



Microbiological Methods

Making Media

Pouring Culture Plates

Sterile Technique

Inoculating Plates and Culture Tubes

Use of a Plate Counter to Estimate Microbial

Population Densities

Culturing Microorganisms

- There are two basic culture techniques used in microbiology:
 1. *Liquid culture*: bacteria, algae, and some fungi can be reared in **culture tubes (test tubes)** in a liquid medium.
 - *Liquid medium* is best when you want to rapidly increase the concentration of the organism or when you want to grow motile cells.

Culturing Microorganisms

- There are two basic culture techniques used in microbiology:
 2. ***Culture Plates***: Liquid medium is solidified using **agar** (agarose) and poured as a thin layer in the bottom of a culture dish (also sometimes called **petri plate**)
 - *Culture plates* are used when you want to test (1) antibiotic sensitivity, (2) estimate culture concentrations from environmental samples, or (3) isolate individual colonies from environmental samples.

Sterile Technique



Sterile Technique

- When culturing bacteria or other microorganisms, it is important to keep your work area as clean as possible.
- This prevents the introduction of other microorganisms from the environment into your culture.
- The techniques used to prevent contamination are referred to as **sterile techniques**.

Sterile Technique

1. Start by washing your down your work or lab benches with a surface disinfectant. The most commonly used disinfectants for lab use are:
 1. 10% bleach (recommended by the CDC)
 2. 85% ethanol

Sterile Technique (2)

2. Turn off any forced air heating or air conditioning units that create strong air current in your work area.
3. A small room or closet that can be closed off is worth the effort to set-up if you will be doing a lot of microbial culturing.
4. You can install a UV bulb in a fluorescent light fixture to surface sterilize your work bench if you have an enclosed area. Remember to leave the area when you turn on the UV light source!

Sterile Technique (3)

5. All glassware should be cleaned and sterilized before you begin.
6. All pipettes, spatulas, and test tube (culture) racks should also be sterilized.
7. You can purchase sterile, disposable culture tubes, petri dishes, and pipettes to minimize the quantity of glassware that you have to sterilize.

Sterile Technique (4)

8. Don't forget to wash your hands after you finish cleaning and put on a pair of sterile disposable gloves before you begin.
9. Once your work area is clean, your hands are clean, and your glassware is clean and sterile, don't contaminate the work area by placing "dirty items" such as pencils, pens, notes, or books in the sterile work area.



Media Preparation

Microbiological Media

- The type of growth medium that you use is a function of the organisms that you want to culture. Use a reference book (there are many) to determine the type of medium that is best suited for your organism of interest.
- Common media include *Luria Broth* (LB), *Nutrient Agar*, *Potato-Dextrose Agar* (PDA), *Bold's Basal Medium* (BBM)....

Luria Broth

Liquid Medium

10 g Bacto-Tryptone
5 g Bacto-yeast extract
5 g NaCl
Distilled H₂O to 1 l volume
Adjust pH to 7.0
Sterilize for 45 minutes using
autoclave or pressure
cooker

Plate Medium

10 g Bacto-Tryptone
5 g Bacto-yeast extract
5 g NaCl
Distilled H₂O to 1 l volume
20 g agarose
Adjust pH to 7.0
Sterilize for 45 minutes using
autoclave or pressure
cooker

Luria Broth

Things to remember:

- ✓ The volume of media (liquid or plate) should be roughly $\frac{1}{2}$ the volume of the container in which it is placed for sterilization realizing that the liquid expands under increased heat and pressure during the sterilization process.
- ✓ Estimate plate quantities (how many you need to make) as a function of 15-20 ml per plate.

Assemble all of your chemicals in your work area before you begin.

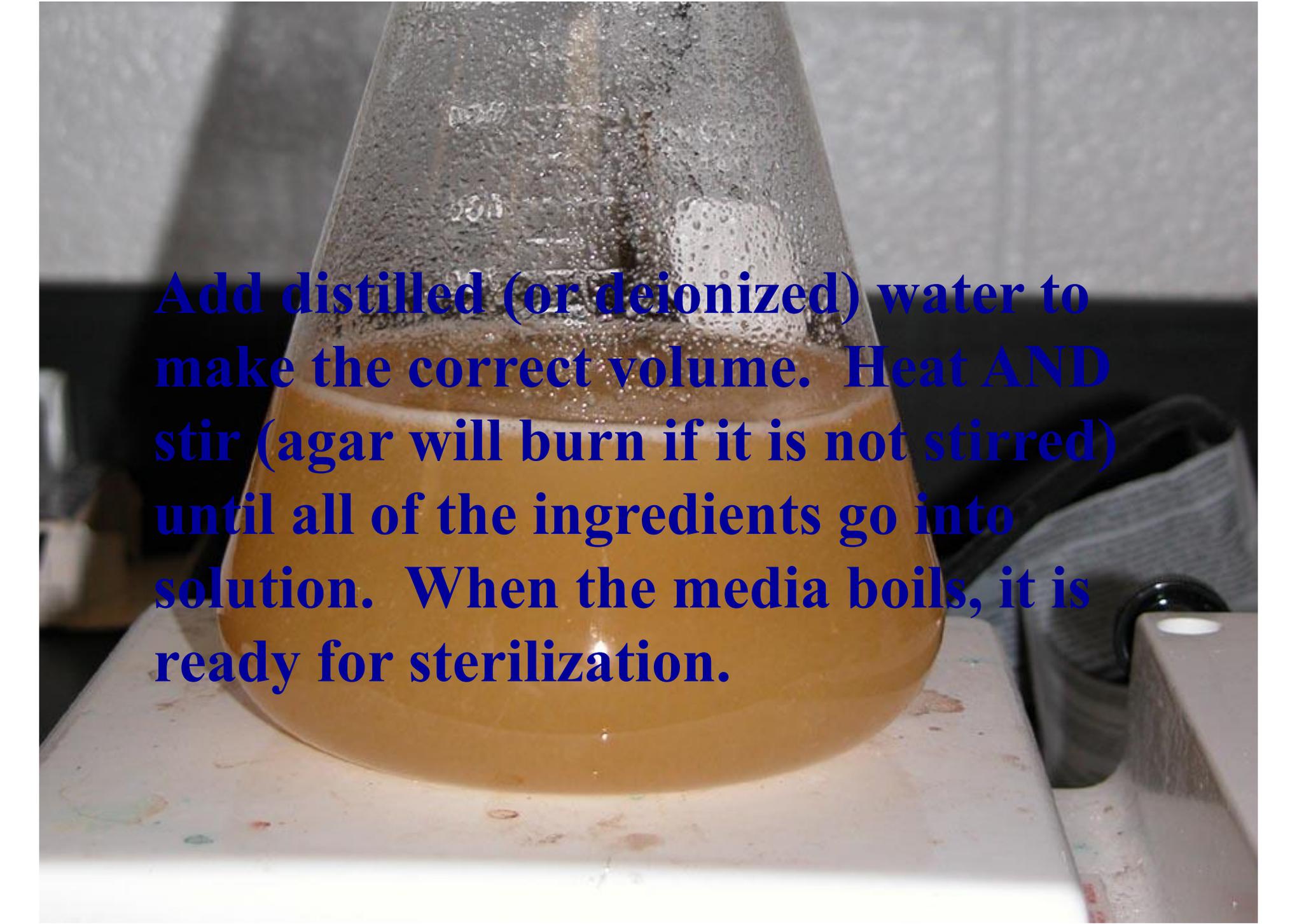


Accurately weigh each of the dry ingredients in your culture media.





Add each dry culture medium ingredient to the culture flask.



Add distilled (or deionized) water to make the correct volume. Heat AND stir (agar will burn if it is not stirred) until all of the ingredients go into solution. When the media boils, it is ready for sterilization.

Media Sterilization



Media Sterilization

- There are two reliable methods used to sterilize microbial culture media:
 1. autoclave
 2. pressure cooker
- When using an autoclave, use the “wet” setting for sterilizing liquids (flasks, bottles, culture tubes, etc), and use the “dry” setting when sterilizing empty containers, stoppers, etc.

Media Sterilization (2)

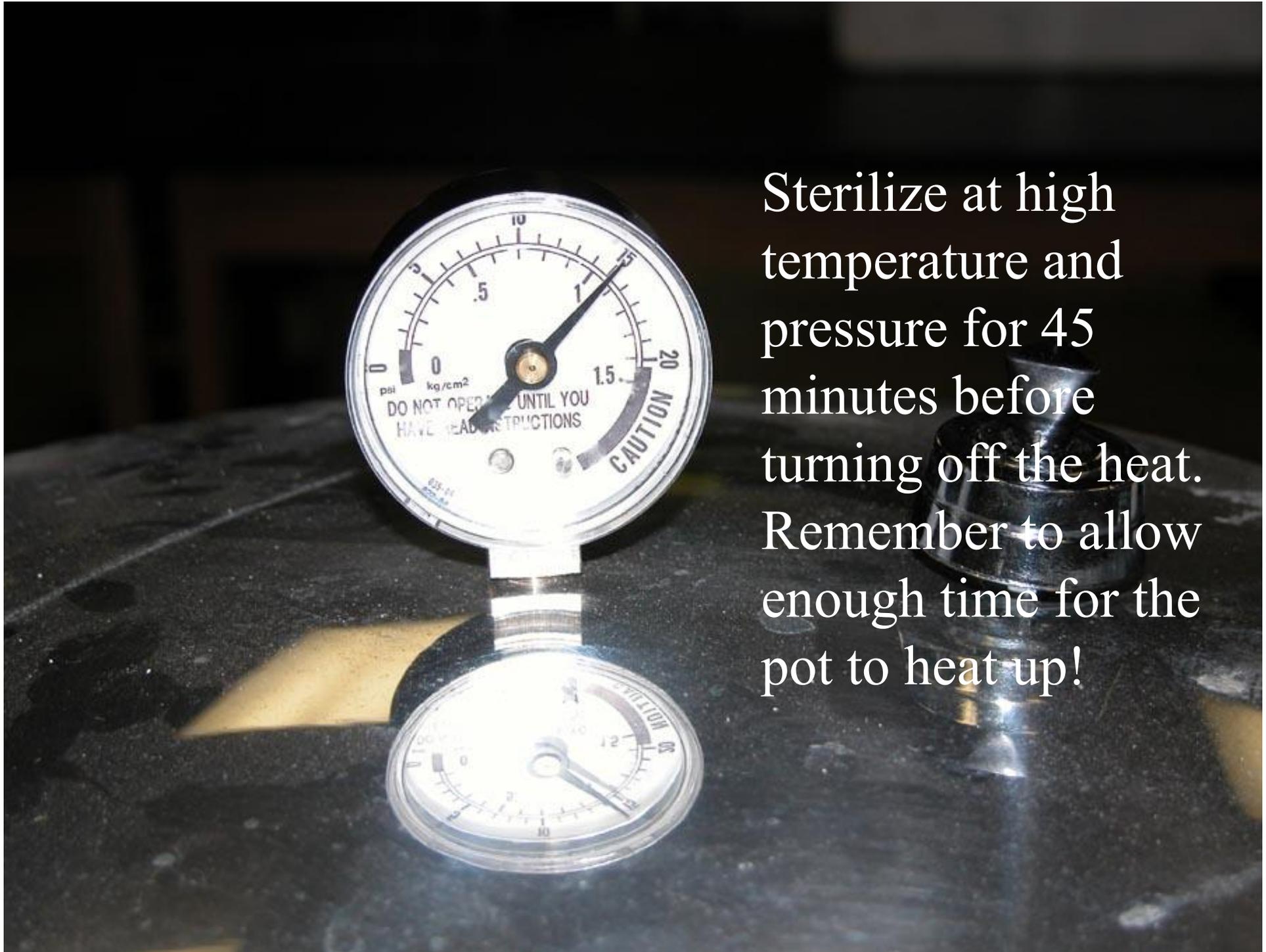
- All liquid media should be sterilized for a minimum for 45 minutes at high temperature and pressure. Autoclaves will cycle automatically, but if you use a pressure cooker, set a timer.
- Remember not to tighten the cap or seal on any container; it will explode under high pressure and temperature!



Sterilize for 45 minutes using the wet cycle (autoclave) or at maximum pressure in a pressure cooker. Remember to cover the top of the flask or jar with aluminum foil to prevent contamination when as the media cools.

A top-down view of a silver pressure cooker. Inside, a clear glass container with a red ring around its neck is placed on a perforated metal steamer basket. The glass container has a white label that reads "1000 mL PYREX®". The top of the glass container is wrapped in aluminum foil. The pressure cooker is filled with liquid, and the steamer basket is partially submerged. The background is dark, suggesting a kitchen setting.

When using a pressure cooker, don't over fill the cooker, and remember to weight your containers so they don't fall over!



Sterilize at high temperature and pressure for 45 minutes before turning off the heat. Remember to allow enough time for the pot to heat up!

Plate Pouring Tips

- Line empty plates along the edge of the work bench.
- Open the petri dish lid at about a 30-45° angle to allow the hot liquid to cover the bottom of the dish. The thermal current created by the hot media prevents bacteria and fungal spores from landing in your clean dish.



Line your sterile petri plates along the edge of the table. Transfer hot media to a small sterile container and pour 15-20 ml of the plate media into each petri plate. The petri plate lid should be open slightly, but not completely open as this increases contamination.

Plate Pouring Tips

- As the plates are poured, move the filled plates to the back of the table until the plates cool and congeal.
- Once the plates have cooled and the media is firm, store the plates media side-up (bottom) with the lid securely taped or the plates restacked in the manufacturer's plastic sleeve.
- To increase the shelf-life of the plates, store in a cool, dry environment until they are used (refrigerator).

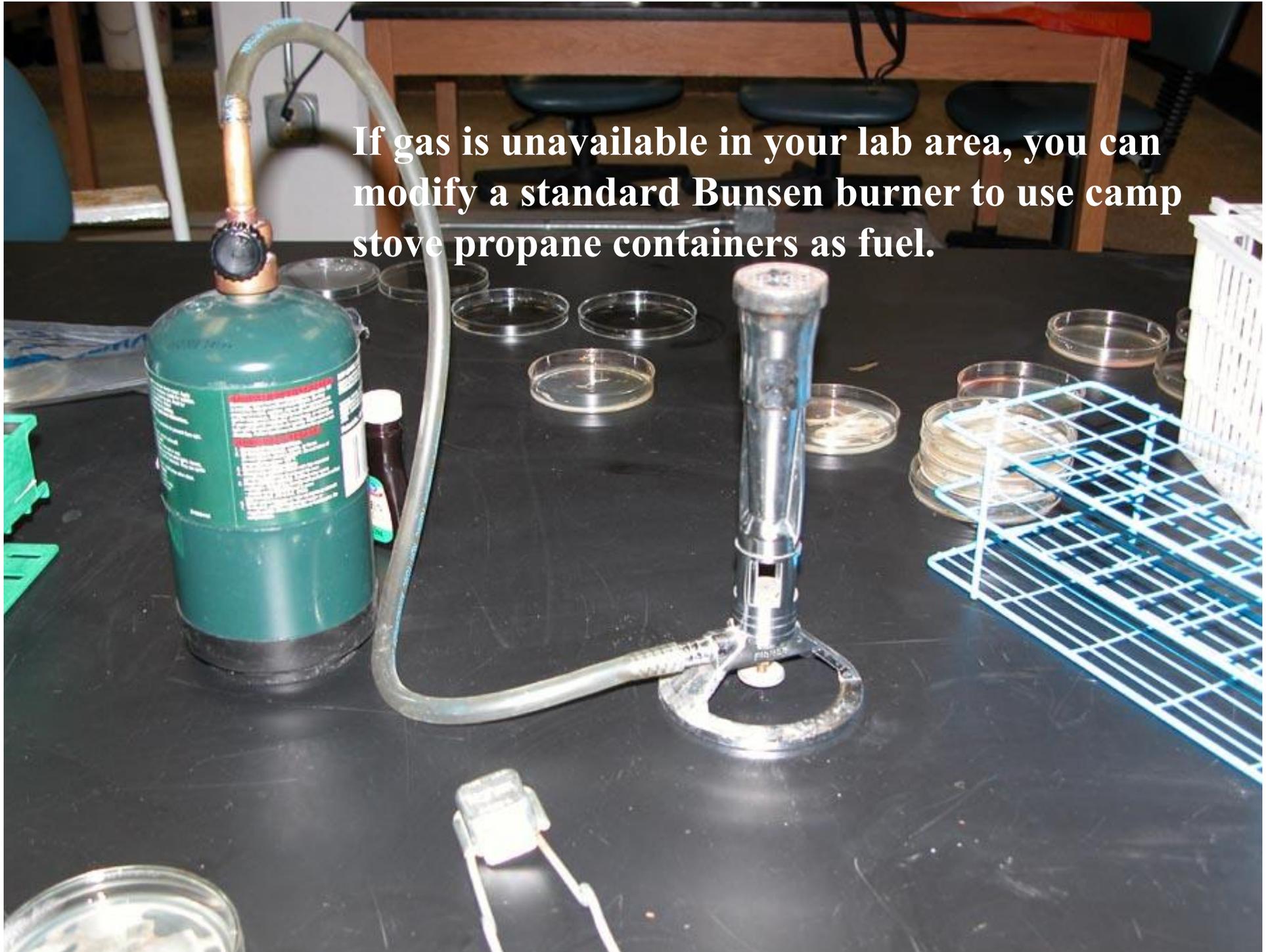


**Inoculating Plates and
Culture Tubes**

Inoculation of Culture Plates and Tubes

- ✓ Clean and surface sterilize your work area as detailed in the section on *Sterile Technique*.
- ✓ Use either disposable inoculation loops or a metal loop that can be heat sterilized to inoculate plates, slants, and liquid culture tubes.
- ✓ If using a metal loop, be sure to cool the loop by touching the sterile cooled liquid media or the sterile culture plate **before** the placing the loop in your live culture. Failure to cool the loop will kill your active microbial cultures!

If gas is unavailable in your lab area, you can modify a standard Bunsen burner to use camp stove propane containers as fuel.



Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 1: Remove the culture tube stopper or cap with one (do not set it down) and flame the mouth of the tube to surface sterilize the mouth. The heated tube surface will generate a thermal current that prevents contamination of the culture.



Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 2: Without setting any of the culture materials on the bench, place the sterile inoculation loop in the culture.

Step 3: Replace cap on the culture tube with the active microbes and put it in the test tube rack.

Step 4: Without setting the loop down, pick-up a sterile fresh culture tube with media with one hand, and remove the cap with the other hand.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 5: Flame the mouth of the clean culture tube.

Step 6: Place the inoculation loop containing the microbes in the fresh media and swirl the loop in the media to ensure even dispersal in the media.

Step 7: If using a solid media slant tube, follow steps 1-5 and then zig-zag the inoculation loop across the slanted surface of the solid media in the tube.



Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 8: Flame the mouth of the newly inoculated culture tube and replace the cap.

Step 9: Place the culture tube in test tube rack.

Step 10: Repeat until all of the sterile tubes have been inoculated. Use a fresh disposable culture loop for each tube or flame the metal loop after each tube has been inoculated.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 11: Incubate the culture at the recommended temperature (check with your supplier for growth requirements). If using environmental samples, incubation at room temperature will avoid the accidental culture of human pathogens.

Step 12: Dispose of all culture materials in a biohazard bag and sterilize all old cultures before pouring out cultures and washing culture tubes. Disposable culture dishes should be melted in an autoclave or pressure cooker prior to disposal.



Inoculating Petri Plates

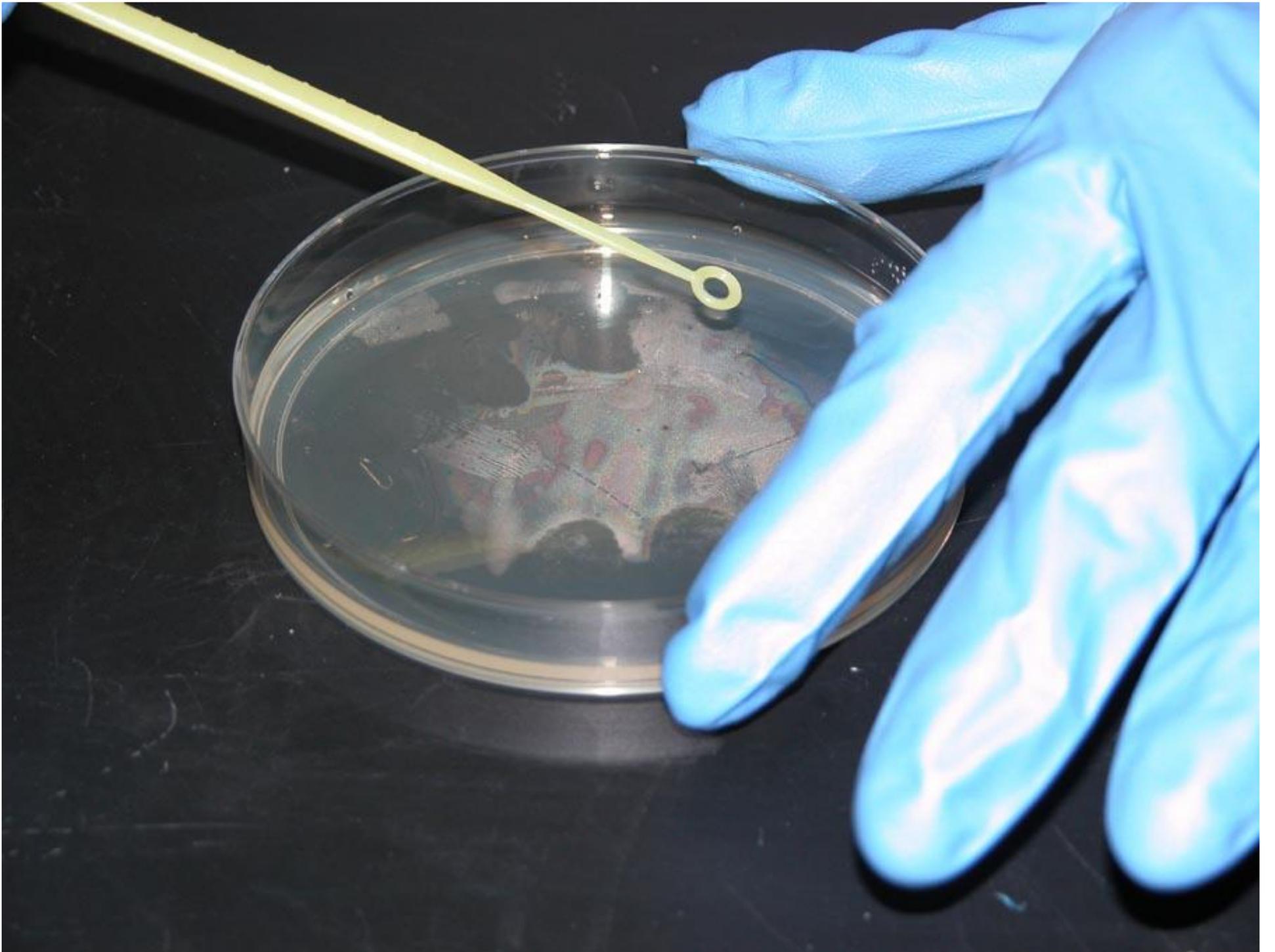
Step 1: Remove the culture tube stopper or cap with one (do not set it down) and flame the mouth of the tube to surface sterilize the mouth. The heated tube surface will generate a thermal current that prevents contamination of the culture.

Step 2: Without setting any of the culture materials on the bench, place the sterile inoculation loop in the culture.

Step 3: Replace cap on the culture tube with the active microbes and put it in the test tube rack.

Inoculating Petri Plates

Step 4: Holding the petri dish lid at an 30-45° angle, work the inoculating loop from the outside of the plate toward the center in a zig-zag pattern that covers approximately 25% of the plate surface (think pie or pizza slice!).



Inoculating Petri Plates

Step 5: Turn the petri plate 90° to the right, dragging the inoculation loop through the last section of the plate, moving from the outside to the inside in a zig-zag motion.

Step 6: Repeat this process twice more until the entire plate surface is covered.

NOTE: If you are trying to isolate individual colonies, each turn of the dish will give you fewer microbes so that you can distinguish individual colonies.



Use of a Plate Counter for Estimating Microbial Populations

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FUNCTION CONTROLS:

- YELLOW INDICATOR: Zero count
- RED INDICATOR: Manual counting
- BATTERY FLUID SOCKET: Recharge
- PHONE JACK: Mating pair of telephone handset
- POWER SWITCH: Illuminates when count is on

NOTE: For handset-counting feature which requires the other handset-count probe to be plugged in phone jack.

bantex

MODEL 900A

COLONY COUNTER



Serial Dilution of Environmental Samples or Commercial Cultures

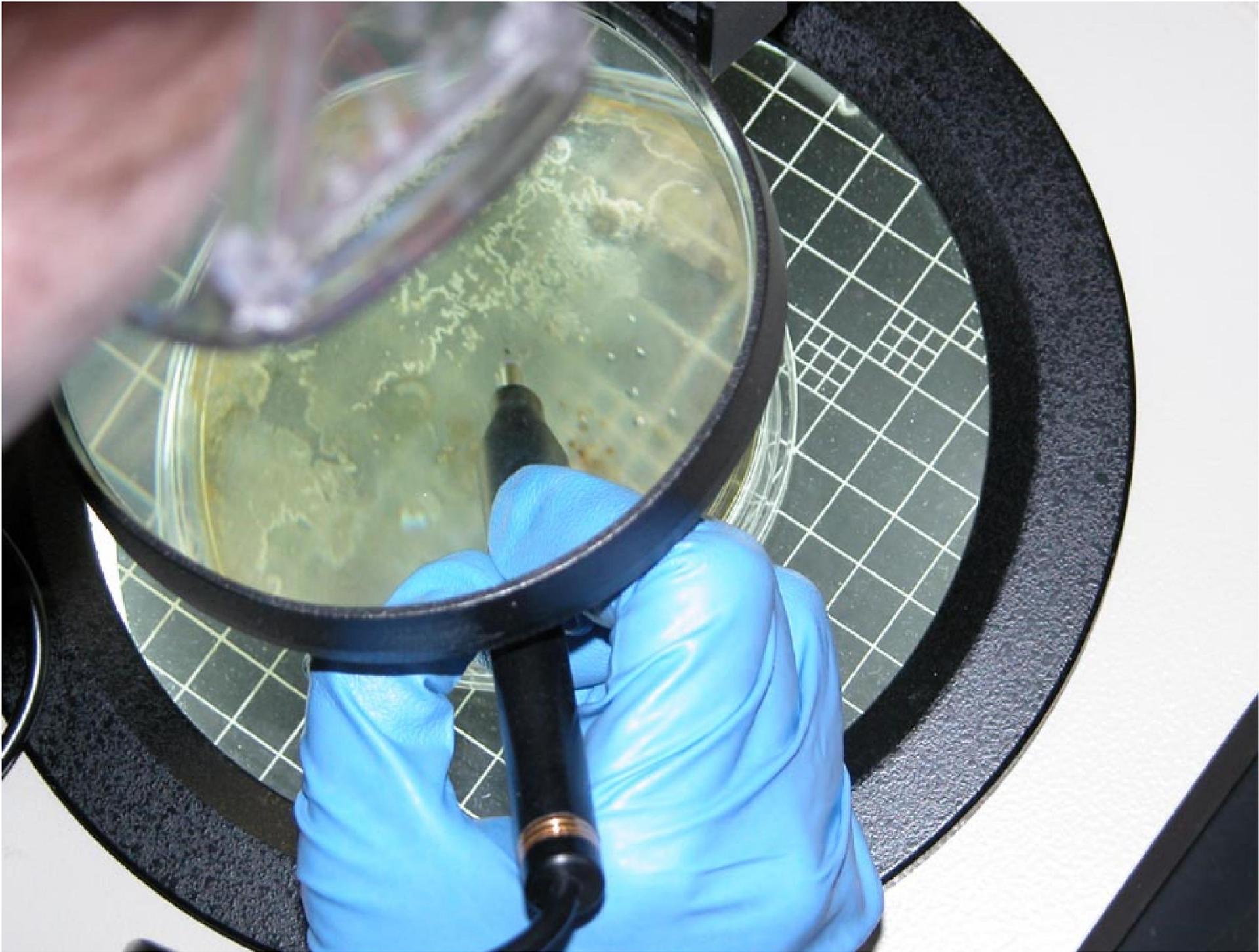
- **Serial dilution** techniques should be used in the estimation of microbial population sizes.
- **Serial dilution** involves the use of a known amount (in ml or μl) in a known volume of liquid media.
- A one in ten dilution is made in a new liquid culture tube, and this process is usually repeated several times. The resulting cultures are dilutions of 1/10, 1/100, 1/1000, 1/10,000, for example, of the original sample.
- These cultures are plated on petri plates and incubated at the recommended temperature.

Estimating Microbial Population Size

- After the inoculated plates are incubated for the appropriate time period, the number of colonies per plate are counted.
- Population estimates are obtained by multiplying the dilution factor by the number of colonies per plate. The resulting number is a rate (function) of the initial weight or initial volume used from the environmental sample or culture (per gram soil, per ml or μl of culture).

Counting Plates

- ✓ If a commercial plate counter is not available, you can Xerox 1 mm square graph paper and use it as a grid for colony counting. You would need to estimate the total surface area (in mm^2) by counting the number of squares in a dish.
- ✓ If using a commercial plate counter, touch each colony on the plate with the pen, and the cumulative number of colonies will appear on the display.



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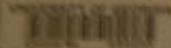
FUNCTION CONTROLS:

YELLOW PLATE BUTTON: Zeroes meter
RED PLATE BUTTON: Manual
counting
BATTERY FLUID SOCKET: Recharge
count probe
TACHO JACK: Mounting point for
optional tachometer probe
POWER SWITCH: Illuminates
when count is on

NOTE: For recharge-counting
feature which requires the
optional recharger probe
to be plugged in, phone
cable

bantex

MODEL 900A
COLONY COUNTER



Summary

- Different media are used to culture microorganisms, be certain that you are using the appropriate media for your organism.
- Always use sterile technique to prevent contamination.
- Choose the type of media (liquid or plate) appropriate for your investigation or application.
- Sterile liquid culture tubes and media plates can be prepared in advance and stored in the refrigerator for later use (2 weeks for liquid culture tubes, 2 months for media plates).

Summary

- Liquid culture tubes, solid slant tubes, and petri plates can be used to culture microbes.
- Media and lab materials should be sterilized prior to use; an autoclave or a pressure cooker can be used in the sterilization process.
- Serial dilution and plate count techniques are used to estimate microbial populations from environmental or commercial cultures.