Aseptic Technique

Module 2, Lesson 1

What is Aseptic Technique?

Aseptic technique is a method that involves target-specific practices and procedures under suitably controlled conditions to reduce the contamination from microbes. It is a compulsory laboratory skill to conduct research related in the field of microbiology

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7123386

Basically it is a set of techniques used to keep the microorganisms we are growing from getting contaminated by any other microorganisms in the environment.

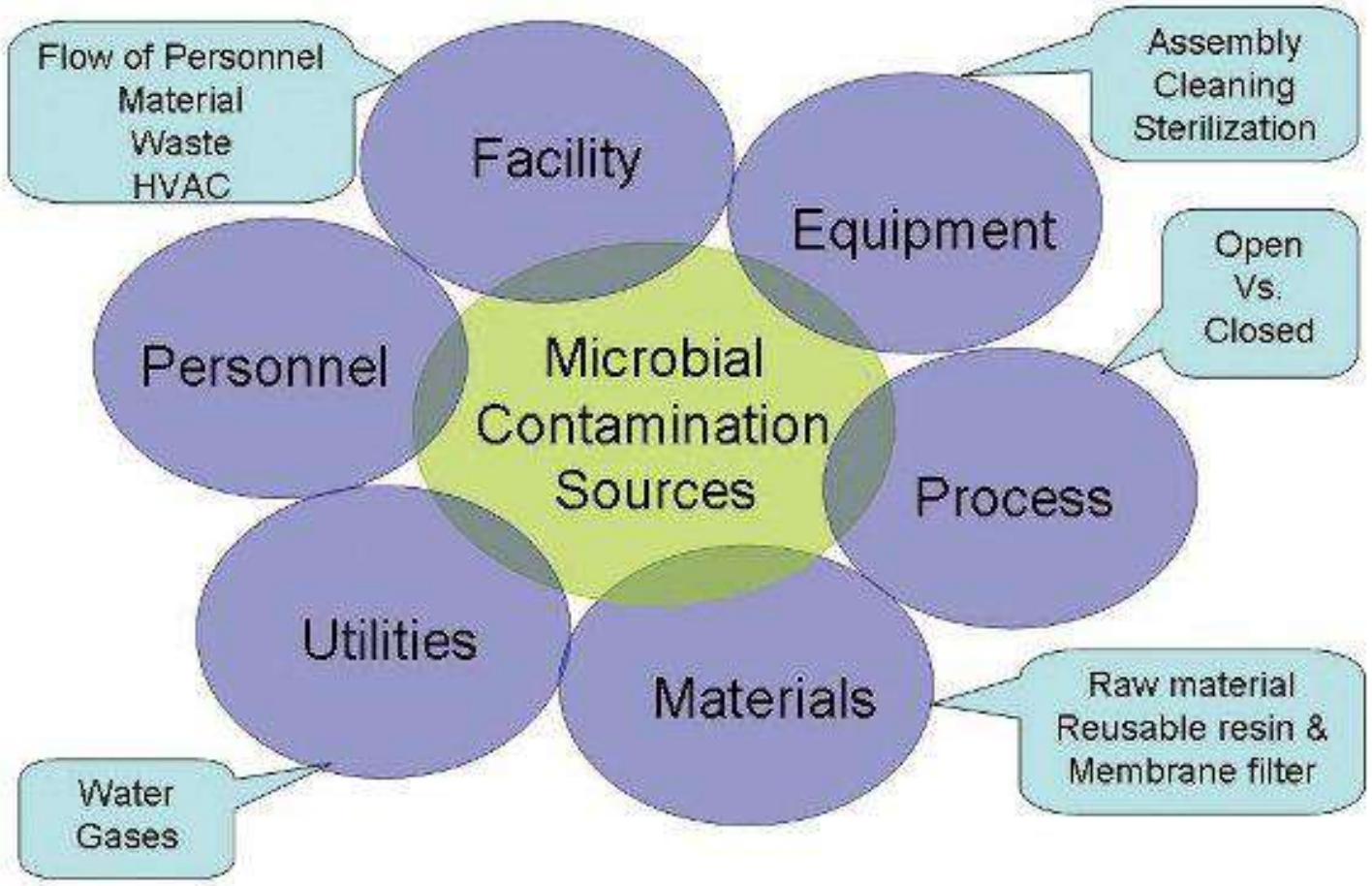
Why Use Aseptic Technique?

When culturing bacterial cells we use aseptic technique to:

- Prevent contamination of the specific microorganism we are working with.

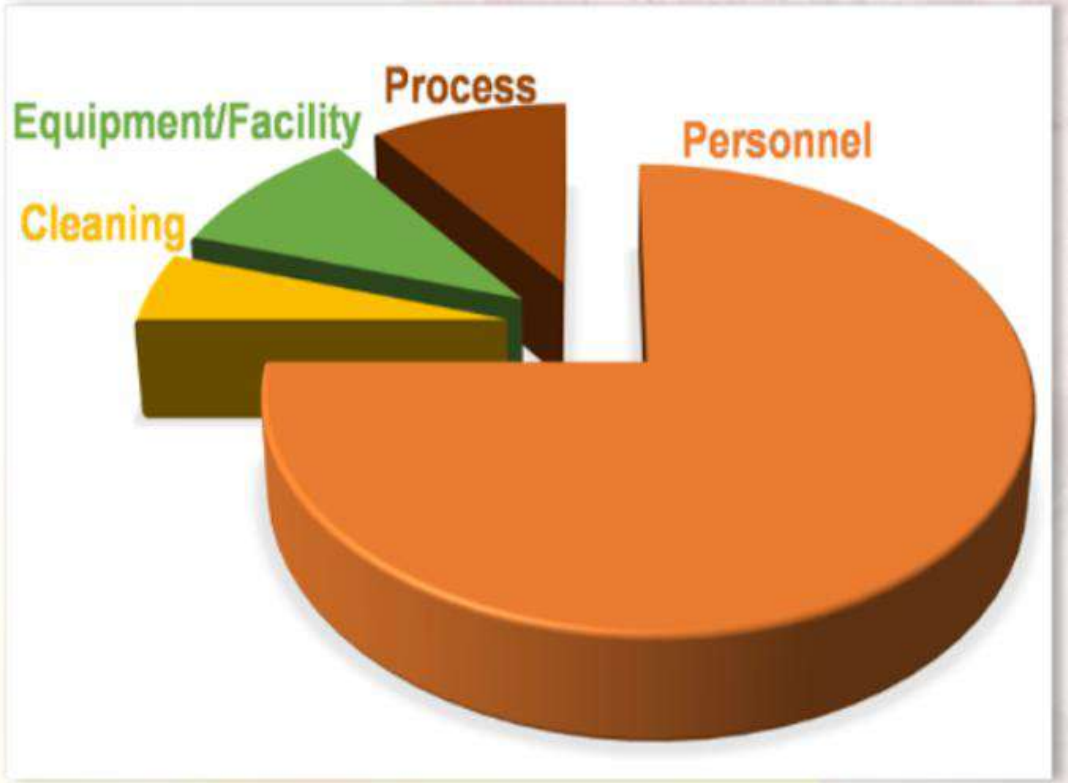
- Prevent contamination of the room and personnel with the microorganism we are working with.

Sources of Contamination



https://www.americanpharmaceuticalreview.com/Featured-Articles/36755-Case-Studies-of-Microbial-Contamination-in-Biologic-Product-Manufacturing/

Sources of Contamination



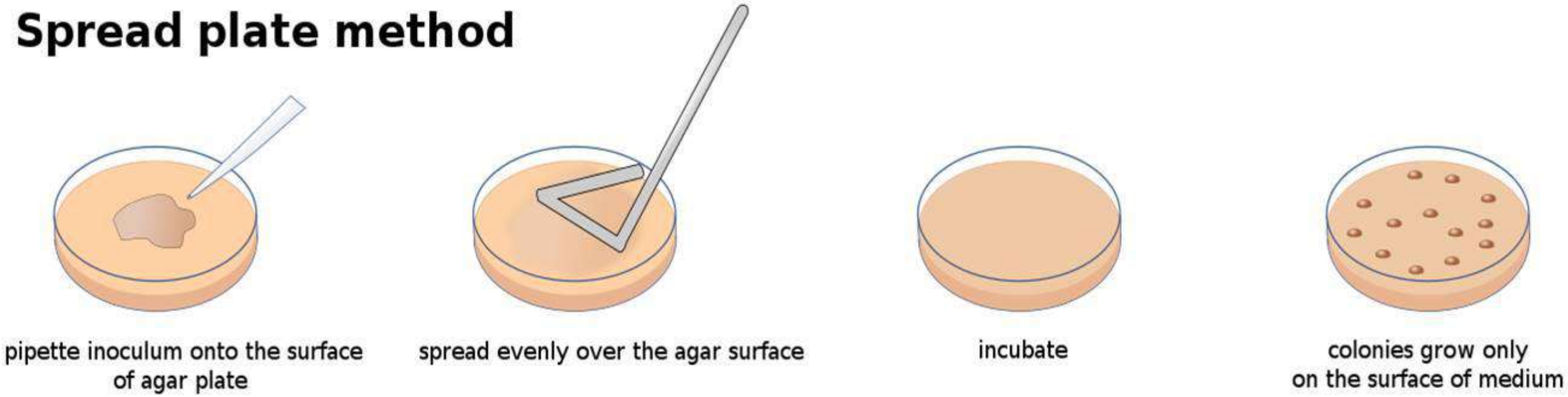


https://www.crambridge.com/portfolio/manufacturing/cleanrooms-and-contamination/

Guidelines for Performing Aseptic Technique

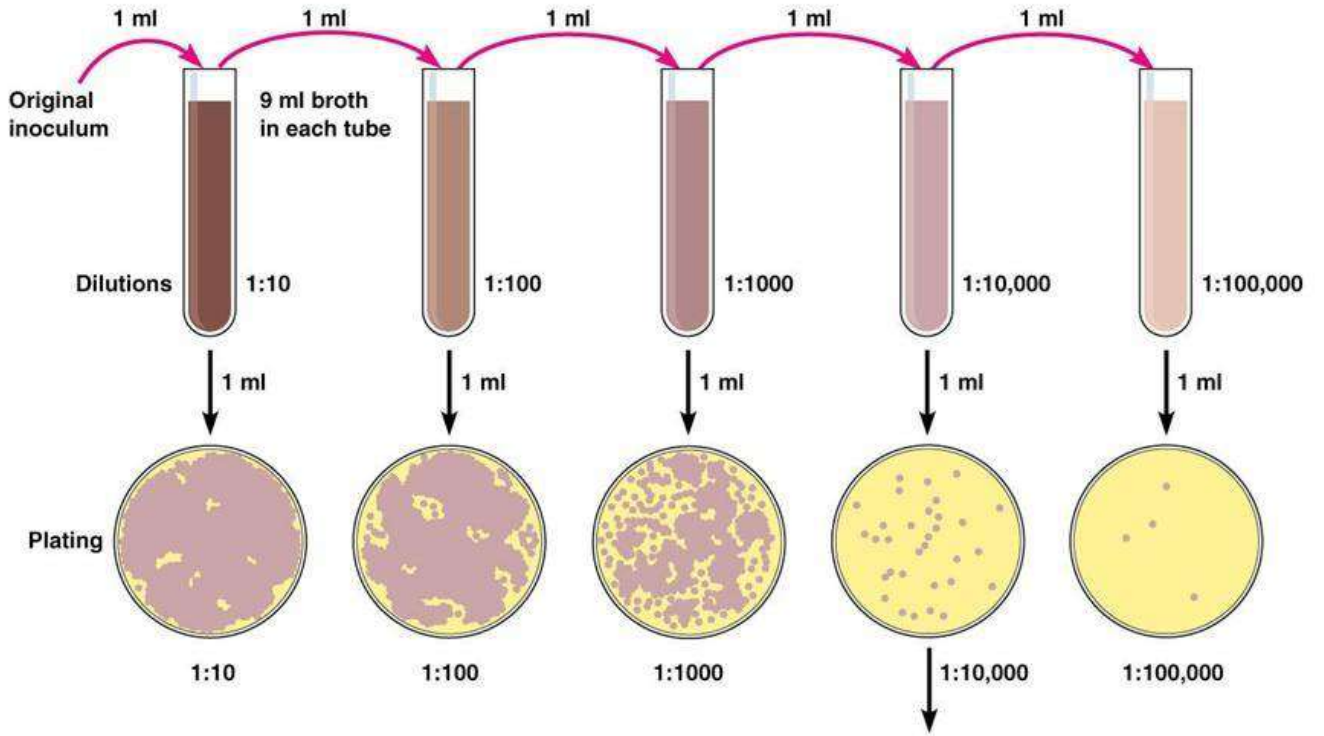
1. Close doors and windows in the lab
2. Wash your hands
3. Put on personal protective equipment (PPE) – lab coat, gloves, safety glasses
4. Long hair should be pulled back
5. Sterilize lab bench with 70% ethanol
6. Organize your lab bench so the things you will be using are within easy reach
7. Sterilize the outside of bottles with 70% ethanol
8. Sterilize your gloves with 70% ethanol
9. Use only sterile containers, pipettes, pipette tips etc
10. Use only sterile solutions
11. Never leave bottles open on the bench – open them only when you need to and recap as soon as possible
12. Open bacterial plates away from you
13. Do not pass your hands or arms over bottles, plates etc when working
14. Work with a partner
15. Be careful not to talk, sing, or whistle when you are performing sterile procedures.
16. Perform your experiments as rapidly as possible to minimize contamination.

How Can We Count Bacteria?



https://noteshippo.com/spread-plate-method-in-microbiology-principle-procedure-uses-and-limitations/

How Can We Count Bacteria?



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

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Spread Plate Method

The spread plate method is a technique to plate a liquid sample containing bacteria **so that the bacteria are easy to count and isolate**. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate.

The spread plate technique involves **using a sterilized spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate**.

The plate needs to be dry and at room temperature so that the agar can absorb the bacteria more readily.

https://microbiologyinfo.com/spread-plate-technique-principle-procedure-and-uses/

Testing the Sterility of LB Media Using Aseptic Technique

Prepare Yourself

Put on Personal Protective Equipment



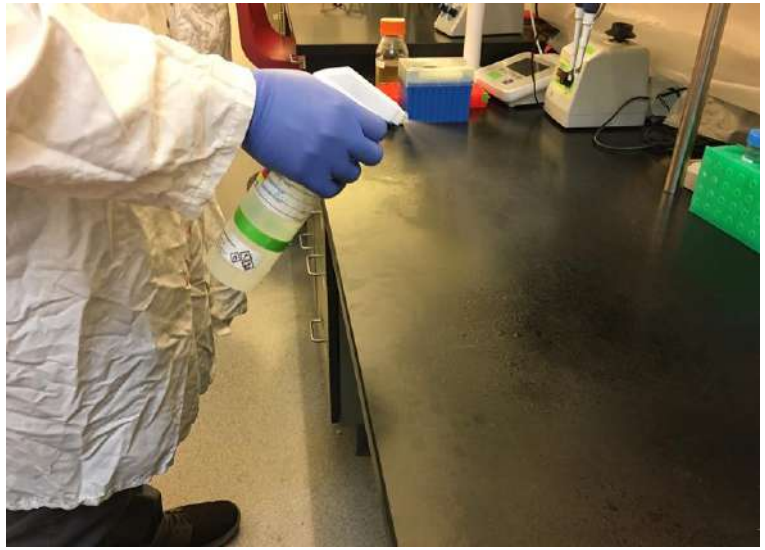
Lab coat
Gloves
Safety glasses

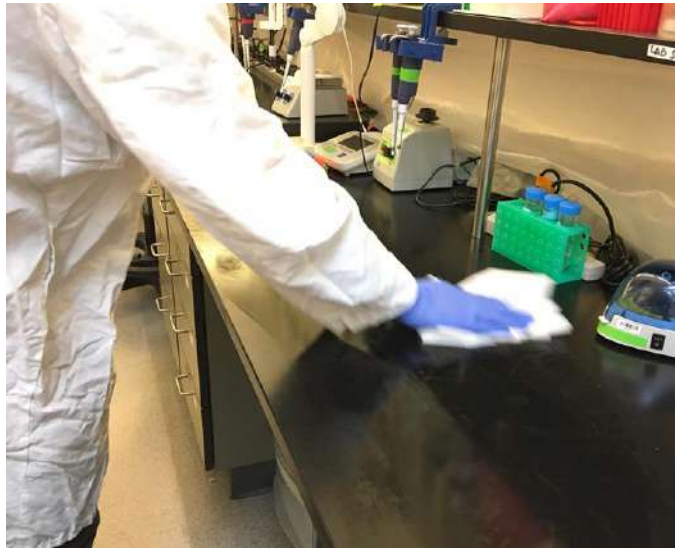


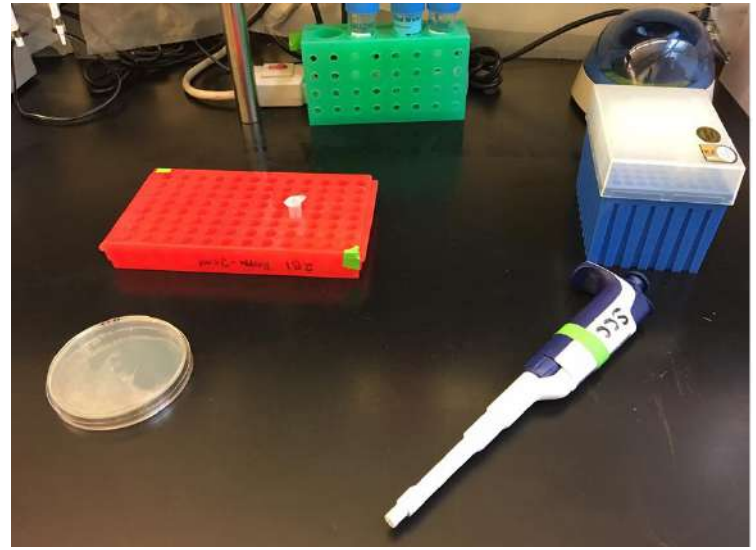
Close-toed shoes

Prepare the Lab Bench

Clear and sanitize the lab bench. Arrange all reagents and supplies.

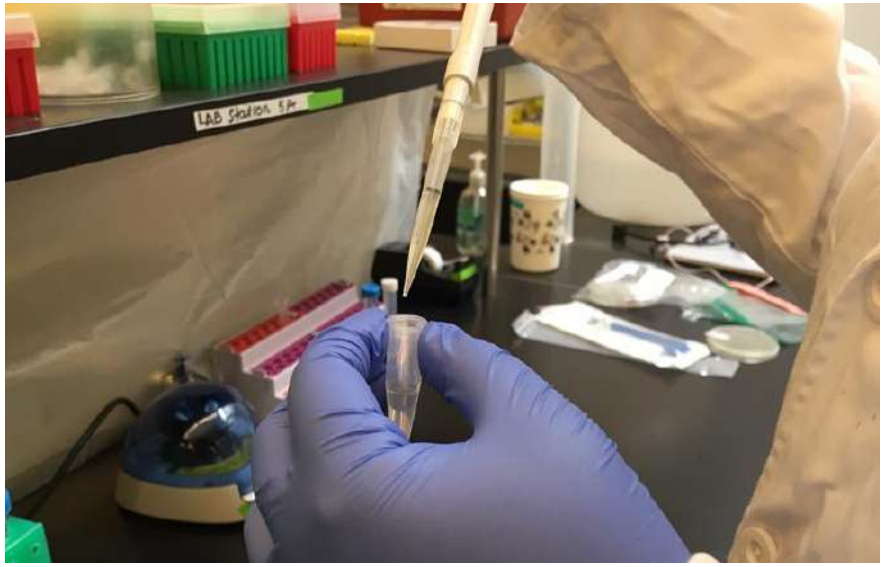






Remove Media From the Container

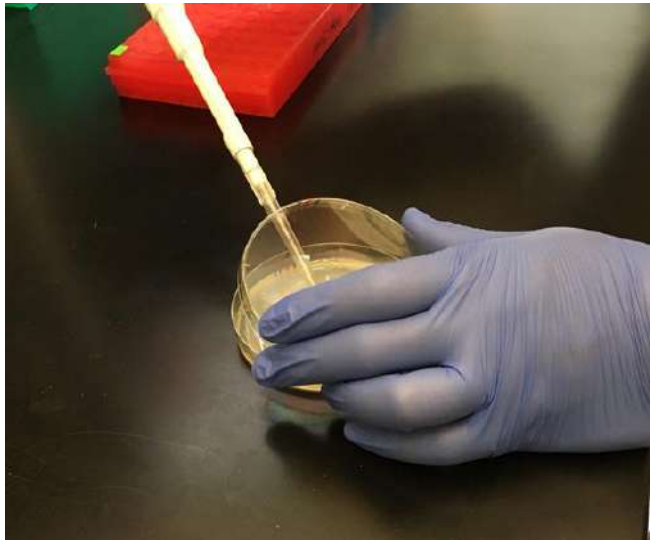




Dispense Media onto the LB Plate



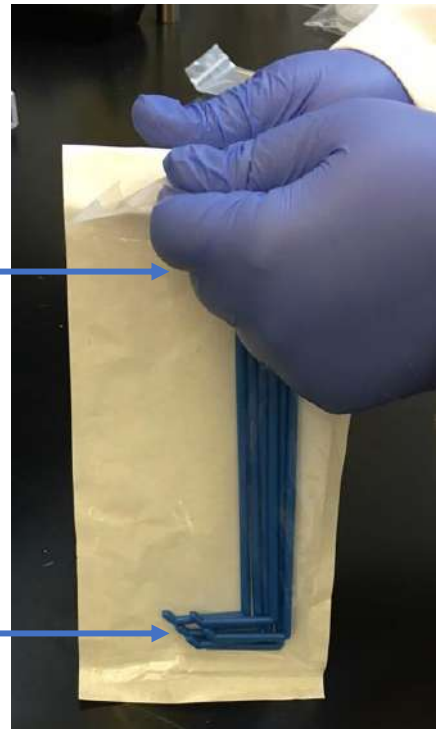
Open the plate using the ‘clamshell’ method.
Open the plate only on one side.
Open the side of the plate furthest from you.



Dispense the media onto the agar surface.
It may be easier to have one person open the plate and a second person dispense the media.

Spread the Media on the Plate

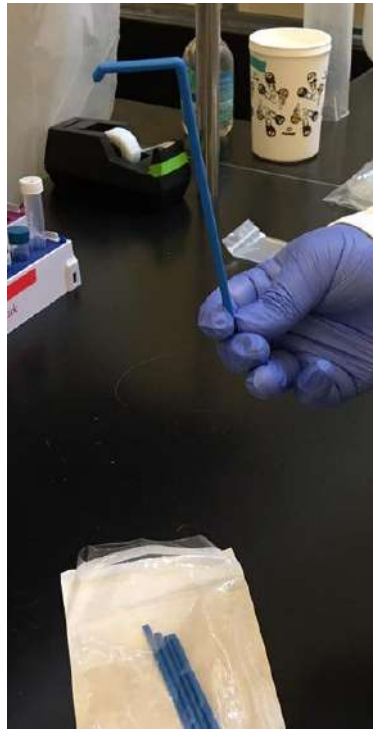
Remove a Sterile Spreader



Open the package from the ‘handle’ end.



Remove one spreader.



Do not touch the spreader end with your fingers or any surface.

Spread the Media on the Plate

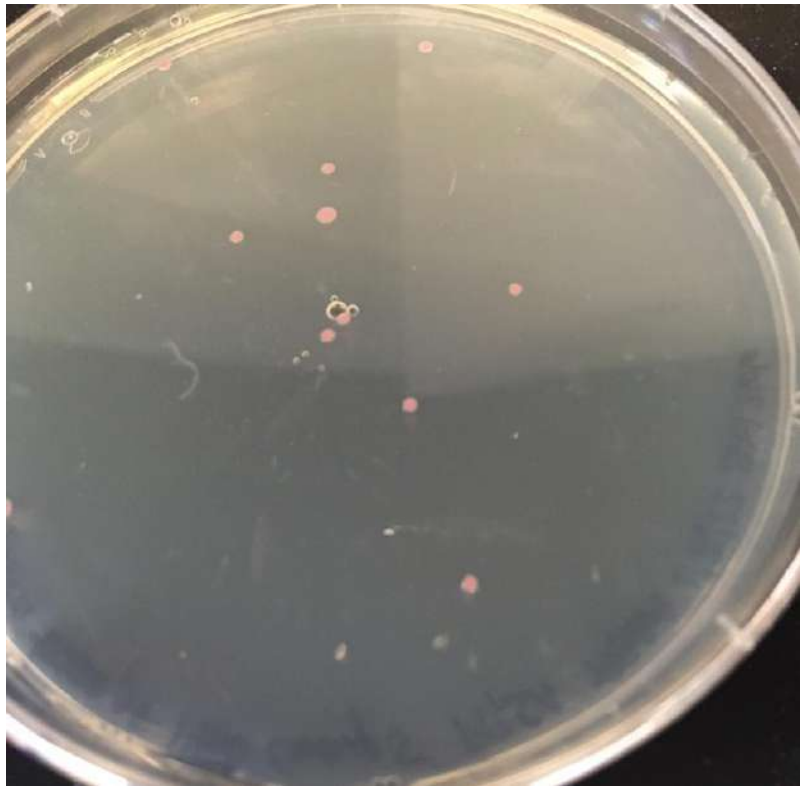




Open the plate using the ‘clamshell’ method.
Spread the media using the spreader.
Do not push down too hard.

Bacterial Inoculation
Using Aseptic Technique

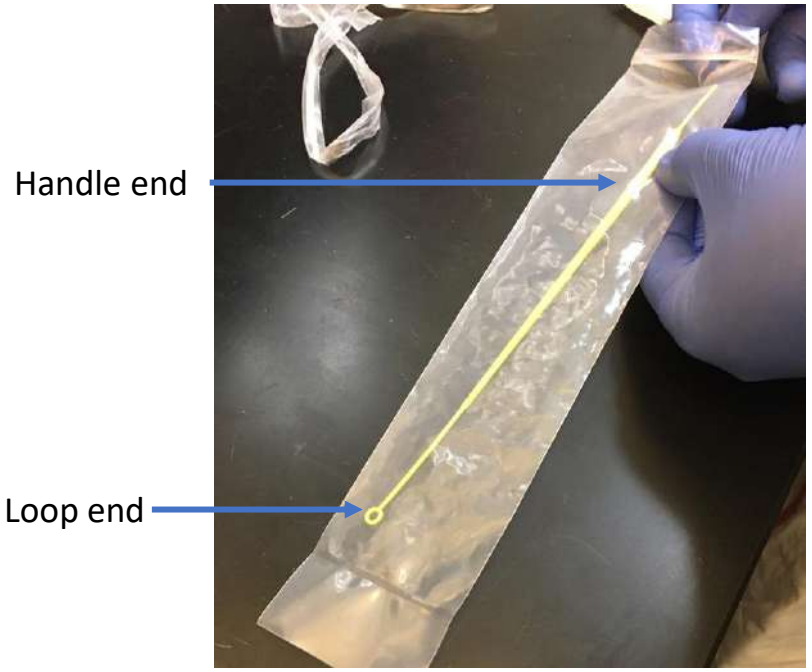
Pick an RFP+ or GFP+ Bacterial Colony



RFP+ bacterial colonies

Pick an RFP+ or GFP+ Bacterial Colony

Remove a sterile inoculating loop.



Open the package from the ‘handle’ end.

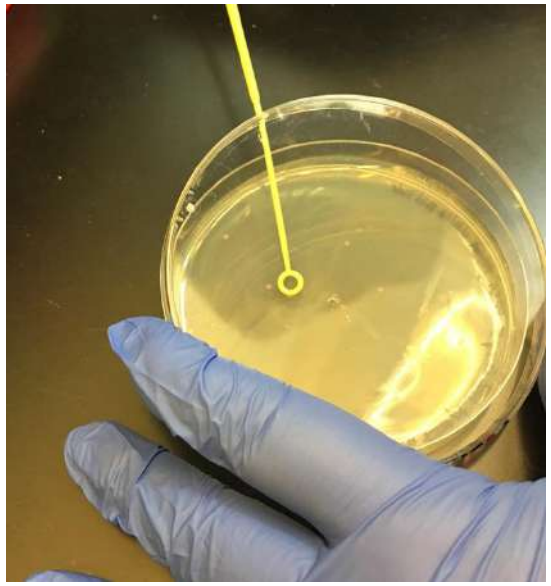


Remove one loop.



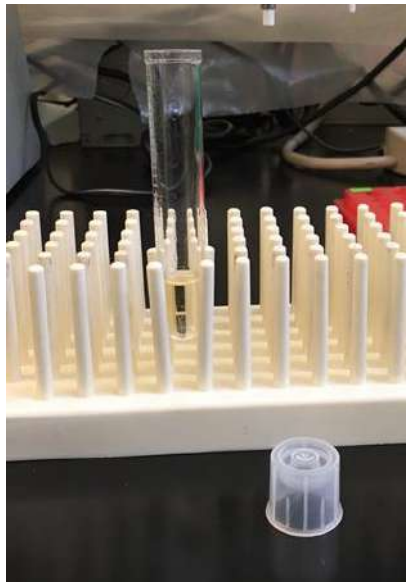
Do not touch the loop end with your fingers or any surface.

Bacterial Inoculation

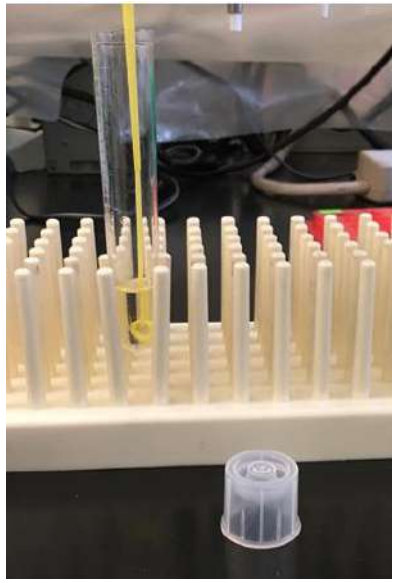


Open the plate using the ‘clamshell’ method.
Pick a colony using the sterile loop.

1



2



3



1. Open the tube that contains the LB.
   Set the lid on the sanitized bench top.
2. Insert the loop into the tube and swirl gently.
   Remove the loop.
3. Cap the tube to the loose setting.
   Insert tube in shaker/incubator.

Proper Disposal of Bacterial Waste

Biohazard Bags – Waste is autoclaved

For solid waste such as:

- Bacterial spreaders
- Inoculating loops
- Bacterial plates
- Micropipette tips
- Microfuge tubes
- Test tubes





10% Bleach Solution – Sits 10 min
Pour down the sink and flush with water

For liquid waste such as:

- Liquid bacterial cultures
- Unused liquid media (LB)

