

# EXERCISE 11

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## Culture Media Preparation

### OBJECTIVES

At the conclusion of the exercise, you should...

1. understand the uses for culture media.
2. know how to make a general type of culture medium.
3. know how to perform the calculations necessary to make different amounts of culture media.
4. be able to differentiate special types of media.
5. understand how autoclaving results in sterilization.
6. learn how to keep contaminants out of sterile media.

### INTRODUCTION

Bacteria need nutrition to grow. Media (*medium*, singular) is the food that is used for culturing bacteria and other microorganisms. It can be in liquid, solid, or semisolid form. Media must contain water, carbon, nitrogen, minerals, and growth factors. In addition, media must be the right pH, as well as sterile. In this exercise, you will prepare a general-purpose type of medium: nutrient agar plates. You will also be introduced to some of the other media that you will be working with. Refer to Appendix L (Selected Media Descriptions) as new media is introduced.

### MATERIALS

Demonstrations:

EMB plate with *E. coli* (lactose-positive, Gram-negative rod)  
BAP plate with *E. coli* and *S. aureus*

Media:

Nutrient agar powder (Difco)

## Supplies:

Weighing balance (digital)  
Weighing paper (or dishes)  
Tongue depressor (or spatula)  
100 mL graduate cylinder  
250 mL flask  
Stirring rod  
5 sterile Petri plates  
RO (reverse osmosis) water

## PROCEDURES

### Day 1

#### *Technical background*

Nutrient agar is a **general-purpose** medium, consisting of beef extract, peptone, and agar. Difco is a company that provides pre-mixed, **dehydrated** culture media. Before media-making companies existed, the microbiologist had to weigh out the different ingredients.

**Selective** media will allow only certain types of bacteria to grow. They usually have inhibitory substances that restrict the growth of other unwanted bacteria. An example of this is Columbia (CNA) media, which restricts the growth of some Gram-negative rods, while permitting Gram-positive bacteria, such as staphylococci and streptococci, to grow.

**Differential** media contains various substances that cause some bacteria to take on a different appearance from other species. **EMB** (eosin methylene blue) agar is an example of a differential (and selective) medium used for the isolation of a specific group of Gram-negative rods, called the enteric bacteria. It contains the milk sugar lactose and allows organisms to be differentiated by their ability to ferment lactose

**Sterilization** of culture media is done to eliminate contaminating microorganisms from the environment. Complete sterilization occurs at 250°F (121.6°C) at 15 pounds per square inch (psi) of steam pressure. Autoclaves provide this type of sterilization.

#### *Preparing Culture Media*

Work as a table, assigning the different tasks, as needed.

1. Weigh out the appropriate amount of nutrient agar for making 100 mL. This amount is written on the side of the bottle, along with the ingredients. (You will have to calculate the correct number of grams to weigh, based on the instructions on the side of the bottle of media. In this case, nutrient agar is 23 grams per liter. You will have to set up a ratio to calculate the required amount to make 100 mL.)
2. Place the powder into a 250-mL flask and add 100 mL water.
3. Swirl the flask until the media is hydrated. (Note: The only time it is necessary to boil the agar to dissolve it is if it is being dispensed in tubes and then autoclaved.)
4. Adjustment of the pH usually is not necessary. (FYI: It should be around 7.0.)
5. Label the flask.
6. Sterilize the flasks in the autoclave.

7. Turn on two water baths to 50°C.
8. Place the sterilized flasks in the 50°C water baths.
9. Set up 5 sterile Petri plates on your lab bench.
10. When the flask is cool enough to touch, pour the plates.
11. Pour about 20 mL into each of 5 sterile Petri plates. This can be judged by pouring enough to cover the bottom of the plate, plus a little more.
12. Allow the plates to cool on your bench until the agar has solidified
13. Remove the lid of one plate while it is cooling. Label this as “Opened.” Allow it to remain open for the remainder of the lab period.
14. Each student should save one plate in his or her lab locker. You will use these later. Designate someone to store the “opened plate” for your table. Store all plates closed and upside down!
15. Clean up! Dirty and unused glassware should go into the decontamination tray Weighing paper should go in the regular trash. Caps should be put back on the jars of nutrient agar.

## PROCEDURES

Day 2

### *Preparing Culture Media, Cont'd*

1. Examine the growth (if any) on the opened plate.
2. Make Gram-stained smears from one or two of the colonies. If there is an overgrowth of Fungi, DO NOT OPEN THE PLATE! Examine with the Stereoscope through the top of the Petri plate.
3. Examine your unopened plate to make sure it is not contaminated.
4. Store the unopened plate in your locker for later use.
5. Describe your results in the evaluation portion of the Data section.

6. As you are introduced to new media, add them to the media table below using the Difco manual as needed. (See examples below.)

Name of Media	Type	Organism & Description
Eosin Methylene Blue (EMB)	Selective,	General-purpose Gram-negatives; Differentiates lactose fermenters
Nutrient Agar (NA)	General purpose	Large variety
Blood agar (BAP)	General purpose differential	Most bacteria Hemolysis
Chocolate agar (CHOC)	Enriched	Growth of <i>Neisseria</i> sp. Includes <i>Branhamella</i> and <i>Moraxella</i>
Trypticase Soy agar (TSA)	General purpose	Large variety

## EVALUATION OF RESULTS (EXERCISE 11: CULTURE MEDIA PREPARATION)

Purpose

Data

Show your calculations for the amount of agar you weighed to make 100 ml of nutrient agar

Describe the surface of the agar on the opened plate and on the unopened plate (number of colonies, including color, size, and shape). Record the appearance and interpretation of the Gram stain for the colonies on the opened plate:

Colony	Number of this type	Color	Size	Shape	Gram stain
1					
2					
3					



## CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. What did you expect the opened plate to look like? Why?
2. Did the unopened plate(s) remain sterile during the media making? If not, list some reasons why not.
3. If you were given a bottle of nutrient broth powder (8 g/l) and a bottle of agar, how much agar would be needed to make 100 mL? (Note: A broth can be made into agar by adding enough agar to make a 1.5% (w/v) solution, or 15 g/l).
4. Why is agar a good ingredient for converting liquid media to solid media?
5. Explain what selective media is and how it is used.
6. Describe three other types of sterilization techniques that are used in the microbiology laboratory.
7. What is a “contaminant”?

