approximate size of the field.
8. Describe how to manipulate the diaphragm and condenser to improve light and define the object.
9. Explain how to take care of a microscope.
10. Describe how a microscope should be carried.
11. Explain how the different powers of your objectives are identified on the microscope.
12. Calculate the approximate size of a yeast cell.

DISCUSSION

Using a microscope correctly is invaluable in studying microbes because of the size of the microorganisms. You can become an expert microscopist only with practice. Module 4, "Compound Microscope for the Study of Microbes," will show you how to use a microscope.

As a beginning microbiologist, the amount of skill you develop in using the microscope can determine whether you find the course interesting or boring. How the microscope functions is not as important as what you are supposed to see through it. If you wish to learn the mechanism of the microscope, refer to any microbiology text. They contain detailed explanations of resolving power, numerical aperture, real image, virtual image, and refractive index. If you plan to major in or go onto advanced work in microbiology, you will need to learn these functions of the microscope. It is the purpose of this module, however, to allow you to enjoy microbiology by teaching you to use your microscope to its optimum.

The microscope illustrated is an Olympus CHS/CHT series binocular microscope, in which focusing is done by moving the stage. Other makes are similar. Study Figures 4-1 and 4-2. (Figures 4-3 and 4-4 show the Reichert-Jung [AO Spencer] series 150 microscope, in which focusing is done by moving the nosepiece. Familiarize yourself with all the labeled parts and explain their functions. (See Table 4-1.) Study the figures and table. (If your microscope is monocular, all parts are the same except the number of oculars and the interpupillary distance scale.)

Each of the objectives on a microscope bears a magnification number: 10×, 40×, or 100×. The number indicates how many times the object is magnified, or enlarged, by that objective. The 10× objective is the low-power objective, magnifying the object 10 times its actual size. The 40× objective (high power) magnifies it 40 times its actual size, and the 100× objective (oil immersion)

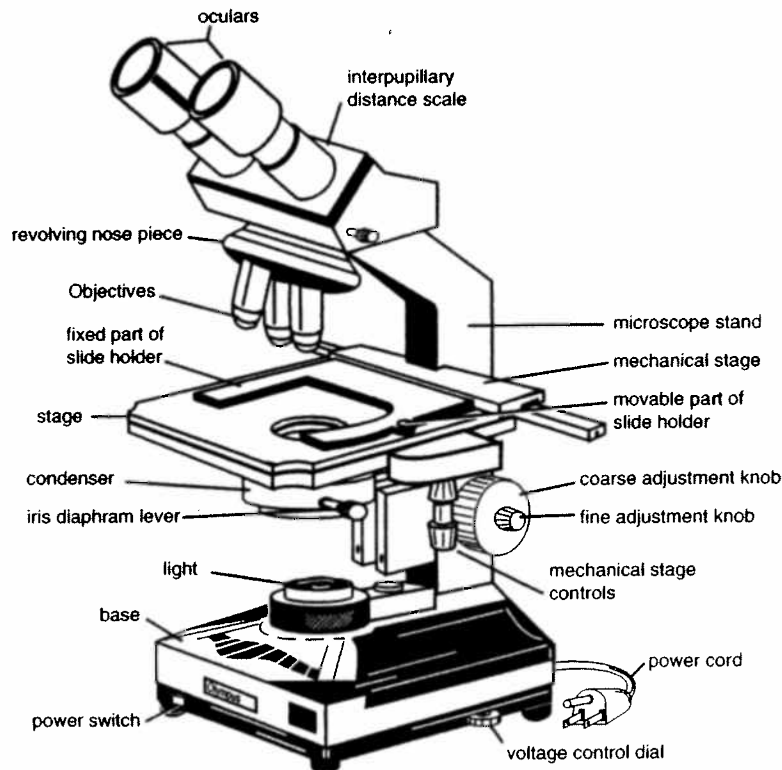


FIGURE 4-1
Olympus CHS/CHT binocular microscope (front view).

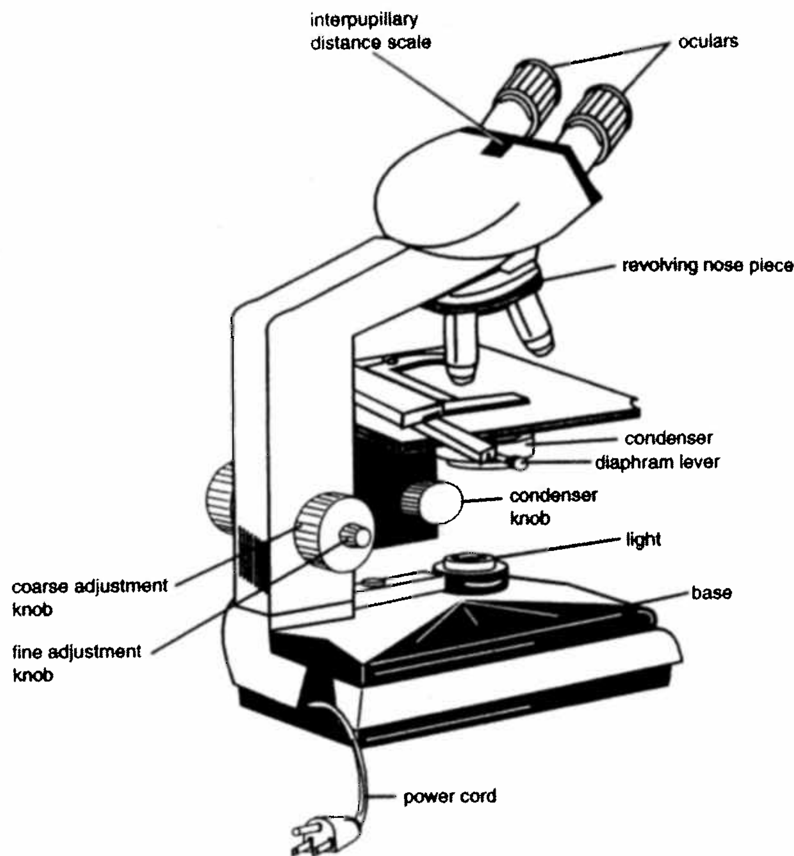


FIGURE 4-2
Olympus CHS/CHT binocular microscope (rear view).

magnifies it 100 times the actual size. Some microscopes are equipped with objectives that vary from the usual 10×, 40×, and 100× combination. The most common variation is a 10×, 43×, and 97× combination. Some microscopes are equipped with a 4× (or 3.5×) scanning lens in addition to the other three objectives.

Objectives are often marked with incised bands around the lower part to allow you to distinguish one from another without seeking the magnification number. These bands are especially important when using the high-power and oil-immersion objectives, which are nearly the same length. The high-power objective (40×) is used dry, and the outer lens can be damaged if immersed in oil. The 10× usually has one band, the 40× two bands, and the oil-immersion objective (100×) three bands. The bands may be colored for still easier recognition, in which case the oil-immersion objective is usually banded in red.

The ocular, or eyepiece, also magnifies, usually 10 times, which means that what you see at eyepoint is magnified 10 times more than the magnification marked on the objective you are using.

The objectives on your microscope, and on most microscopes, are *parfocal*. That means they are mounted so that when an object is in sharp focus with one objective, it will be in approximate focus with the other objectives when they are rotated into working position. If an object is in sharp focus on low power, you can rotate the high-power objective (40×) and achieve sharp focus with only a slight turn of the fine adjustment knob.

Despite the fact that the objectives are parfocal, the novice microscopist frequently "loses" the object when switching to a higher-power objective because each increase in magnification *decreases* the microscopic field by about half. The illuminated field *appears* to be the same size at all powers, but the area being

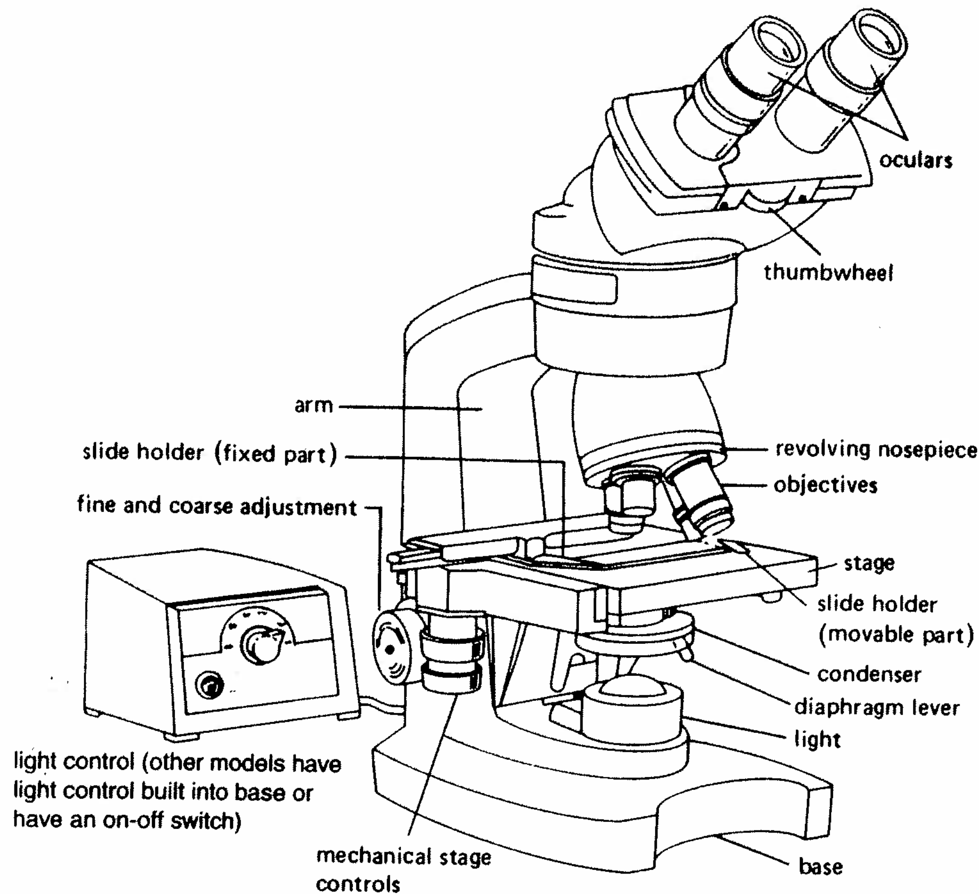


FIGURE 4-3
Reichert-Jung (AO Spencer) series 150 binocular microscope (right front view).

magnified shrinks each time you switch to an objective that magnifies at a higher power. Field diameter is inversely related to power of magnification. That is, when the magnification doubles, the field diameter halves. If the object is not in the center of the field when you switch to a higher power, you can easily lose it from the resulting diminished field. Center the object before you switch to a higher power.

As magnification increases, more light must enter the optical system. Activity 1 will help you master light control, which is critical. Repeat Activity 1 until you master it.

Care of Your Microscope

Read and memorize the following instructions and precautions and you will become a skilled microscopist sooner.

1. Use both hands to carry the microscope. Grasp the stand firmly with one hand and lift it carefully. Place the other hand under the base as you carry it. Keep the microscope vertical because if tilted the oculars could fall out.
2. Clean the optical system (ocular lens, objectives, and condenser lens) before and after each use. This is especially necessary if you share the microscope with a student in another lab section. Use optical lens tissue *only* to clean the optical system. To remove oil or dust from other portions, use a soft cloth or facial-type tissue. Keep the microscope immaculate!
3. Never remove a part of the microscope without consulting your instructor.

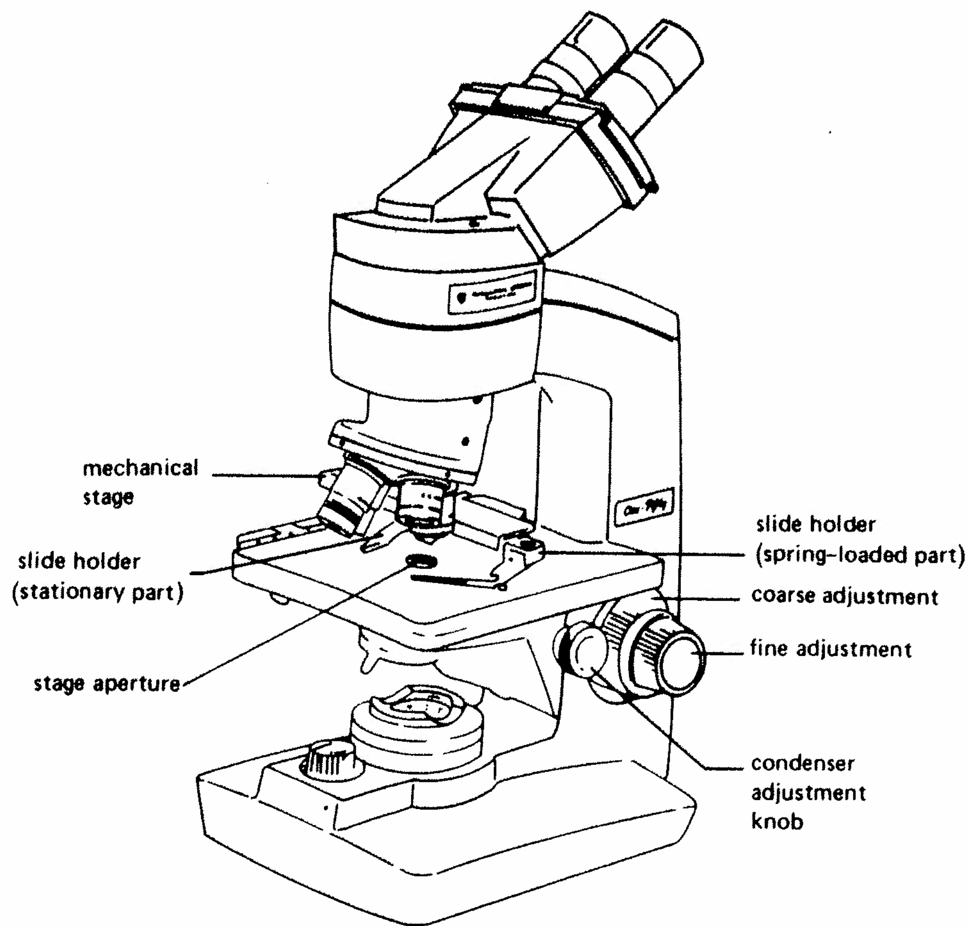


FIGURE 4-4
Reichert-Jung (AO Spencer) series 150 binocular microscope (left front view).

4. When you finish using the microscope and have cleaned it, if the microscope does not have an autofocus stop, place the low-power objective into working position. It is shorter than the other two objectives and less likely to be damaged if it strikes the mechanical stage. Replace the dust cover before you return the microscope to the storage cabinet.

5. If the microscope does not have an autofocus stop, never focus downward while looking through the eyepiece. To prevent breaking slides and damaging the objective, turn the objective to its lowest point while watching from the side before you look through the eyepiece and focus. Do not touch the lenses of the eyepieces because oils in the skin can mar the polished glass surface of the lens.

ACTIVITIES

Activity 1: Practice for Light Control

1. Turn on the light source to maximum intensity.
2. While looking through the oculars, adjust eyespan by gently pushing the oculars closer together or farther apart (use interpupillary distance scale) until you see a single illuminated microscopic field.
3. While looking through the oculars, open and close the iris diaphragm.

TABLE 4-1 The Binocular Microscope

Part	Function
Oculars (eyepieces)	A series of lenses that usually magnify 10 times.
Interpupillary distance scale	Adjusts to your eyespan.
Revolving nosepiece	Rotates for changing from one objective to another.
Objectives	Usually three magnifications (if no scanning lens is present): 10×, low power; 45×, high dry power; and 100×, oil immersion. Powers of the objectives are distinguished by colored bands.
Slide holder	Spring-loaded portion allows for placing the microscope slide in the mechanical stage, which holds it tightly.
Stage	Rises and lowers in focusing.
Diaphragm lever	Opens and closes the diaphragm to control the amount of light that strikes the object.
Condenser	Condenses light waves into a pencil-shaped cone, preventing light from escaping. Controls the intensity of light when raised and lowered.
Condenser adjustment knob	Raises and lowers the condenser.
Mechanical stage	Allows the slide to be moved on the stage.
Mechanical stage controls	Move the slide on two horizontal planes, that is, back and forth and side to side.
Base	Supports the entire microscope.
Power switch	Turns light on and off.
Voltage control	Controls the intensity of light.
Microscope stand	Supports upper half of the microscope.
Coarse adjustment	Moves the stage up and down quickly for approximate focusing.
Fine adjustment	Moves the stage up and down slowly for definitive focusing

- Determine the effect the diaphragm opening has on the brightness of the microscopic field.
- Open and close the diaphragm several times, making a mental note of optimum brightness. Optimum brightness does not necessarily mean maximum brightness.

4. Using the condenser knob, raise and lower the condenser while looking through the oculars.
   - Note what effect the position of the condenser has on the intensity of light. Do this several times, adjusting the condenser to optimum illumination. Do not hesitate to open and close the diaphragm or raise and lower the condenser.
   - Once the condenser is adjusted to optimum light intensity, do not change the setting. The amount of light must now be controlled with the iris diaphragm.

Repeat this practice activity several times. Light control is crucial to good microscopy.

Activity 2: Diameter of the Field

1. Place a flat, clear plastic millimeter ruler on the stage and focus the millimeter marks with your 10× objective.

TABLE 4-2 Magnification at Eyepoint

Ocular		Objective	Magnification at eyepoint
10	×	10×	100×
10	×	40× (43×)	400×(430×)
10	×	100× (43×)	1000×(970×)

2. Position the scale so that one of the unit marks is at the edge of the field and the edge of the ruler runs across the center of the field.
3. Estimate the diameter of the field in millimeters. Record your observation on the worksheet.
4. Convert this figure to micrometers (μm), also called microns, by multiplying by 1000. Record your calculation on the worksheet.
   - The micrometer (micron) is the preferred unit of measure for very small microscopic objects. For example, a red blood cell is about 7.5 μm in diameter.
5. The field of view on high dry power and oil immersion is too small to measure with a ruler. You can, however, calculate the size of the fields using the 10x objective measurement as a basis.
   - High dry power is 4.0 times the magnification of low power. (See Table 4-2.)
   - Because magnification and size of field are inversely related, the field diameter of high dry power must be 4.0 times less than the field diameter of low power.
6. Calculate the size of the oil immersion field.
7. Record the figures for the diameter of the fields on the worksheet.
8. You can determine the approximate size of an object in the microscopic field by estimating the number of objects that would fit across the diameter of the field of view. Then divide the diameter of the field by that number.
   - You estimate that an animal cell is about $^1/_5$ the diameter of the field. That is, about 5 of these cells would fit across the field diameter of the low-power field (10×). The size of the cell is approximately:

Activity 3: Low-Power Observation of Salt Crystals

1. Place a small drop of concentrated salt solution on a microscope slide, spread it out, and allow it to dry.
2. Place the slide in the slide holder as shown in Figure 4-5.
   - Be sure your slide is held against the arm of the mechanical stage.
3. Position the crystals under the objective by using the mechanical stage controls.
4. Using the coarse adjustment, move the 10× objective as close to the slide as possible, that is, until you reach the autostop. Do not force the objective further.
5. Looking through the ocular, turn the coarse adjustment back slowly, moving the objective away from the slide until the salt crystals come into focus.

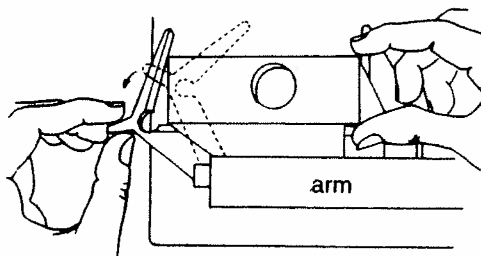


FIGURE 4-5
A slide is placed in the slide holder. Though there are variations of the slide clip, all work on the same principle: spring-loaded clamps place tension on the slide, keeping it in place as it moves with the mechanical stage. (Be sure your slide is held against the arm of the mechanical stage.)

6. Adjust the optical system as you did in Activity 1 until you obtain the best definition of salt crystals.
   - Adjust and readjust the iris diaphragm and condenser.
   - It will probably be necessary to reduce the light intensity. Experiment with the iris diaphragm and condenser until you find the optimum position of each that allows you to see the most definition of the salt crystals. Do not be satisfied with just any image; get the best image you can.

Make a composite drawing of a few salt crystals on the worksheet. Using the mechanical stage, select one crystal from several different fields.

Activity 4: High-Power Observation of a Stained Blood Smear

1. Place a prepared slide of a stained blood smear in the slide holder.
   - Be sure the smear side faces upward.
2. Position the smear under the objective by using the mechanical stage.
3. With the 10× objective in place, adjust the iris diaphragm and condenser for optimum light.
   - Repeat Activity 2 until the blood cells are in sharp focus.
4. Rotate the high-power objective (40×) into working position.
5. Using the fine adjustment, adjust to the sharpest possible focus.

Sketch representative blood cells at 400× on your worksheet. Do not ponder the different types of cells. In the next activity you will study cell types.

Activity 5: Demonstration of Parfocality Using Oil-Immersion Objective

1. With the stained blood smear from Activity 3 still in focus and the 40× objective still in place, adjust for maximum light.
2. Rotate the revolving nosepiece until the space between the 40× and the 100× objective is directly over the center of the smear. No objective is in working position yet.
3. Place a large drop of immersion oil on the smear, being careful that none gets on the microscope stage.
4. Rotate the nosepiece until the 100× objective clicks into working position.
   - The tip of the objective should be immersed in the oil but not quite touching the slide. If the objective is not submerged in the oil, you will not obtain sharp definition of the object.
5. Look through the oculars and focus with the fine adjustment.

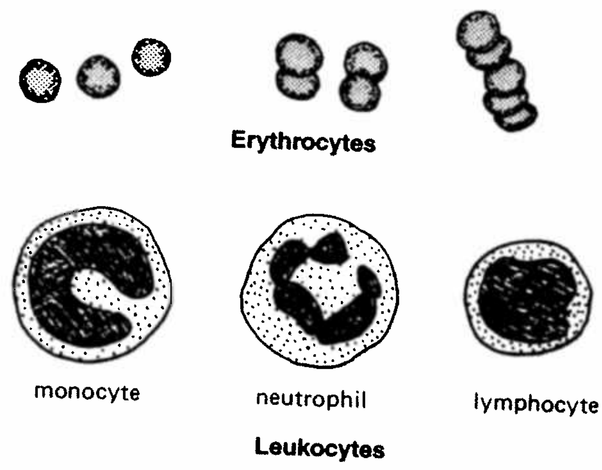
Draw several blood cells on your worksheet as they appear at 1000×. See Figure 4-6, charts, and color plates for blood cell types. Include erythrocytes and leukocytes. Calculate the approximate size of a leukocyte.

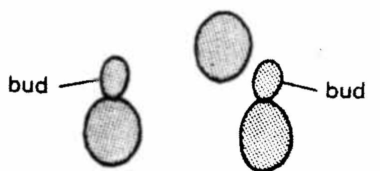
Repeat this activity using a stained smear of yeast and of bacteria (*Bacillus* sp.) as shown in Figure 4-6.

After you have become proficient in employing parfocality to use the oil-immersion objective, and after you are satisfied that you are getting the maximum amount of definition by using the optimum amount of light, proceed to Activity 6.

Activity 6: Direct Use of the Oil-Immersion Objective

The more expert you are as a microscopist, the more you will use only the oil-immersion objective to study bacterial structures. Modern microscopes have an autofocus stop, so after applying oil to the slide, you can lower the coarse adjustment gently to its positive stop without danger of breaking the slide. The object will come into focus with a slight adjustment of the fine adjustment knob.





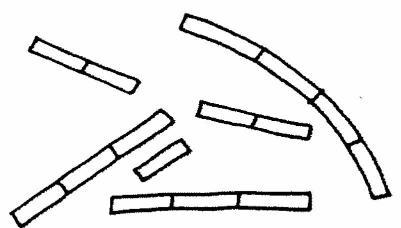


FIGURE 4-6
Blood cell types, yeast cells, and bacterial cells as viewed with an oil-immersion objective (not sized to scale). Use a photographic plate for accuracy. See color insert.

1. Place a simple stain of yeast cells in the mechanical stage.
2. Put a large drop of oil on the slide as described in Activity 5.
3. Using the coarse adjustment, immerse the 100× objective into the oil until the stop is reached.
4. Do not force the coarse adjustment knob downward.
5. Looking through the oculars, slowly turn the coarse adjustment backward; that is, move the oil-immersion objective slowly away from the object until it comes into partial focus.
6. When you can see the object vaguely, use the fine adjustment for definitive focus.

Precaution: The fine adjustment should be forward or in the middle of its track before you begin this activity. It should not be turned toward you, where there is no more focus adjustment.

Adjust the light for optimum definition. Draw several yeast cells on the worksheet.

Repeat the procedure using stained smears of *Bacillus* sp., *Escherichia coli*, and a yeast–*E. coli* mixture. Don't hesitate to reread the instructions. If you take precautions and time now, you will become expert sooner. Time, practice, and mastery of the microscope will make microbiology more rewarding.

Draw numerous cells from the *Bacillus* sp., *E. coli*, and the mixed slide on your worksheet. Calculate the approximate sizes of a yeast cell and an *E. coli* cell.

When you are ready to make your drawings of *E. coli*, ask your instructor to look through your microscope to check your illumination. It is good to know if the organisms you are observing have the maximum visibility.

Activity 7: Continued Practice Using the Oil-Immersion Objective

Examine a prepared smear of mixed bacterial types or other available smears with the oil-immersion objective. This activity repeats Activity 6 and offers more practice in finding the object with the oil-immersion objective.

Permanent, commercially prepared slides have cover slips over the smear. Place the immersion oil directly on the cover slip, and immerse the oil-immersion objective carefully.

Draw several representatives of each bacterial cell type on the worksheet. Figures 4-6 and 5-1 may help you identify cell types.

When you think you are well acquainted with the microscope and its parts, take the post test. If you are not satisfied with your results, review this module before going on. Your success in this course depends on your being honest with yourself.

Phonetic Pronunciation

Bacillus = buh-sill´-us

Escherichia coli = esh-ur-eek´-ee-uh koe´-lee

Name________________________________

Lab Section___________________________

MODULE 4: COMPOUND MICROSCOPE FOR THE STUDY OF MICROBES

Activity 2: Diameter of Field

Diameter of 4× field (scanning) [40× at eyepoint]: measured

________ mm = ____________ µm

Diameter of 10× field (low power) [100× at eyepoint]: measured

________ mm = ____________ µm

Diameter of 40× field (high power) [400× at eyepoint]: calculated

Calculations:

________ mm = ____________ µm

Diameter of 100× field (oil immersion) [1000× at eyepoint]: calculated

Calculations:

________ mm = ____________ µm

Sodium Chloride Crystals

Stained Blood Smear

Direct Use of Oil-Immersion Objective

Continued Practice Using Oil-Immersion Objective

Rank the cells in order of size from small to large when observed with the oil-immersion objective.

yeast	Smallest	______________________
Bacillus sp.		______________________
E. coli		______________________
leukocyte		______________________
erythrocyte	Largest	______________________

MODULE 6

Ubiquity of Microorganisms

PREREQUISITE SKILL

Completion of Module 5, "Cleaning Microscope Slides and Preparing a Wet Mount."

MATERIALS

sterile tube of nutrient broth (1)*
wet mount materials
sterile nutrient agar plates (7)
tryptic soy agar plates (TSA) (1)
tube containing two sterile swabs and 2 ml sterile saline
sterile nasopharyngeal swab
beaker of disinfectant
For related experiences:
- sterile nutrient agar plates (2)
- surgical scrub pack†

* Prepared by the student if the instructor so indicates, otherwise, a tube of broth from Module 2, "Preparing and Dispensing Media," can be used.
† Usually obtained by student from place of employment.

OVERALL OBJECTIVE

Show that microbes are present everywhere in the environment.

Specific Objectives

1. Demonstrate the presence of microorganisms in different areas of your environment, such as in the soil, air, on the benchtop, your hands, in your mouth, and under your fingernails.
2. Describe, with examples, the ubiquity of microorganisms in the environment.
3. Define the terms ubiquitous and omnipresent.
4. Describe the location of labels on petri dishes and the position of culture plates in the incubator.
5. State the temperature of incubation for organisms that make up the normal flora in your body.

DISCUSSION

Module 6, "Ubiquity of Microorganisms," shows that microbes are everywhere. It also introduces the necessity of using aseptic technique when working with bacteria. Module 7, "The Aseptic Transfer of Microbes," will show you how to transfer bacteria from one tube to another. In that module you will manipulate living bacteria without fear of contaminating yourself or a pure culture.

After you complete Module 6 you will connect the omnipresence of microorganisms with the importance of aseptic technique to transfer bacteria. The need for aseptic technique will become even more apparent to you after you grow different kinds of microorganisms from various sources in your environment (Specific Objective 1). You will see beyond a doubt that microbes are truly ubiquitous, or omnipresent, meaning they are everywhere! They are at the bottom of the ocean, on ice-capped mountaintops, in hot sulfur springs, in milk, in drinking water, every place on the planet.

When you realize you are surrounded by bacteria, fungi, protozoans, and other microorganisms, you will become bacteria-conscious. The activities in this module will prove to you that tiny, invisible, ever-reproducing, living microbial cells are around you, on you, and inside you.

Gather together the materials you will need to perform the following activities.

Precaution: **You will be working with sterile petri dishes containing two different agar media that look alike. Do not remove the plates from the supply area until you label the bottoms. This way you will be able to distinguish between the two media.** Petri dishes are labeled on the bottom instead of the lid to prevent errors caused by interchanging the lids.

ACTIVITIES

Activity 1: Growing Microorganisms from Environmental Sources

A. Growing Microorganisms from Soil

1. Label a tube of nutrient broth with your name, date, and activity number.
2. Step outside the lab and obtain a pinch of moist soil.
3. Drop the soil in the tube of sterile nutrient broth.
4. Put the soil-broth tube in a coffee can or culture tube basket.
5. Incubate at 30°C for 48 hours or at room temperature for a longer period.
6. After incubating, make a wet mount (review Module 5, "Cleaning Microscope Slides and Preparing a Wet Mount"). Observe with your microscope.
7. Draw and describe your observations on the worksheet.

B. Growing Microorganisms from the Air

Microbial cells are so small and so light that they are constantly being wafted around by air currents, or they can hitch a ride on dust particles. To demonstrate their presence in the air, do the following:

1. Label a sterile nutrient agar plate as you did the nutrient broth tube.
   - Label the bottom of the petri dish.
2. Open the nutrient agar plate for 20 minutes.
   - When you expose the microbial nutrient, airborne bacterial and fungal cells will settle on it and develop into colonies after incubation. Place the nutrient agar plate where air currents are maximal. The lab worktable is also a good place to find bacteria. Decide where you would like to open the plate.
3. After 20 minutes, close the agar plate and place it upside down in the 30°C incubator for 48 hours.
   - Always incubate culture plates in an inverted position unless otherwise instructed.

In your next lab session, draw several different colony types. Not all colonies are bacteria. Light, fluffy, cotton ball-like colonies are fungi.

C. Growing Microorganisms Obtained from Your Benchtop

After you have grown bacteria obtained from your benchtop, you will understand why you should wipe your working area with disinfectant at the beginning and at the end of each laboratory period.

1. Label the bottom of a nutrient agar plate with your name, date, activity number, and type of medium.
2. Remove one saline-soaked swab from the tube and rub the cotton tip on your tabletop before it is disinfected.
   - Include areas such as those near gas jets and corners where dust accumulates.
3. Use the contaminated swab to inoculate the nutrient agar plate.
   - It does not matter how you inoculate the nutrient; simply rub the cotton swab gently over the surface of the agar. Use as much of the surface of the nutrient as you can.
4. Return the cover to the plate and incubate inverted in the 30°C incubator for 48 hours.
5. Disinfect the benchtop and repeat this activity.
6. Dispose of the used swabs in a container of disinfectant in the decontamination/ discard area of the lab.

D. Growing Bacteria Found on Yourself

You can grow bacteria from any surface of your body at any time. To demonstrate this:

1. Place your fingers lightly on the surface of the sterile nutrient agar in a petri dish, and drag your fingers gently back and forth across the plate two or three times.
2. Close the plate and incubate it upside down at 30°C for 48 hours.
3. Wash your hands with soap and rinse well, but do not dry them.
4. Repeat steps 1 and 2 with slightly moist fingers on a second plate.

In your next lab session, draw the growth of these skin bacteria before and after washing your hands.

E. Growing Bacteria Found Inside You

Label a tryptic soy agar (TSA) plate. TSA is preferred to NA as a nutrient medium for this activity because it is especially formulated to support the growth of strepto-cocci and other organisms commonly present in your body that have complex nutritive requirements. Such bacteria are also called fastidious organisms.

All orifices of your body contain numerous types of microbes. To demonstrate the bacteria in one of your body openings:

1. Touch your tongue to the sterile surface of a TSA plate.
   - The more surface area of your tongue touching the agar, the more growth you can expect.
2. Close the plate and incubate it at 37°C for 48 hours.
   - Note that 37°C is a higher temperature of incubation than that used for skin bacteria. This higher incubation temperature is used because microbes that flourish inside you grow best at body temperature.

In your next lab session, draw colonies that arise from microorganisms in your mouth.

Activity 2: Growing Other Bacteria Found on Yourself

1. Press your slightly open lips against the surface of a nutrient agar plate. Incubate for 48 hours at 30°C.
   - Are bacteria transmitted while kissing?

2. Remove material from under your fingernails with a sterile nasopharyngeal swab.
    a. Before you remove the material from under your nails, moisten the swab by dipping it into the sterile saline remaining from Activity 1C.
    b. Rub the swab over the surface of a sterile NA plate, rotating it as you rub. Incubate the plate.

    After incubating, draw the growth on the plates.

Activity 3: Conclusions about the Ubiquity of Microorganisms

After growing microbes from several sources, summarize your findings in short written form on your worksheet.

Take the post test. Be completely satisfied with your results before you proceed to the next module.

Related Experience

Repeat Activity 1D, "Growing Bacteria Found on Yourself," after a surgical scrub of your hands. Did the number of bacteria drop?

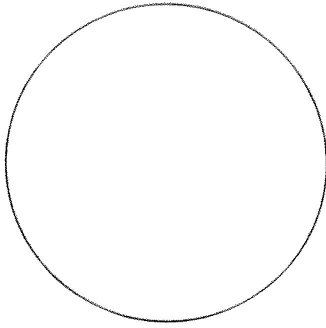
Name________________________________

Lab Section____________________________

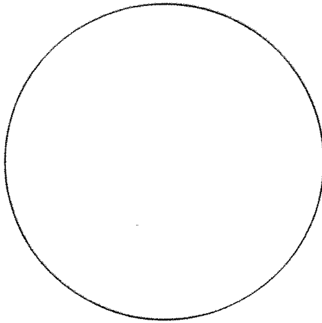
MODULE 6: UBIQUITY OF MICROORGANISMS

Activity 1

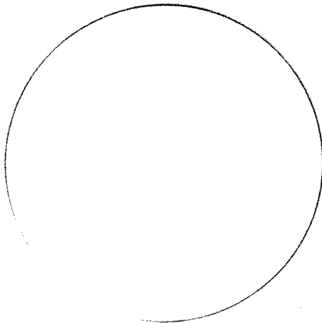
A. Microorganisms from soil



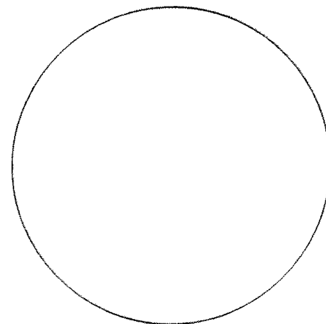
B. Microorganisms from air



C. Microorganisms from benchtop

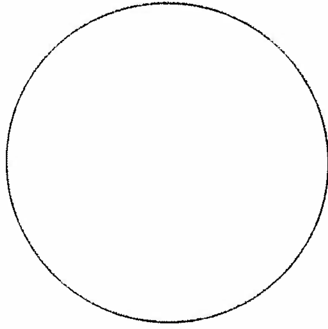


Before disinfection

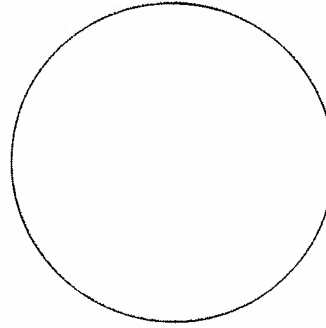


After disinfection

D. Bacteria found on you

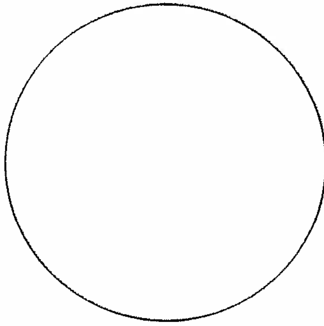


Unwashed dry hands



Washed wet hands

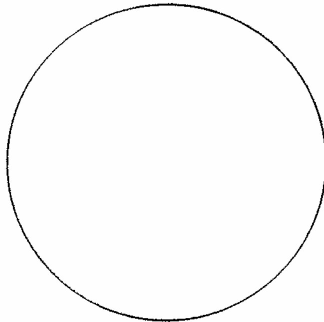
E. Bacteria found inside you



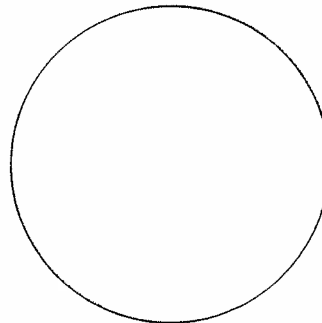
Tongue

Activity 2

Other Bacteria Found on Yourself



Kiss



Fingernails

Activity 3

Conclusions

____

____

____

____

Why does the amount of bacterial growth differ on washed compared with unwashed hands?

MODULE 9

Aseptically Dispensing Agar into Petri Dishes

PREREQUISITE SKILL

Successful completion of Module 7, "Aseptic Transfer of Microbes."

MATERIALS

culture tube rack
Bunsen burner
burner striker
sterile petri dishes (7 or 8)
tubes of melted agar medium (2), deeps*
screw-cap bottle or flask of melted nutrient agar, 100 ml*

*Prepared by the student if the instructor so indicates, otherwise, media from Module 2 "Preparing and Dispensing Media," can be used.

OVERALL OBJECTIVE

Pour a plate by aseptically transferring sterile, melted agar from a culture tube, flask, or screw-top bottle to a sterile petri dish.

Specific Objectives

1. Demonstrate aseptic plate pouring in a dry run.
2. Demonstrate the technique used in the dry run with sterile, melted agar and a sterile petri dish.
3. Demonstrate the plate-pouring technique by pouring several plates from a container with a large amount of medium.
4. Describe the reasons agar plates are used in clinical laboratories.
5. Define the terms holding temperature, holding water bath, pouring temperature, solidifying temperature, and agar.

DISCUSSION

To grow bacteria to study colony morphology or to count numbers of colonies, you grow the bacteria on or in a solid medium in a petri dish. Module 9, "Aseptically Dispersing Agar into Petri Dishes," teaches how to get the sterile, melted medium into a sterile petri dish without introducing any airborne contaminating bacteria.

The figures in the module are nearly self-explanatory. Accompanying them is a checklist of the correct step-by-step procedure. Read it while you study the figures.

After you have read the checklist, studied the figures, and mastered Module 7, "Aseptic Transfer of Microbes," you will be ready for the first activity in this module.

Checklist

1. Assemble the materials you will need as in Figure 9-1.
2. Take the culture tube of melted agar (or the empty dry run tube substituting for it) and place it in your left hand.*
3. Remove the closure with the little finger of your right hand, as in Figure 9-2.
4. Transfer the tube from your left to your right hand, as in Figure 9-3.
5. Flame the neck of the tube, as in Figure 9-4.
6. Simultaneously lift the lid of the sterile petri dish and pour in the melted medium, as in Figure 9-5.
7. If the medium does not cover the bottom of the plate, rotate the plate on your table in a circle 6 to 8 inches in diameter, as in Figure 9-6.
8. Recap the empty culture tube, return it to the tube rack, and allow the agar plate to solidify, as in Figure 9-7.

*Reverse if you are left-handed.

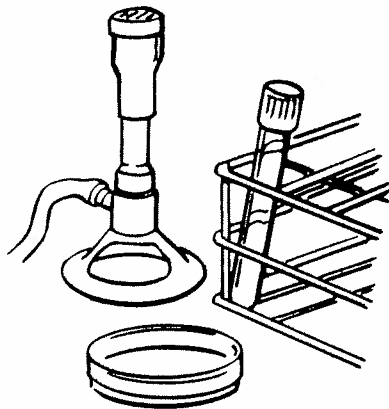


FIGURE 9-1
Equipment needed to dispense sterile agar aseptically into a sterile petri dish.

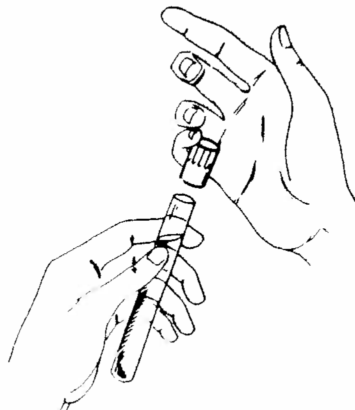


FIGURE 9-2
Removing the closure.

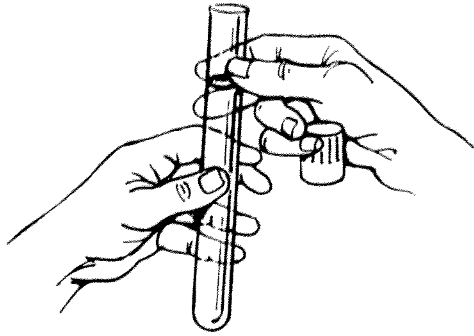


FIGURE 9-3
Transfer the tube to your right hand. Continue to hold the closure with the little finger of your right hand.

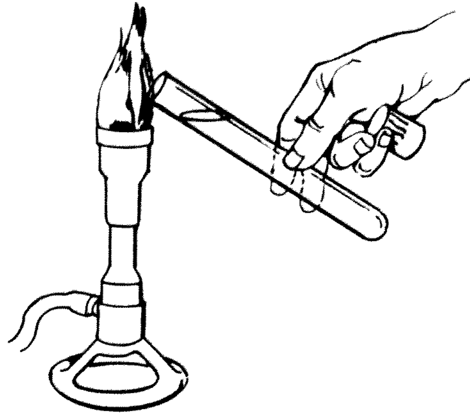


FIGURE 9-4
Flame the neck of the culture tube.

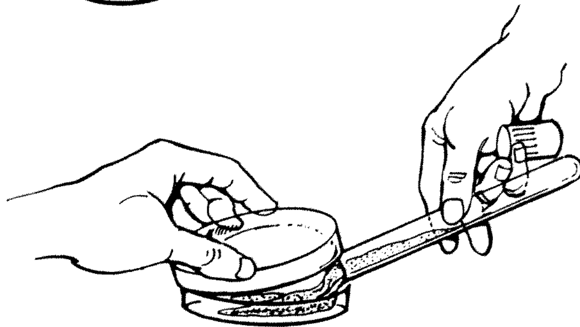


FIGURE 9-5
Lift the lid of the sterile petri dish while you pour in the melted agar. Hold the lid over the plate to protect it from contamination by airborne bacteria.

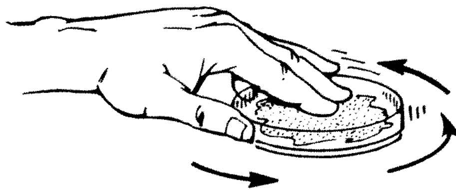


FIGURE 9-6
If the melted agar does not cover the bottom of the petri dish, rotate the plate on the tabletop in a circle 6 to 8 inches in diameter. Once the agar covers the bottom, do not move the plate again until the agar has solidified.

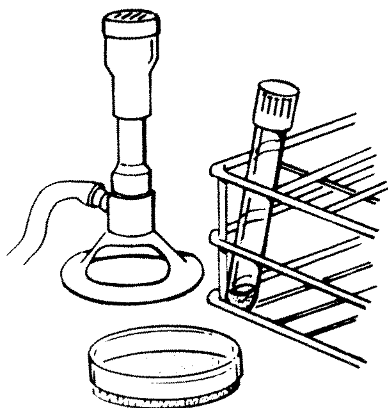


FIGURE 9-7
Replace the closure on the empty culture tube and return it to your tube rack. Allow the plate to solidify.

ACTIVITIES

Activity 1: Plate-Pouring Practice

In this dry run you use an empty culture tube and a nonsterile petri dish. Pretend you have melted medium in the empty tube and proceed through the checklist, referring to the figures.

Repeat this practice run as often as necessary to become so familiar with it that you can pour into a plate without the checklist or figures. Have a classmate check your ability to perform this activity.

Activity 2: Pouring a Plate Using a Tube of Melted Agar

After you successfully complete Activity 1 you are ready for the real thing, that is, you can pour sterile, melted medium into a sterile petri dish. The medium you use must first be autoclaved to sterilize it. If it has been sterilized and allowed to cool, it must again be brought to boiling to reliquefy it for pouring. After autoclaving, place the liquid medium in a holding water bath set at 50°C. This is called the holding temperature for media since agar-agar, a nonnutritive extract of seaweed used as a solidifying agent in media, remains liquid at 50°C and becomes solid at 40°C. Sterile agar media can be transferred only as liquids. Once you remove the tubes of melted medium from the holding water bath and place them in the culture tube rack, you must work quickly before the agar in the medium solidifies. Note that the holding temperature is only 10° higher than the solidifying temperature of agar. It does not take long for media to cool 10° and solidify when they are at room temperature.

The average temperature between the holding water bath and solidification is called the pouring temperature, approximately 45°C. Pour media containing agar at approximately 45°C because (1) agar medium solidifies at 40°C (its solidifying temperature), (2) if the medium is just beginning to solidify while you pour it, lumpy agar plates result, and (3) medium poured above 50°C results in too much moisture condensing on the petri dish lid. The moisture can drop onto the surface of the solidified medium and prevent the separation of bacterial cells and formation of pure colonies.

1. Keeping time in mind, retrieve the two tubes of medium from the holding water bath and carry them in your culture tube rack.
2. Wipe off any excess water on the outside of the tubes.
3. Pour the liquid medium into your sterile plate using the plate-pouring technique you mastered in Activity 1.
   - If the medium covers the bottom of the plate and no lumps of agar are present, you have poured a perfect plate.
   - If the medium does not cover the bottom of the petri dish, place your fingers flat on the lid of the closed plate. Rotate the plate on the tabletop in a circle 6 to 8 inches in diameter, as shown in Figure 9-6. Rotating the plate moves the melted medium around so it covers the bottom of the petri dish. Do not get the medium on the petri dish lid. The limited amount of medium the tube holds will result in a thin agar plate. From now on, pour thicker plates.
   - *Precaution:* When the medium covers the bottom of the plate, do not move it again until it solidifies. The medium becomes opaque when it is solid.
4. Using the second tube of melted agar, pour another plate using the same technique.
5. After the agar plates have solidified, label them on the bottom and incubate them for 24 hours in the 30°C incubator.

The plates must always be inverted (turned upside down) when incubating to prevent any excessive condensation on the lid from dropping onto the agar surface. This would cause the bacteria to float and the colonies to run together.

Observe the plates in your next lab period. If no colonies of bacteria or fungi have formed, you have successfully mastered pouring a plate of melted agar. Congratulations on performing another basic microbiological technique!

Refrigerate the noncontaminated plates so they can be used in a technique you will learn in Module 12, "Streaking for Isolation." Place a label on the bottom of the plates and refrigerate them upside down.

Perform the following variation of the plate-pouring technique, using a bottle or flask containing large amounts of sterile medium and several sterile petri dishes. You will be using this method to pour melted sterile agar into plates, rather than from culture tubes, for the remainder of the course.

Activity 3: Use of a Large Container to Pour Several Plates

A frequently used variation employs larger containers that hold enough agar to pour several plates. To perform this variation, you pour a larger amount of medium (20 ml per plate) into a flask or screw-top bottle to be sterilized and liquefied. For example, if you wish to pour 10 plates, you will need about 200 ml of sterile melted medium in a screw-top bottle. You will find a ready-to-pour bottle of sterile liquid agar medium in the holding water bath. Be sure the water in the bath covers the medium in the bottle.

Reminder: Do not pour agar when it is too hot or too cool.

1. Label and line up five or six sterile petri dishes.
2. Pour five or six plates using the same principles you learned in Activity 2.
   - Once you flame the neck of the container of medium, you can pour four to six plates before reflaming the neck if you work quickly.
3. After the plates have solidified, incubate all the plates upside down for 24 hours to check on your aseptic technique.
   - If colonies are present after incubation, repeat the module.
   - If the plates are not contaminated, refrigerate them as you did in Activity 2.

Take the post test.