Pouring Plates from Prepared Bottled Media

Primary Hazard Considerations

Never purchase living specimens without having a disposition strategy in place.

When pouring bottles, agar is **HOT**! Burning can occur. Always handle hot agar bottles with heat-protective gloves. For added protection wear latex or nitrile gloves when working with bacteria, and always wash hands before and after with hot water and soap.

Availability

Agar is available for purchase year round.

Information

- **Storage:** Bottled agar can be stored at room temperature for about six months unless otherwise specified. Never put agar in the freezer. It will cause the agar to breakdown and become unusable. To prevent contamination keep all bottles and Petri dishes sealed until ready to use.

Pouring Plates

Materials Needed:

- Draft-free enclosure or Laminar flow hood
- 70% isopropyl alcohol
- Petri dishes
- Microwave or hot water bath or autoclave

1. Melt the agar using one of the following methods:

a) **Autoclave:** Loosen the cap on the agar bottle and autoclave the bottle at 15 PSI for five minutes. While wearing heat-protective gloves, carefully remove the hot bottle and let it cool to between 75–55°C before pouring. This takes approximately 15 minutes.

b) **Water Bath:** Loosen the cap on the agar bottle and place it into a water bath. Water temperature should remain at around 100°C. Leave it in the water bath until the agar is completely melted. While wearing heat-protective gloves, carefully remove the hot bottle and let it cool to between 75–55°C before pouring.

c) **Microwave:** Loosen the cap on the agar bottle before microwaving. Heat in one minute intervals on low power until all of the agar is melted. Between intervals, gently swirl the bottle to make sure the agar is melting evenly. While wearing heat-protective gloves, carefully remove the hot bottle and let it cool to between 75–55°C before pouring.

Figure 1

2. Working in a clean, draft free area, wipe the work surface with isopropyl alcohol or bactericidal cleanser. Take care to use sterile handling techniques to prevent contamination.
3. Each Petri dish consists of a wider/shallower cover, and a narrower/deeper bottom portion. Remove the Petri dishes from the package; do not remove the covers from the dishes yet. Lay out all the Petri dishes that you will be using, making sure the cover is on top. Re-seal the bag if it contains any plates that will not be used at this time.
4. Working on one plate at a time, carefully tilt open the cover (***See Figure 1***) and pour about 15–20 mL of liquid into the bottom portion (it should cover about ⅔ of the plate's surface). Gently swirl the plate to spread out the liquid media so that it covers the entire bottom surface of the plate. The layer should be about 3–4 mm deep. Once you have enough liquid in the plate, place the top back on.
5. Allow plates to cool before use. This takes about one hour. You can use a refrigerator to speed up this process, but do not put agar in a freezer.
6. Plates can be used to subculture bacteria or fungus once they have cooled. Unused plates can be stored in the refrigerator for 2–4 weeks.

Disposition

- Unused agar plates can be disposed of in the regular trash.
- Used agar plates containing bacteria can be disposed of by one of the following methods:
- Autoclave @ 121°C for 15 minutes in an autoclavable bag (Petri dishes will melt in the autoclave—do not autoclave unbagged).
- Use a 20% bleach solution to kill bacteria in the plate, and then dispose in the regular trash.
- Use 70% isopropyl alcohol to kill bacteria, and then dispose in the regular trash.