Microbiological Media Preparation

Overview

Growth medium or culture medium is a gel or liquid designed to support the growth of microorganisms or cells. There are different types of media for growing different types of organisms or cells. One commonly used type of media is nutrient broth or agar. Some organisms, termed fastidious organisms, require more specialized types of media.

Applicability

This procedure applies to all University Faculty, Staff, and Students and other university employees who are involved with the preparation of microbiological media.

Storage and Use Requirements

- Powders to prepare media are stored in general chemical storage.
- Lab coats, gloves and goggles are worn during the preparation of media.
- Use weigh paper or a plastic weigh dish for weighing media powder. Weigh all media in a fume hood.
- Autoclave gloves are worn when handling hot glassware and when using the autoclaves.

General Procedures for Preparing Media

Agar Petri Plates

- Calculate the amount of media that needs to be made.
  - Each plate requires 25 – 30 mL of agar.
  - If 100 plates are needed, 2500-3000mL of agar is needed.
  - Always add 200mL to the amount required in case of spills or miscalculation.
- Follow package instructions for preparation.
  - Instructions are typically written for 1L (1000mL) of media. If less is desired calculate the amount needed as shown:
    - For example: If the instructions state 23g for 1L and 600mL is desired, use a ratio to calculate the amount needed (in this example 13.8 g is needed for preparing 600mL):
      - 23g/1000mL = Xg/600mL
      - 23 * 600 = 1000X
      - 13800 = 1000X
      - 13800/1000 =X
      - 13.8 g = X
  - Always prepare media in a beaker with 1/3 of empty space. (i.e. prepare 600mL of media in a 1000mL beaker). If the amount of media to be prepared is greater than 1L, prepare it in 500mL aliquots or use a 2000mL beaker.
  - Label the beaker with autoclave tape and state what media is being prepared, the date, and your initials (i.e. Nutrient Agar  8-18-08  KH).
  - Add powder to beaker first, and then fill with necessary amount of water.
  - Stir with a glass stirring rod to mix.
  - Place in microwave and heat at 3-5 minute intervals.
  - Stir between intervals, using caution and allowing the media to sit for 30 seconds in the microwave before stirring.
  - Heat for approximately 10 minutes or until boiling has been achieved.
  - Test the pH of the media to insure that it is within the acceptable range as stated on the package. If the pH needs to be adjusted, add drops of 1N Hydrochloric Acid (HCl) (to make more acidic) or 1N Sodium Hydroxide (NaOH) (to make more basic) as necessary until desired pH is achieved.
  - Cover the beaker with foil and secure with autoclave tape.
  - Autoclave for 20 minutes. Refer to the Standard Operating Procedure for Autoclave Use.
  - While the media is in the autoclave, arrange Petri plates on the counter top.
Once sterilization is complete, open the autoclave and remove the beaker of media. You must wear the autoclave gloves when removing anything from the autoclave.

Allow media to cool slightly, but not for longer than 10 minutes as the agar may solidify.

Poke a small hole in the foil covering the top in order to pour the agar.

Pour approximately 10-15mL of agar into the plates. The bottom of the plate needs to be covered. If necessary, swirl the plate slightly in order to evenly disperse agar.

Allow plates to cool on the countertop overnight to reduce condensation.

When cooled, store upside-down in plastic bags in the refrigerator to prevent the agar from drying out.
- Be sure to seal the plastic bag with masking tape.
- Be sure the bag is labeled with its contents, the date it was prepared, and your initials (i.e. Nutrient Agar 8-18-08 KH).

**Broth Preparation**

- Calculate the amount of media that needs to be made.
  - Each broth tube requires 5-7mL of broth.
  - If 100 tubes are needed, 500-700mL of broth is needed.
  - Always add 200mL to the amount required in case of spills or miscalculation.

- Follow package instructions for preparation.
  - Instructions are typically written for 1L (1000mL) of media. If less is desired calculate the amount needed as shown:
    - For example: If the instructions state 23g for 1L and 600mL is desired, use a ratio to calculate the amount needed (in this example 13.8 g is needed for preparing 600mL):
      - $23/1000mL = X/600mL$
      - $23 \times 600 = 1000X$
      - $13800 = 1000X$
      - $X = 13.8g$
  - Always prepare media in a beaker with 1/3 of empty space. (i.e. prepare 600mL of media in a 1000mL beaker). If the amount of media to be prepared is greater than 1L, prepare it in 500mL aliquots or use a 2000mL beaker.
  - Label the beaker with autoclave tape and state what media is being prepared, the date, and your initials (i.e. Nutrient Broth 8-18-08 KH).
  - Add powder to beaker first, and then fill with necessary amount of water.
  - Stir with a glass stirring rod to mix.
  - Place in microwave and heat at 3-5 minute intervals.
  - Stir between intervals, using caution and allowing the media to sit for 30 seconds in the microwave before stirring.
  - Heat for approximately 10 minutes or until boiling has been achieved.
  - While heating, place test tubes in racks and label with autoclave tape (i.e. Nutrient broth 8-18-08 KH).
  - Test the pH of the media to insure that it is within the acceptable range as stated on the package. If the pH needs to be adjusted, add drops of 1N Hydrochloric Acid (HCl) (to make more acidic) or 1N Sodium Hydroxide (NaOH) (to make more basic) as necessary until desired pH is achieved.
  - Pour the broth into an appropriately sized glass bottle for pipette dispenser use.
  - Before attaching the pipette dispenser, set it to the proper setting for the volume of media required for each tube.
  - Attach the dispenser to the bottle top.
  - While over the sink, test the dispenser to ensure that the liquid media is filling the pipette dispenser.
  - Fill each tube.
  - Place loose caps on filled tubes.
    - For screw caps, leave the caps partially unscrewed to allow steam to enter and escape.
Autoclave for 20 minutes. Refer to the Standard Operating Procedure for Autoclave Use.

After sterilization is complete, remove the test tube rack, tighten all test tube caps and allow the tubes to cool at room temperature.

Once cooled, place in the refrigerator for storage.

**Agar Slant Tube Preparation**

- Calculate the amount of media that needs to be made.
  - Each broth tube requires 7-10mL of broth.
  - If 100 tubes are needed, 700-1000mL of broth is needed.
  - Always add 200mL to the amount required in case of spills or miscalculation.

- Follow package instructions for preparation.
  - Instructions are typically written for 1L (1000mL) of media. If less is desired calculate the amount needed as shown:
    - For example: If the instructions state 23g for 1L and 600mL is desired, use a ratio to calculate the amount needed (in this example 13.8 g is needed for preparing 600mL):
      - 23g/1000mL = Xg/600mL
      - 23 * 600 = 1000X
      - 13800 = 1000X
      - 13800/1000 = X
      - 13.8 g = X
  - Always prepare media in a beaker with 1/3 of empty space. (i.e. prepare 600mL of media in a 1000mL beaker). If the amount of media to be prepared is greater than 1L, prepare it in 500mL aliquots or use a 2000mL beaker.
  - Label the beaker with autoclave tape and state what media is being prepared, the date, and your initials (i.e. Nutrient Broth 8-18-08 KH).
  - Add powder to beaker first, and then fill with necessary amount of water.
  - Stir with a glass stirring rod to mix.
  - Place in microwave and heat at 3-5 minute intervals.
  - Stir between intervals, using caution and allowing the media to sit for 30 seconds in the microwave before stirring.
  - Heat for approximately 10 minutes or until boiling has been achieved.
  - While heating, place test tubes in racks and label with autoclave tape (i.e. Nutrient Agar Slants 8-18-08 KH).
  - Test the pH of the media to insure that it is within the acceptable range as stated on the package. If the pH needs to be adjusted, add drops of 1N Hydrochloric Acid (HCl) (to make more acidic) or 1N Sodium Hydroxide (NaOH) (to make more basic) as necessary until desired pH is achieved.
  - Pour the broth into an appropriately sized glass bottle for pipette dispenser use.
  - Before attaching the pipette dispenser, set it to the proper setting for the volume of media required for each tube.
  - Attach the dispenser to the bottle top.
  - While over the sink, test the dispenser to ensure that the liquid media is filling the pipette dispenser.
  - Fill each tube.
  - Place caps on filled tubes.
    - For screw caps, leave the caps partially unscrewed to allow steam to enter and escape.
  - Autoclave for 20 minutes. Refer to the Standard Operating Procedure for Autoclave Use.
  - After sterilization is complete, remove the test tube rack, tighten all test tube caps, and tilt the tubes of liquid agar on a support that is about ½ inch thick (Plastic weighing dishes work well) or use special white “slant racks”.
  - Allow the agar to solidify (about 25 minutes) and store in the refrigerator.
First Aid

- In case of contact with eyes, immediately wash the eyes with large amounts of water for 15 minutes, while holding eyelids open. Get medical attention immediately. If contact lenses are worn remove them immediately.
- In the event of skin contact, wash the area thoroughly.
- In the event of skin contact with a hot beaker, seek medical attention.
- Report any incident to the laboratory manager.

Disposal Requirements

- Spills can be cleaned up with a paper towel and disposed of in the trash. Use caution with hot media.
- Unused plates can be disposed of in the waste to be autoclaved trash cans.
- See the microbiological waste SOP for further instruction on waste disposal.

References: