

Sterilizing Laboratory Materials for the Classroom

Providing sterile materials for microbiology activities in the classroom requires some effort. This article describes methods for providing sterile media and supplies for microbiology experiments.

Sterilizing Glassware with Dry Heat

1. Cover the openings of flasks or beakers with foil.
2. Make a foil pouch with heavy-duty foil to hold pipettes (glass only; disposable are fine, but the cotton plugs must be removed) with a large flap to fold over the top of the pipettes. Insert pipettes into the foil pouch (tip down) and fold over the flap. The foil pouches can be reused if they are handled carefully.
3. Place materials on a metal tray or cookie sheet.
4. Place glassware in an oven at 350° F. Leave for 2-3 hours. Remove from oven and cool.

Caution!

- Do not put paper or cotton in the oven or it will char.
- Be sure that pipettes are glass, not clear plastic.
- Do not put any plastic in the oven, including plastic baskets or trays to hold glassware.

Sterilizing with Moist Heat and Pressure

Spores of bacteria are very heat resistant and are not killed by boiling at atmospheric pressure. In order to kill spores, liquids must be heated under pressure to 121° C. This is usually done in an autoclave, which is a large pressure cooker. In the classroom, a pressure canner can be used to sterilize liquids. A pressure canner is simply a larger version of a pressure cooker. Pressure canners are available with or without a built-in heating unit. Those without the heating unit are used on a conventional stovetop. These canners have pressure regulators and are safe to use; however, they must be watched and should not be used by students without the direct supervision of an adult.

1. Prepare broth or agar medium in a flask that is about twice the volume of liquid (e.g., 500 ml of medium in a 1-liter flask). Cover the flask with foil.
2. Prepare tubes with broth and cover with plastic or metal caps. If using screw caps, do not tighten.
3. Place flasks or tubes in racks in the pressure canner with water in the canner. Follow the manufacturer's instructions in filling and sealing the canner.
4. **Note:** steam must enter containers in order to sterilize them. Caps must be loose. Do not seal any container that goes into the pressure canner.
5. Bring the pressure up to 20 lb and keep it at that pressure for 30 min. Allow the pressure to come down slowly.
6. Remove the lid carefully, directing the steam away from your face.
7. Use hot mitts to slowly remove the flasks or tubes from the canner.
8. Agar medium should cool to about 50° C before it is poured into petri plates.
9. Test tubes and glassware can also be sterilized in the pressure canner, but they will be wet inside when they are removed and need to dry before they are used.

Caution!

- Do not leave any pressure cooker or canner unattended for long periods of time.
- Remove flasks containing liquid slowly! The liquid can boil over as you remove the flask. Wear good protection on your hands.
- Do not autoclave inexpensive clear plastic (polystyrene) tubes – they will melt.
- Translucent plastic (polypropylene) tubes (including disposables) can be autoclaved, but the screw-caps must be very loose or the tubes will collapse as they cool.

Sterilizing by Boiling

Boiling does not kill bacterial spores; therefore, it is not the best method for preparing microbial media and reagents. However, boiled media is often fine in the classroom if it is not stored for long. If you must make media by boiling, do not make more than you will use in 1-2 days.

1. Prepare broth or agar in a flask about twice the volume of medium. Stir or swirl the contents to mix well.
2. Cover the flask with foil.
3. Heat the flask on a hot plate until the liquid is just boiling. Simmer the liquid for 30 minutes on low heat.
4. Agar medium should cool to about 50° C before it is poured into petri plates.
5. Boiled broth can be dispensed into smaller sterile flasks or sterile tubes using a sterile pipette and good sterile technique.
6. Alternatively, dissolved broth solutions can be dispensed before boiling. The smaller flasks must then be heated to boiling for 30 min. Tubes should be heated in a boiling water bath for 30 min.

Caution!

- Watch boiling broth or agar carefully as it will boil over quickly.
- Move flasks containing hot liquid slowly! The liquid can boil over as you move the flask. Wear good protection on your hands.
- Use medium that has been boiled within 1-2 days.

Sterilizing by Microwave

Do not use a microwave to boil liquid media. Heating is uneven and liquid can boil over long before it is actually heated through. Agar medium often “explodes” in the microwave. Cleaning agar from the top and sides of a microwave is not fun.

A microwave can be used to melt sterile solidified agar medium in flasks, but results are unpredictable. The agar often partially melts and then explodes out of the flask all over the inside of the microwave. A boiling water bath is a better way to melt solidified agar medium in flasks.

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