

SAFETY FIRST IN THE LAB

Preparing for the Lab

- Wash your hands with hot, soapy water. If soap and water are not available, you can use disposable wipes or a gel hand sanitizer.
- Wear disposable safety (protective) gloves.
- Tie back long hair.
- Wear safety goggles or regular glasses for microbiology work.
- If possible, wear a lab coat or apron.
- NEVER EAT, DRINK, OR CHEW GUM IN THE LAB. Keep your hands, pencils, etc. out of your mouth.
- Disinfect all surfaces with a disinfecting bleach solution before beginning a lab (see box above).
- INAPPROPRIATE BEHAVIOR WILL NOT BE TOLERATED AT ANY TIME IN THE LAB!

TIP

Disinfecting Bleach Solution:

20 ml of liquid household bleach (chlorine bleach) in 1 L of tap water.

Safety Gloves

- Wear safety gloves when inoculating Petri dishes and when working with raw meat.
Safety gloves are made using many types of materials, including vinyl and polyethylene. They can be purchased at a local pharmacy, supermarket, or through science supply catalogues.
- When removing safety gloves, be careful not to contaminate your hands, items, or surfaces with any residue that may be on the gloves.
- Throw away used gloves immediately after removing them. Wrap one glove inside the other, then throw both gloves away.
- Wash your hands with hot, soapy water after removing the gloves.

Hot Surfaces

- Use thermal gloves or hot-pad holders when working with hot plates, burners, autoclaves, or any other heat source.

Petri Dishes

- Use Parafilm to seal Petri dishes after inoculating them.
- Never open a Petri dish with organisms growing in it. It could contain/release dangerous pathogens!

Pipettes

- **Never pipette by mouth.** Always use a pipette bulb or pipette aid.
- Be careful when attaching a pipette bulb. Hold your hand close to the end of the pipette where the bulb will be attached. Push the bulb onto the pipette carefully and gently. If you push too hard, the pipette could break and you could cut yourself.

Food in the Lab

- NEVER EAT OR DRINK ANY FOOD OR LIQUID USED IN A LAB.
- Thoroughly wash hands before and after handling and cooking raw meat.
- Wear safety gloves and lab aprons when handling raw meat, as well as safety goggles when cooking raw meat.

Proper Clean-Up

- Wear safety gloves and take appropriate defensive measures when cleaning up cultures and used equipment.
- Wash all glassware and other instruments in hot, soapy water, then sterilize them (see page 8).
- Properly dispose of used Petri dishes and other used equipment.
- Thoroughly disinfect all surfaces, especially those that were in contact with raw meat.
- Before leaving the lab, wash your hands with hot, soapy water or use a gel hand sanitizer.

Disposal of Used Materials and Equipment

- Check your school, local, or state safety regulations for specific information on how to properly dispose of potentially hazardous materials. If there are no guidelines, follow these precautions:

For Raw Meat

- Unless contaminated with a virulent pathogen in the lab, raw meat and other foods can usually be disposed of as regular solid waste. Place the meat in a sturdy plastic bag, seal, and dispose.

For Used Swabs, Petri Dishes, Pipettes, and Other Disposable Equipment

- Use a sturdy plastic bag that won't leak.
- Place the bag in a metal container, such as an empty coffee can. Use one bag/container for each team of students conducting the labs.
- Place used swabs, disposable Petri dishes, pipettes, etc. in the bag.
- At the end of the lab, add enough disinfecting bleach solution (see above) to cover the contents and tightly close the bag.
- Dispose of the closed bag in the trash.

Note: Equipment that will be reused should be cleaned using hot water and soap and then placed in boiling water for 10 minutes or sterilized in an autoclave.

LAB PROCEDURES

Washing Hands

- Use hot water.
- Wet hands and add soap.
- Scrub hands for 20 seconds away from the running water. Thoroughly scrub wrists, under fingernails, around nail beds, and between fingers.
- Rinse hands under running water.
- Dry hands thoroughly with clean paper towels.
- Use the paper toweling to turn off the faucet.
- Dispose of used paper towels in the trash.

Note: If necessary, disposable alcohol wipes or gel hand sanitizers can be substituted for soap and water.

Disinfecting

Disinfecting Bleach Solution: 20 ml of liquid household bleach (chlorine bleach) in 1 L of tap water.

To Disinfect Countertops:

- Put solution in spray bottle and label the bottle, "Disinfecting Solution."
- Wipe off counters to remove any visible soil.
- Spray the disinfecting solution on counters and leave it on for 2 minutes.

Note: Use the solution within 24 hours, then dispose of remaining solution by pouring it down the drain. Solution will lose its effectiveness in 24 hours.

Sterilizing Equipment

(*test tubes, pipettes, etc.*)

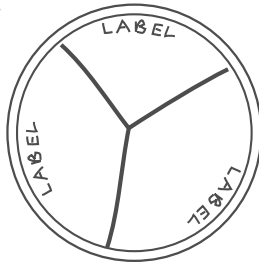
Options:

- Use an autoclave.
- Use dry heat — 160° F to 180° F (71° C to 82° C) for 3 to 4 hours.
- Use chemical agents, such as 5% bleach, ethyl or isopropyl alcohol, commercial disinfectants, or iodine solutions.

Inoculating a Petri Dish

1. Label

- Divide the Petri dish into sections (if applicable), and label the bottom (agar side) of the dish using a permanent marker.
- Label along the outer edges of the dish or the sections, so the labels don't interfere with viewing the colonies.



2. Inoculate

- Use a sterile cotton swab* to wipe the surface or liquid being tested. Hold the cotton swab at one end — do not touch the end that will be used to inoculate the agar.

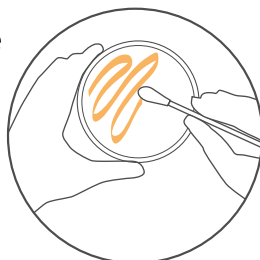
* If you use a control dish, new, untouched cotton swabs are good to use. Inoculate the control dish with a new swab to check for any microbial contamination.

For a Dry Surface

- Wet the swab by dipping it in boiled or sterile water. Then, wring out the swab by wiping it against the inside of the container. (If the swab is too wet, the liquid will flow into other sections and the microbial colonies will run into each other.)
- Swab the dry surface.

For a Liquid

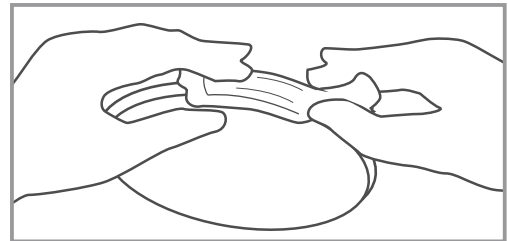
- Dip the sterile cotton swab in the liquid. Then, wring out the swab by wiping it against the inside of the container.
- Inoculate the nutrient agar using a back-and-forth motion, covering the entire area of the dish or section. Do not swab too close to the dividing lines for the next section.



3. Parafilm

Place the cover on the Petri dish and seal it closed using Parafilm.

- Cut a narrow strip and stretch it around the outside edge (along the full circle perimeter) of the covered dish.



4. Incubate

- Place dishes upside down (label side up) in an incubator set at 95° F (35° C) or let the dishes sit at room temperature (away from the sun) for the appropriate amount of time.

TIPS For Viewing Inoculated Petri Dishes

- Use a light box (ask a parent or shop class to make a light box for your class from plywood and Plexiglas®; or, borrow a light box from the photography class). Line up all the Petri dishes and compare the results.
or
- Use an overhead projector. Line up the Petri dishes on the projector and project onto a screen, so the entire class can view the results. This is very effective!
- If neither a light box nor overhead projector is available, simply view the dishes on a light-colored surface.