

- 3 Divide into three or four 500 mL flasks for pouring. Smaller flasks are easier to handle when pouring plates. Don't forget to add a magnetic stir bar and to cover each flask before autoclaving.
- 4 Autoclave for 15 minutes at 121°C to sterilize the medium.
- 5 Remove the sterile agar from the autoclave, and allow it to cool to 50°C while it stirs on a hot plate.
- 6 Dispense approximately 20 mL into sterile Petri plates (Fig. 1.7). Be careful! The flask will still be hot, so wear an oven mitt. While you pour the agar, shield the Petri dish with its lid to reduce the chance of introducing airborne contaminants. If necessary, gently swirl each plate so the agar completely covers the bottom; do not swirl the agar up into the lid. Allow the agar to cool and solidify before moving the plates (Fig. 1.8).
- 7 Store these plates on a countertop for 24 hours to allow them to dry prior to use.

Nutrient Broth

- 1 Weigh the ingredients on a balance (Fig. 1.2).
- 2 Suspend the ingredients in 1 liter of distilled or deionized water in the 2-liter flask. Agitate and heat slightly (if necessary) to dissolve them completely (Fig. 1.3).



1.5 Autoclaving the Media ✦ Media are sterilized in an autoclave for 15 minutes at 121°C. Shown are four racks of tubed media being removed from an autoclave. The tubes on the left are brain-heart infusion broth (BHI) and they will be allowed to stand and cool. Once cool, they will remain liquid. The tubes on the right contain brain-heart infusion agar (BHIA), a solid medium, which is liquid when it is removed from the autoclave but will solidify as it cools due to the agar in it. The position of the tube as it cools will determine whether an agar deep tube or an agar slant is produced (Fig. 1.6). Note the angled sides of the racks that allow them to be tipped so the agar will solidify in a slant. Plated media are autoclaved in a flask and then dispensed into sterile Petri dishes (Fig. 1.7).

- 3 Dispense 7 mL portions into test tubes (or less, depending on your lab customs) and cap loosely (Fig. 1.4). As with agar slants, if your tubes are smaller than those recommended in Materials, add enough broth to fill them approximately 20% to 25%.
- 4 Sterilize the medium by autoclaving for 15 minutes at 121°C (Fig. 1.5).



1.6 Tubed Media ✦ From left to right: a broth, an agar slant, and an agar deep tube. The solid media are liquid when they are removed from the autoclave. Agar deeps are allowed to cool and solidify in an upright position, whereas agar slants are cooled and solidified on an angle.



1.7 Pouring Agar Plates ✦ Agar plates are made by pouring sterilized medium into sterile Petri dishes. The lid is used as a shield to prevent airborne contamination. Once poured, the dish is gently swirled so the medium covers the base. Plates are then cooled and dried to eliminate condensation.

Microbiology Laboratory Theory And Application Lab Answers

Rose Arny



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