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.

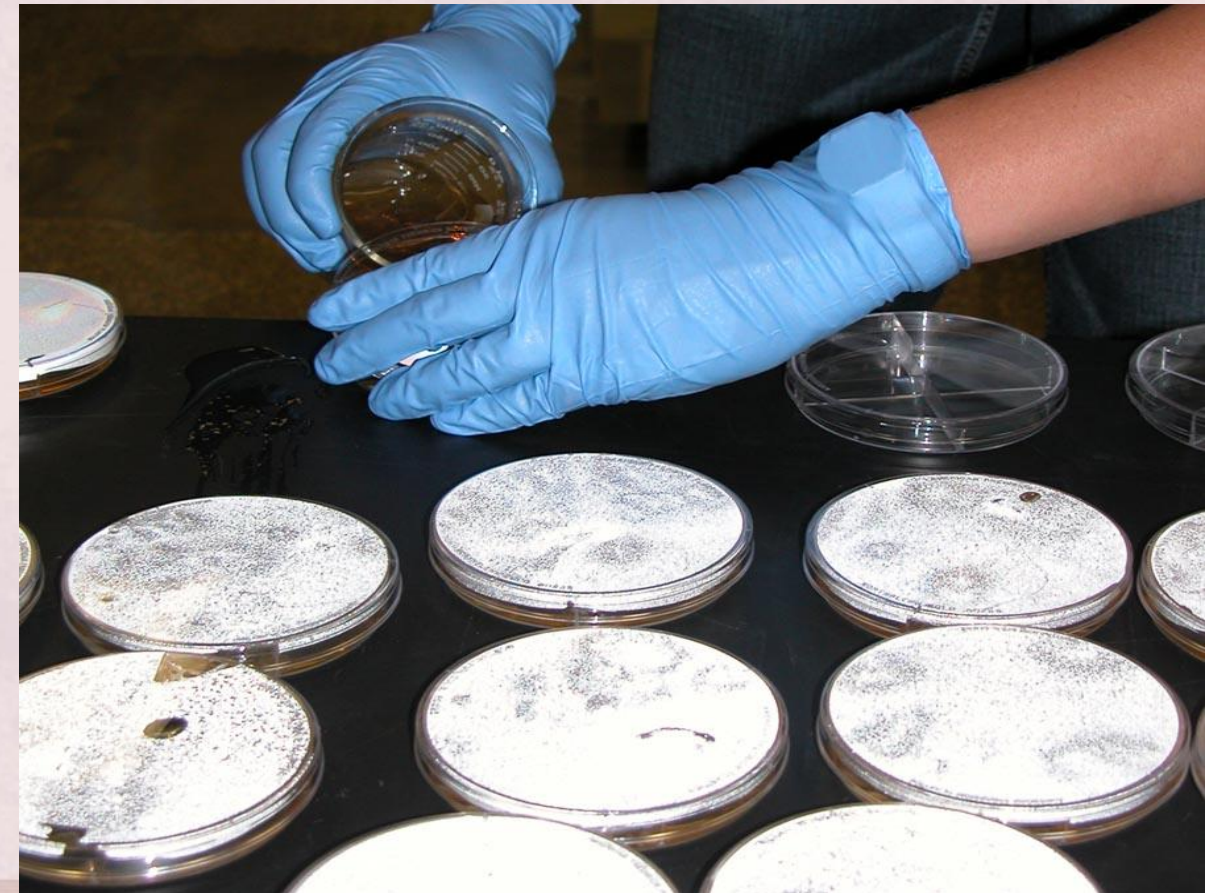
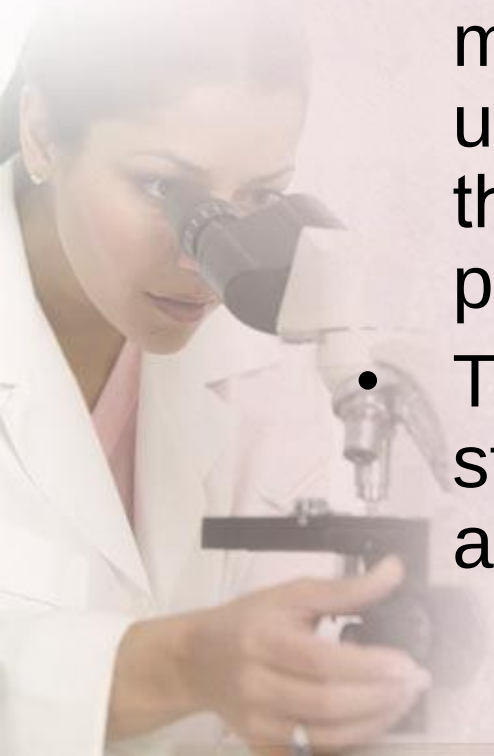


Plate Pouring Tips

- 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.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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.

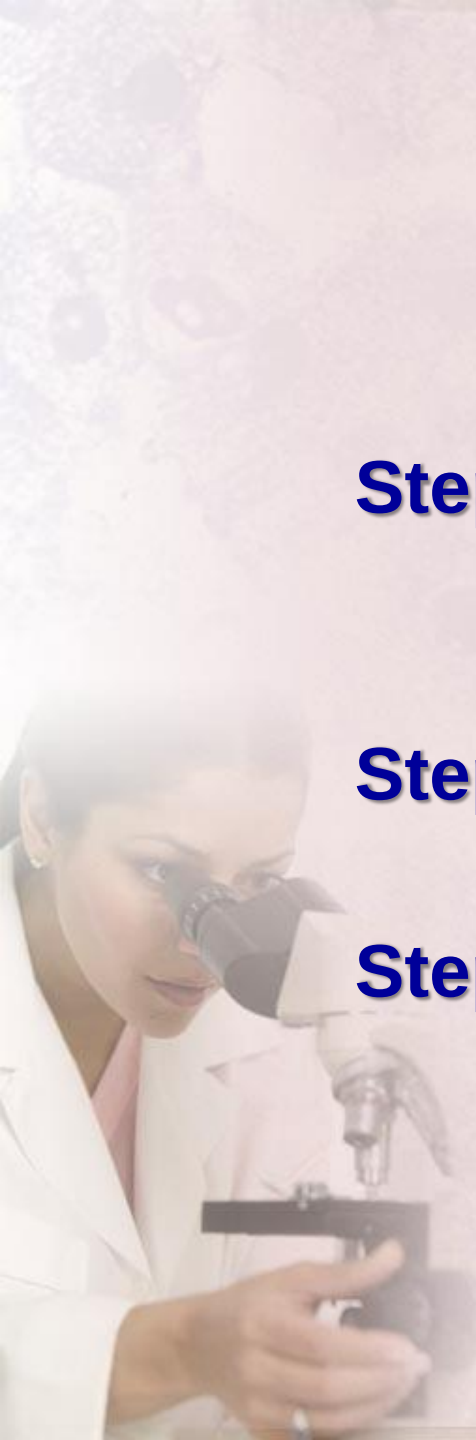
Inoculation of Liquid and Solid (Slant) Culture Tubes

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 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

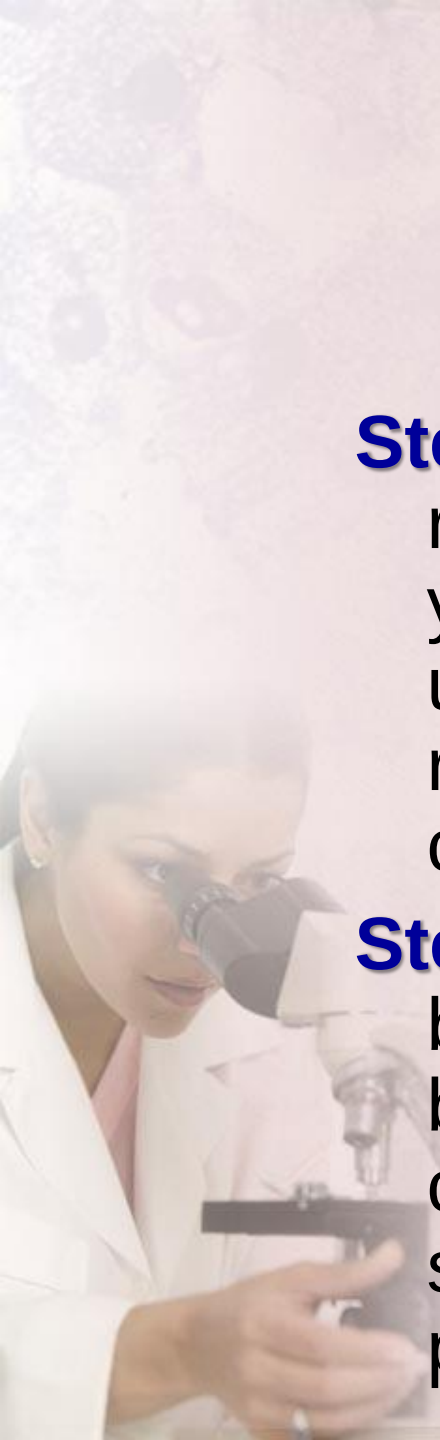


Step 8: Flame the mouth of the newly inoculated culture tube and replace the cap.

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.

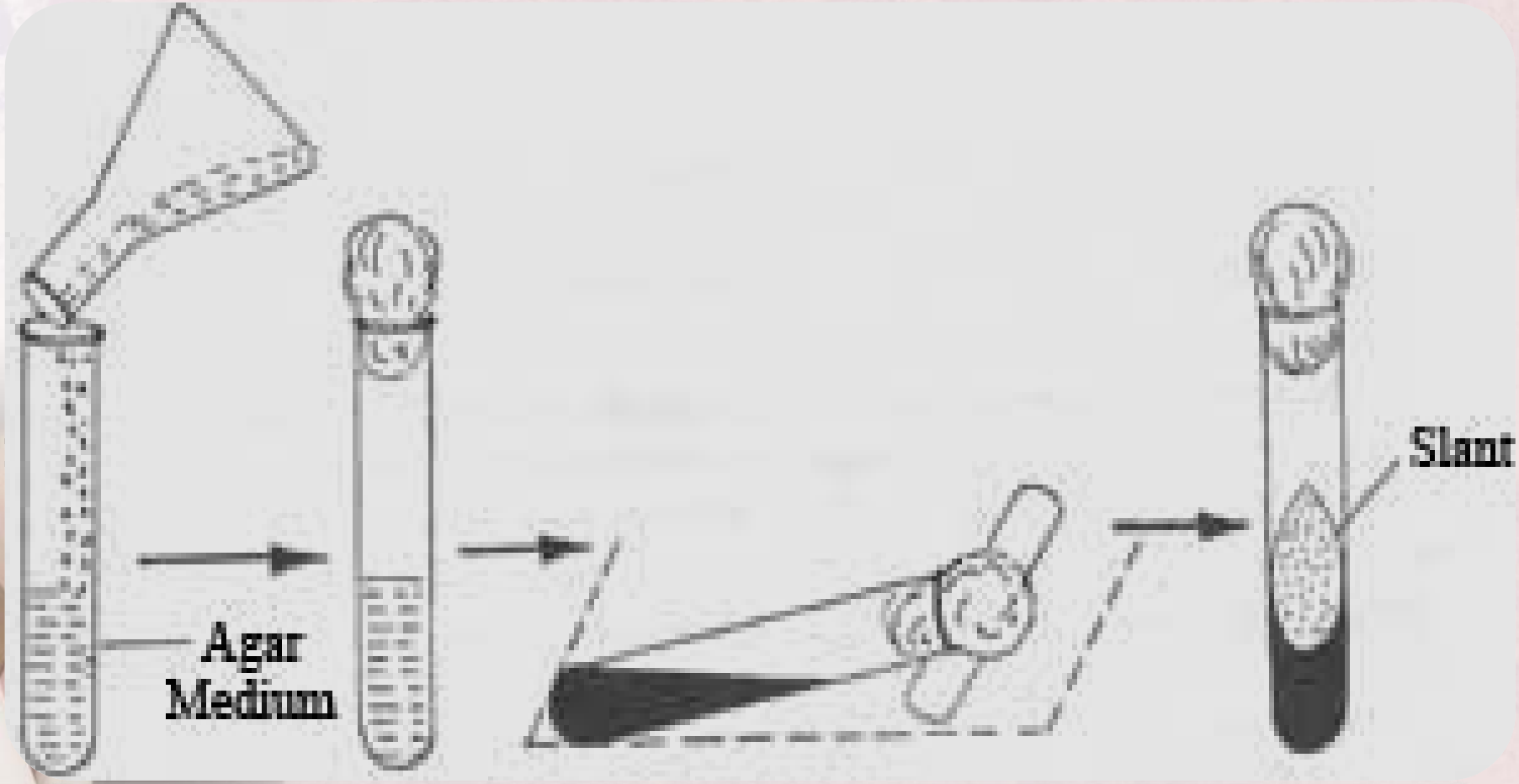
Inoculation of Liquid and Solid (Slant) Culture Tubes



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.

Agar Slant Preparation



Fish

Caution:
materials
prior to
Autoclave Bag

Inoculating Petri Plates

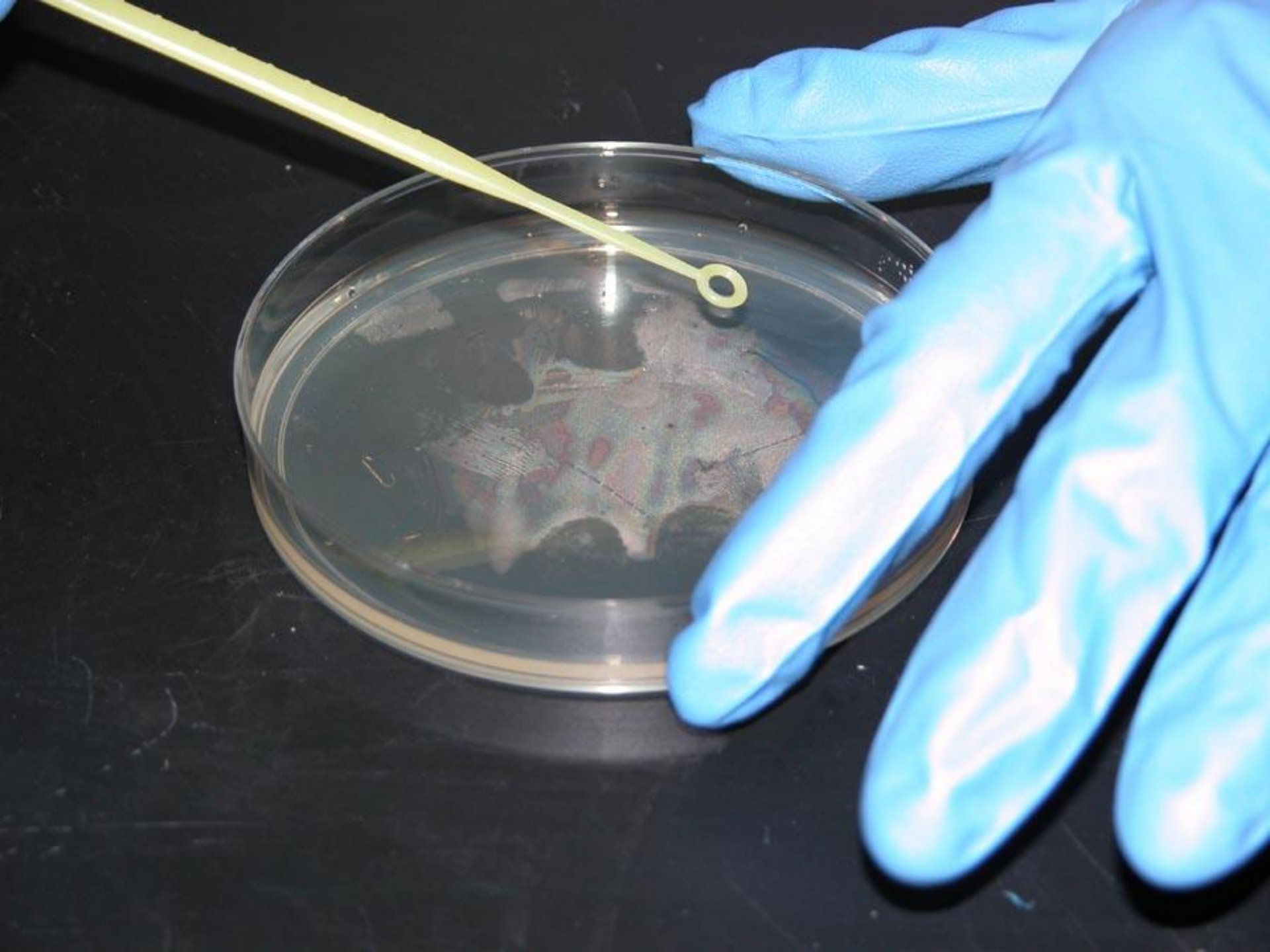
Step 1:Remove the culture tube stopper or cap with one (do not set it down) and flame the mouth of the tube to surface sterilize the mouth. The heated tube surface will generate a thermal current that prevents contamination of the culture.

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!).

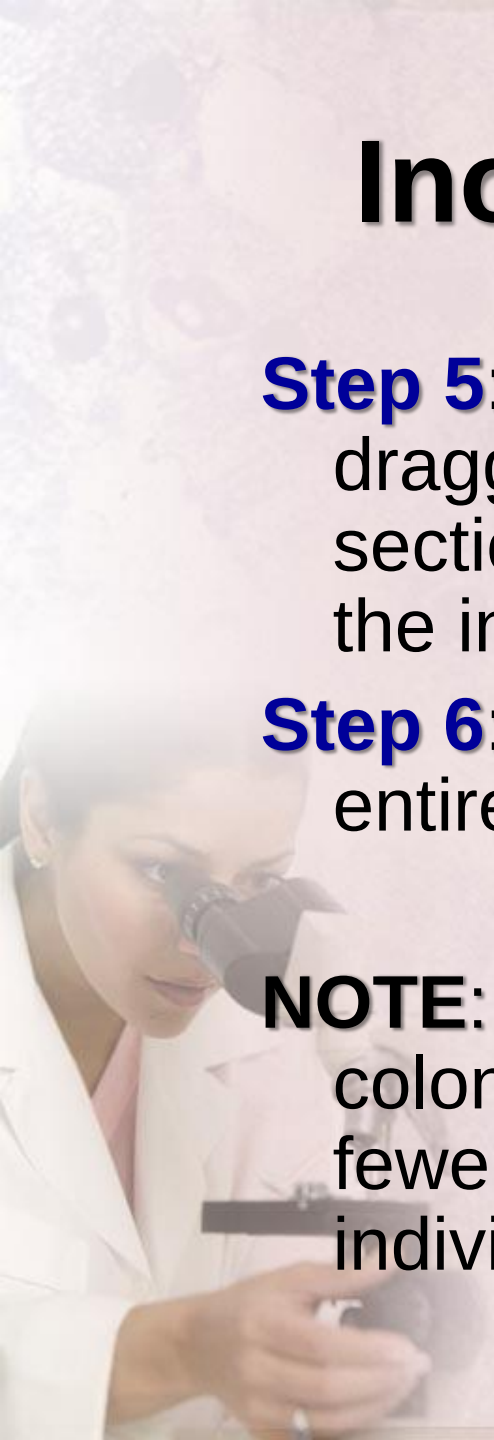


Inoculating Petri Plates

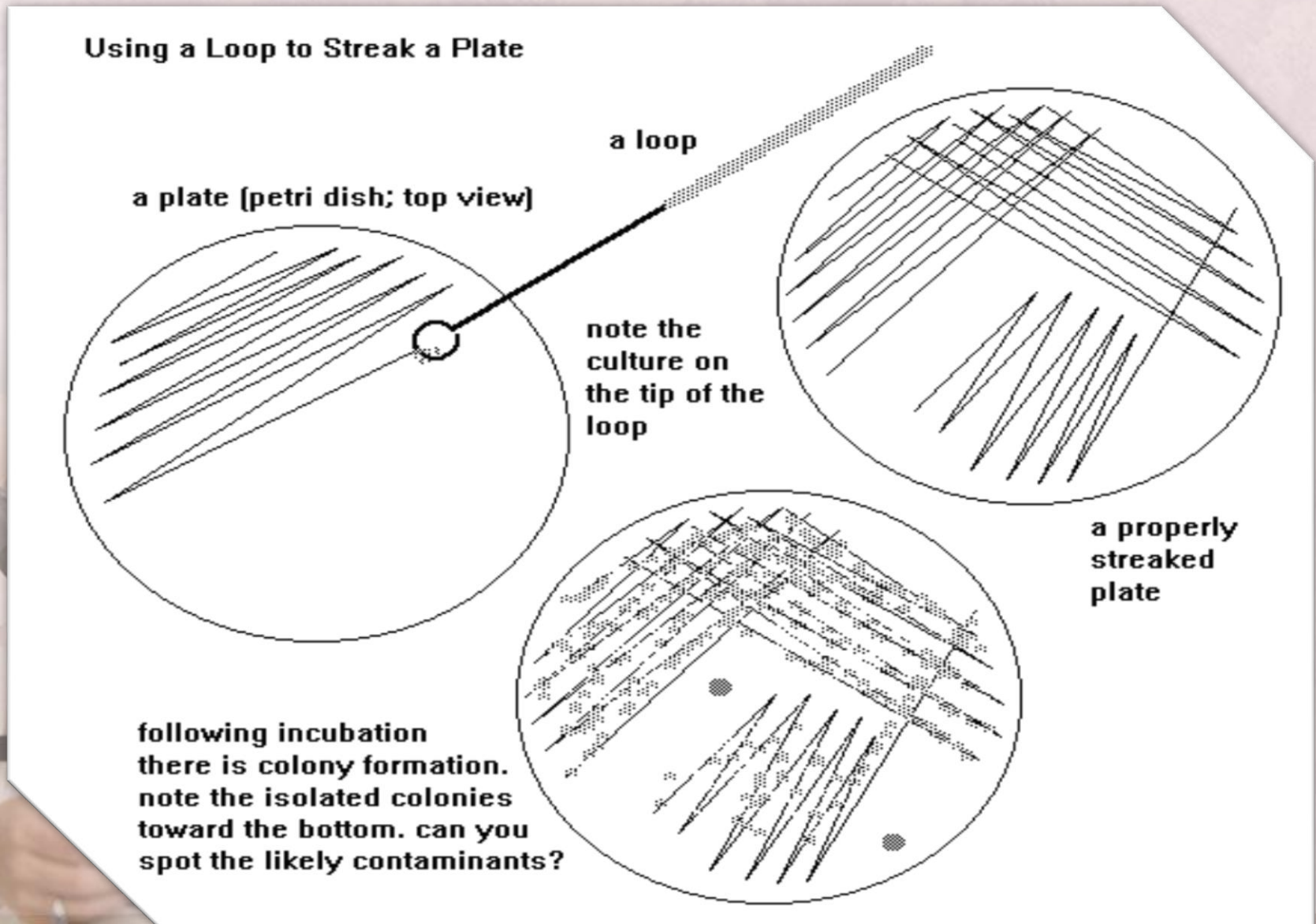
Step 5: Turn the petri plate 90° to the right, dragging the inoculation loop through the last section of the plate, moving from the outside to the inside in a zig-zag motion.

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

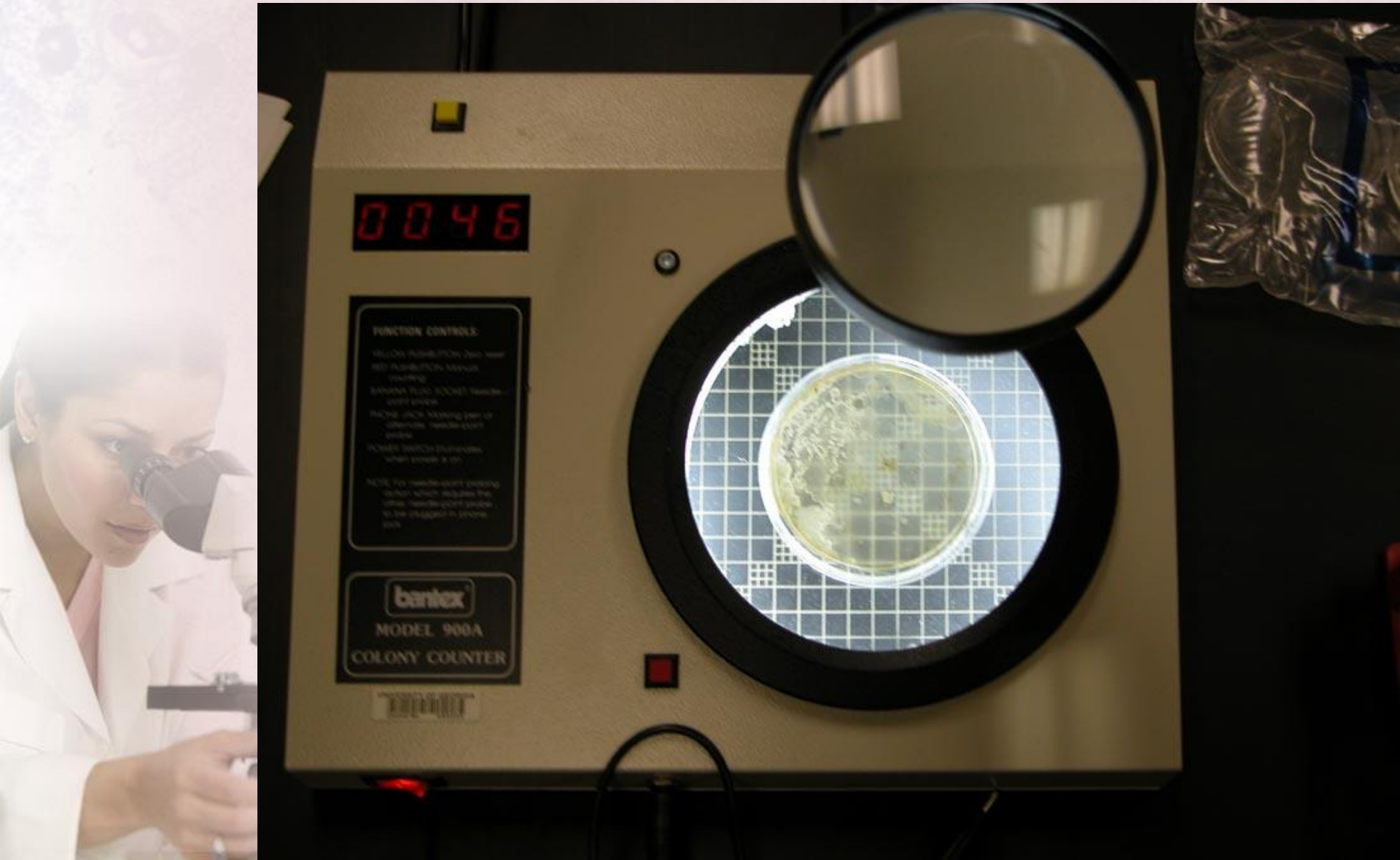
NOTE: If you are trying to isolate individual colonies, each turn of the dish will give you fewer microbes so that you can distinguish individual colonies.



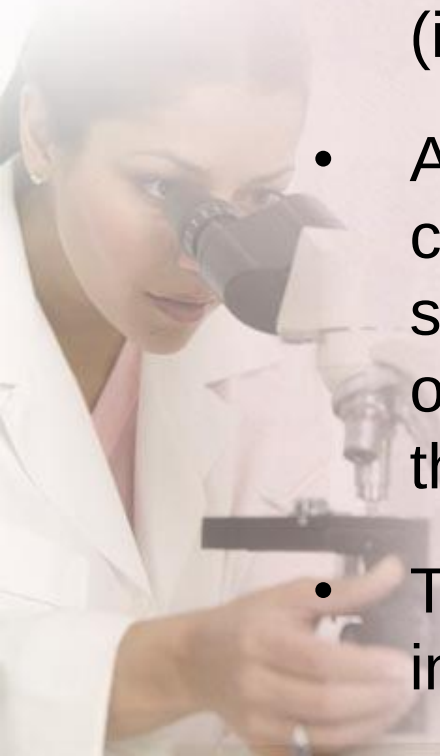
Streak Plate Method



Use of a Plate Counter for Estimating Microbial Populations



Serial Dilution of Environmental Samples or Commercial Cultures

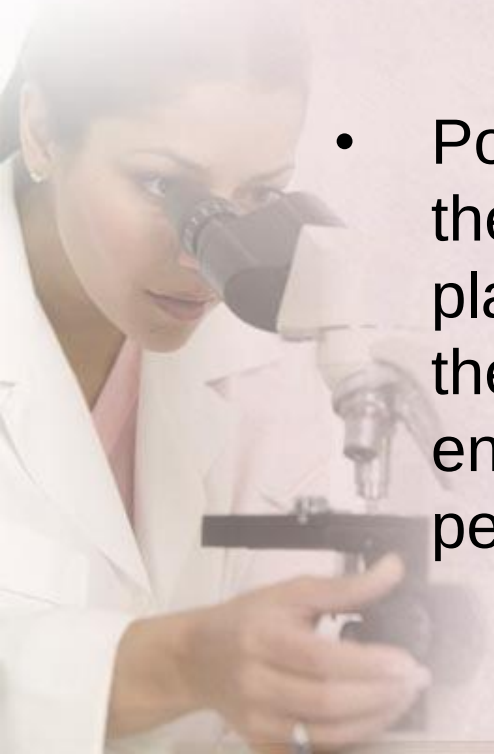


- Serial dilution techniques should be used in the estimation of microbial population sizes.
- Serial dilution involves the use of a known amount (in ml or µl) in a known volume of liquid media.
- A one in ten dilution is made in a new liquid culture tube, and this process is usually repeated several 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.

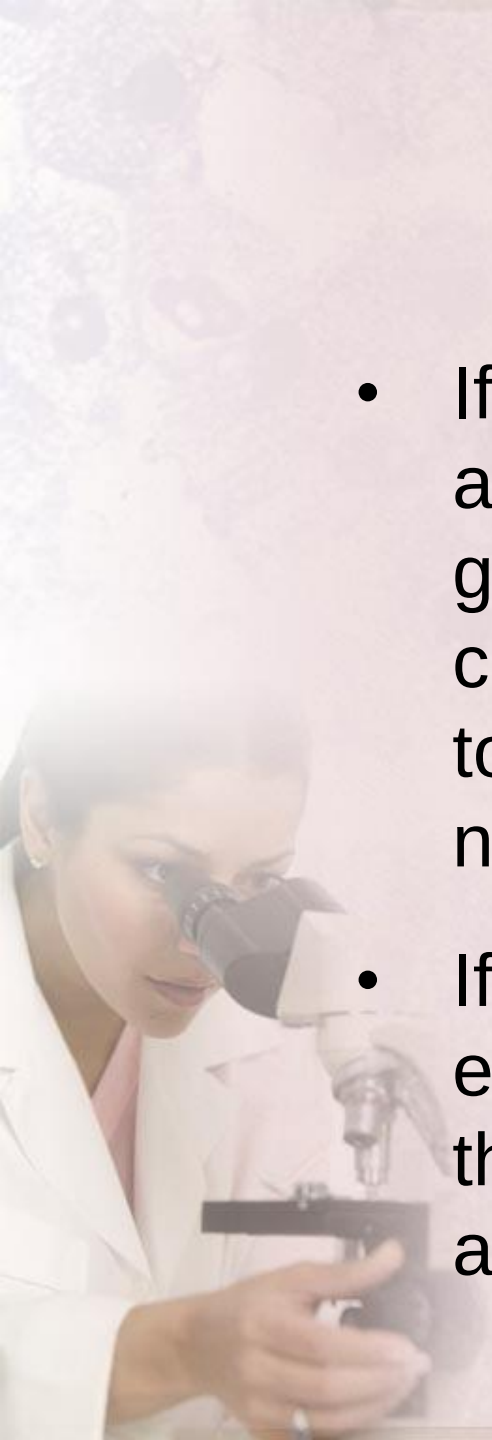
Estimating Microbial Population Size

- 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:
bantex
MODEL 900A
COLONY COUNTER

THE END