# Exercise 4

(Ed. Fall 2010)

## **The Preparation of Culture Media**

For the growth of Bacteria

#### **INTRODUCTION:**

Student Learning Objectives: After completing this exercise students will:

- a. Demonstrate their proficiency in using the metric system for weight and volume measurements.
- b. Demonstrate their ability to reconstitute media per directions on the container.
- c. Demonstrate the use of a graduated cylinder and pipettes to measure liquids.
- d. Prepare and dispense media into the appropriate containers for use.
- e. Understand the purpose for using a particular medium for culturing microorganisms

#### Activities for today:

- Calculating the amounts of powdered medium to be reconstituted in a particular volume of water.
- Calculate the amounts of the medium need to make a specific number of plates or tubes.
- Prepare assigned culture media and dispense in bottles or tubes as instructed by your instructor
- Prepare a list of all the media which will be used in the lab. As a homework assignment, prepare a table which describes the classification, content, and purpose of each medium in the isolation of microorganisms.

#### **Materials**

Work in groups of 4 per table. This is a group activity. Each group needs the following:

Electronic balance Hot plate stirrer Magnetic stir bar Stir bar retriever Weighing paper, weighing boats Spatula Beaker/flask Graduated cylinder Pipettes and Pipettors	Bottles/flasks Screw-cap tubes Standard tubes with closures Tube rack/Kimrack Distilled/Deionized water Dehydrated Media (powder)
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#### **1.** Calculating amount of media to be reconstituted with water

The culture medium is prepared by measuring a specific amount of the powdered mix and mixing it with deionized/distilled water, heating it to a boil to dissolve the contents completely. The mix is then aliquoted into tubes or bottles to be sterilized in the autoclave, and used as needed. Label all media accordingly. Calculating the correct amount of powder to be dissolved in the correct amount of water is essential to yield an ideal medium for culturing microorganisms. These specific concentrations are necessary for successful growth of these organisms. This is done by reading the directions on the (powdered mix) media bottle as described by the manufacturer, which are standardized to making one liter of the medium. However, if less or more media needs to be prepared, then you will need to learn to recalculate the specific amounts based on the final volume needed. For example, if the bottle indicates mixing 40 grams per liter of water, and you only need half a liter, then you must divide the amount of media and water in half (ie. 20 g/500 ml). Practice your calculations with several amounts of a particular medium mix to determine the amounts that you need.

Bottle Label Grams/Liter	Water	Media mix	Final volume
42g/L	500 ml		500 ml
	500 ml	25g	500 ml
40g/L		10g	
50g/L	400 ml		400 ml

Show your calculations below:

## 2. Preparation of Nutrient Agar (NA)

- 1. Using a graduated cylinder, measure the amount of distilled/deionized water desired and pour it into a beaker or flask.
- 2. Slowly and carefully, place a magnetic stir bar in the container. Let it slide down the glass wall to avoid cracking the bottom.
- 3. Using the electronic digital scale and a weighing boat/paper, weigh the amount of dehydrated Nutrient Agar based on the calculations from the formula on the bottle.
- 4. Carefully and gently, place the beaker/flask on the hot stirrer plate and turn on the stirrer knob until the water swirls.
- 5. Turn on the temperature knob to a reading of about 400.
- 6. Add the dehydrated Nutrient Agar to the swirling water.
- Heat to a rolling boil while stirring, allowing the mixture to dissolve completely. The mixture will become clear when the medium is completely dissolved. Keep an eye on the mixture to avoid a boil-over.
- 8. Turn off the temperature knob only, but allow the stirrer to remain on.
- 9. Remove from the hot plate and allow to cool down for a few minutes.
- 10. Dispense in 250 ml bottles to be autoclaved for later use.
- 11. If making slants, using a pipette, dispense in 10 ml amounts in tubes to be autoclaved for later use. (do not forget to cap the tubes)
- 12. If making deeps, using a pipette, dispense in 12 ml amounts in tubes to be autoclaved for later use. (do not forget to cap the tubes)
- 13. Label all media before autoclaving to prevent confusion errors.

## 3. Preparation of Nutrient Broth (NB)

- 1. Using a graduated cylinder, measure the amount of distilled/deionized water desired and pour it into a beaker or flask.
- 2. Slowly and carefully, place a magnetic stir bar in the container. Let it slide down the glass wall to avoid cracking the bottom.
- 3. Using the electronic digital scale and a weighing boat/paper, weigh the amount of dehydrated Nutrient Broth based on the calculations from the formula on the bottle.
- 4. Carefully and gently, place the beaker/flask on the hot stirrer plate and turn on the stirrer knob until the water swirls.
- 5. Turn on the temperature knob to a reading of about 400.
- 6. Add the dehydrated Nutrient Broth to the swirling water.
- 7. Heat while stirring, until the mixture is comletely dissolved. The mixture will become clear when the medium is completely dissolved. The medium does not need to come to a boil.
- 8. Turn off the temperature knob only, but allow the stirrer to remain on.
- 9. Remove from the hot plate and allow to cool down for a few minutes.
- 10. Dispense the amounts desired into tubes using a pipette.
- 11. Close the tubes and place them in a basket for autoclaving.
- 12. Label all media to prevent confusion errors.

#### 4. Preparation of other media

Follow the instructions on the product bottle/container, and prepare the media as director by your lab instructor. Remember that while most media will require sterilization by autoclaving, there are some that should not be exposed to heat or excessive heat, and other means of sterilization or reconstitution may be required. Working with sterile media is extremely essentail for the isolation of pure cultures, and identification of microorganisms.

Category	Volume	Container	Purpose/Usage
Broth	3 – 10 ml	Tube	Growing pure cultures, determining oxygen requirements, growth, and biochemical/enzymatic characteristics of bacteria, as well as arrangement of cells
Agar Slants	10 ml	Tube	Growing and maintaining pure cultures for long periods of time with minimal water loss. Slant allows for an increased surface area of growth.
Agar Deeps	10 – 12 ml	Tube	Growing and maintaining cultures, determining oxygen requirements and motility.
Agar Plates	20 – 25 ml per plate	Bottle or Flask	Isolating and growing bacteria, selecting for growth and differentiating between bacteria, determining the biochemical/enzymatic characteristics of bacteria.

### 5. Preparing agar plates

After sterilizing the meduim by autoclaving at 121 C at 15 PSI, the media containers are placed in a waterbath at 50 C to prevent the agar from solidifying and maintain it in the liquid state. Agar usually solidifies at about 40 - 45 C, and will have to be remelted.

- 1. Disinfect the table surface you will be working on.
- 2. Prepare a specific number of sterile Petri dishes based on the amount of media you have prepared; each Petri dish requires about 20 25 ml of medium.
- 3. Do not discard the plastic sleeve packaging; retain it to store the poured dishes in the refrigerator.
- 4. Remove the container (bottle or flask) from the waterbath and wipe dry with a paper towel. Place it on your desk with the lid closed. Do not pour the agar if it is too hot or too cool.
- 5. Open the container and flame the neck with the Bunsen burner.
- 6. Open the top lid of the dish half way and aseptically pour the medium to about a depth of 4 6 mm. This should be equivalent to about 20 25 ml.
- 7. Close the lid immediately, and move to the next dish. Reflame the neck of the container every 5 plates or so, and continue pouring the agar until finished.
- 8. After the agar has cooled down and solidified, label the bottom of the Petri dishes with the name or letters of the medium.
- 9. Incubate the plated media upside down (inverted) at 37 C for 24 hours to check for contamination. This will minimize the amount of condensation formation.
- 10. Plates must be stored in the refrigerator, inverted, and preferably in the same plastic sleeves they came in. Label with medium name and date of production.

Chapter Assignement: