SQBI 1303 MICROBIOLOGY

Basic Laboratory Techniques for Isolation, Cultivation and Cultural Characterization of Microorganisms

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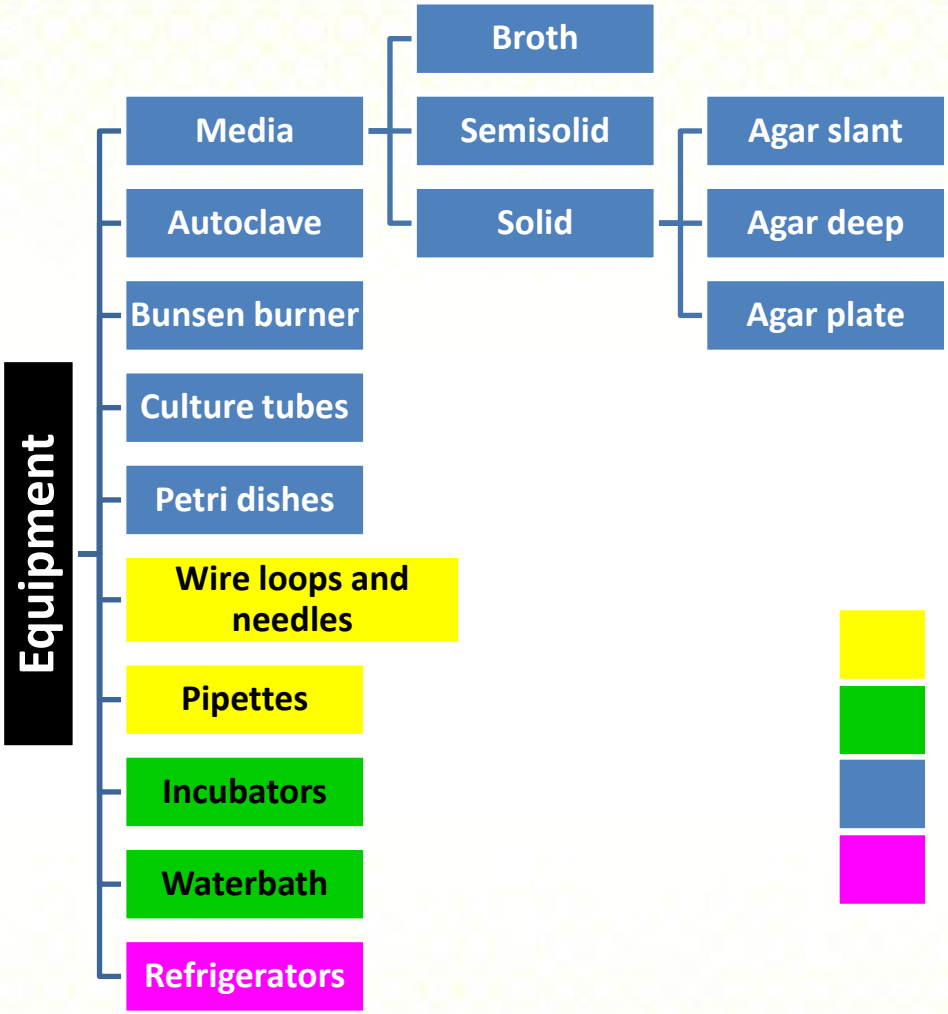
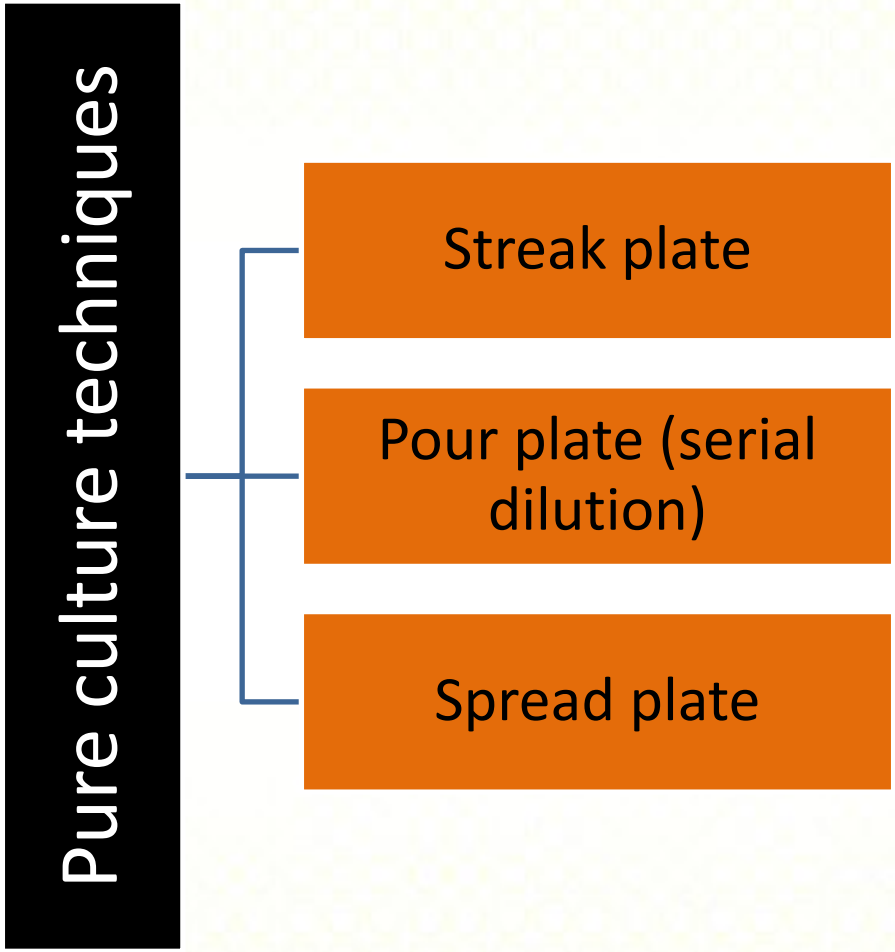
Introduction

- Microorganisms are **ubiquitous** (meaning??)
- Microbiologist separates mixed population of microorganisms into individual species for study.
- To isolate and study microorganisms in pure culture, the microbiologist requires basic laboratory apparatus in the application of specific techniques

Laboratory Apparatus



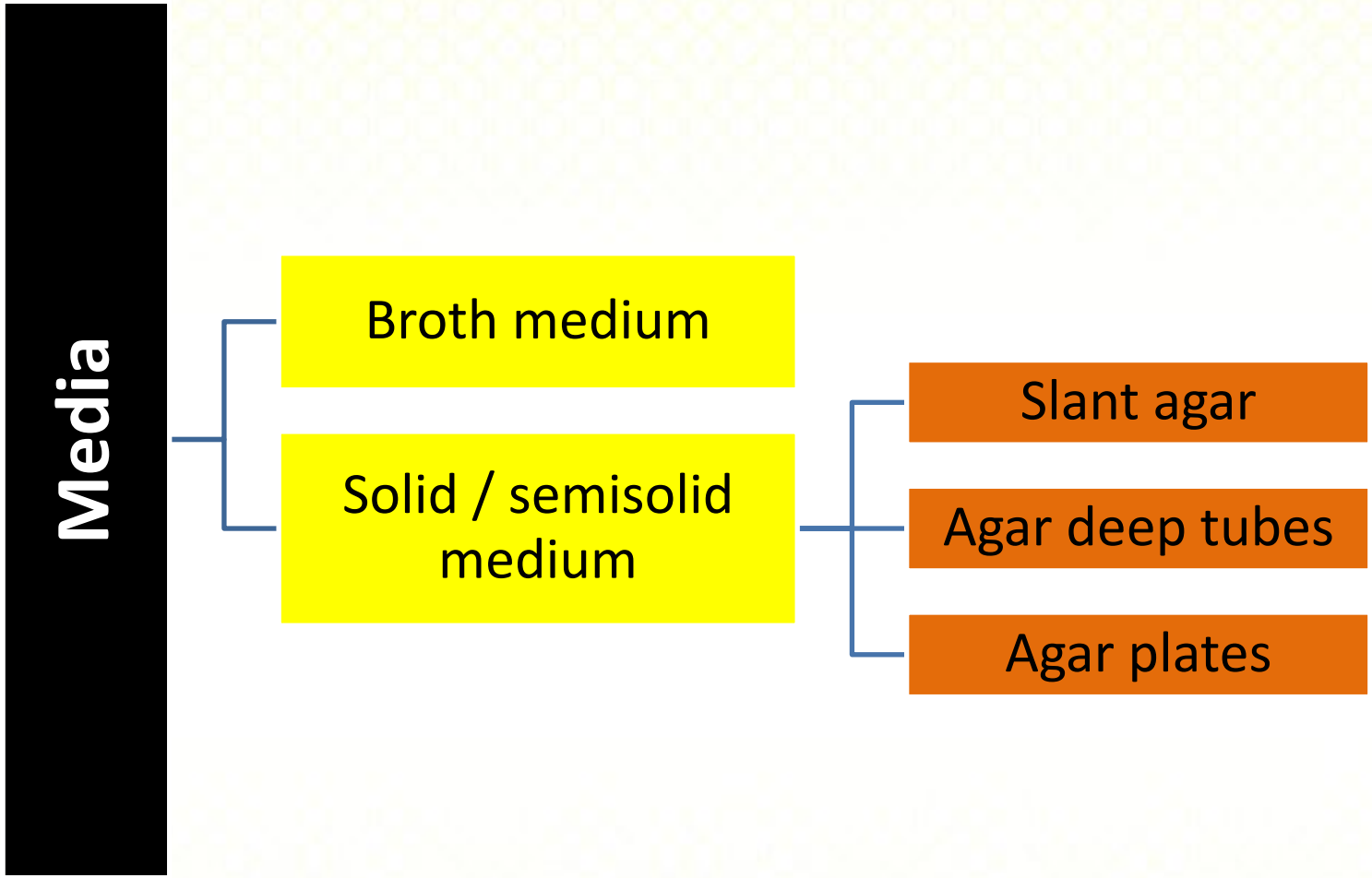
Culture Techniques



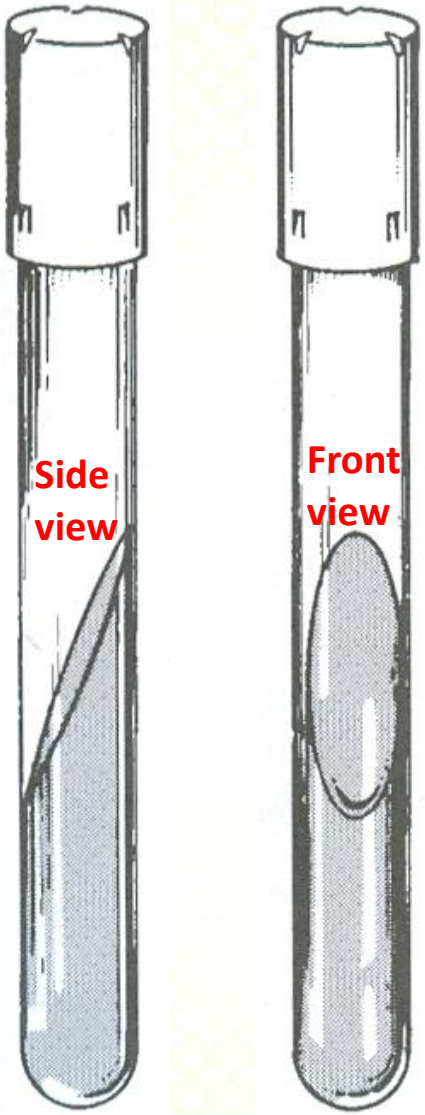
What will you need to do?

- Prepare growth media
- Know your culture tubes/vessel and Petri dishes
- Perform sterilization
- Know your microbial transfer instruments
- Perform microbial transfer aseptically

Growth Media

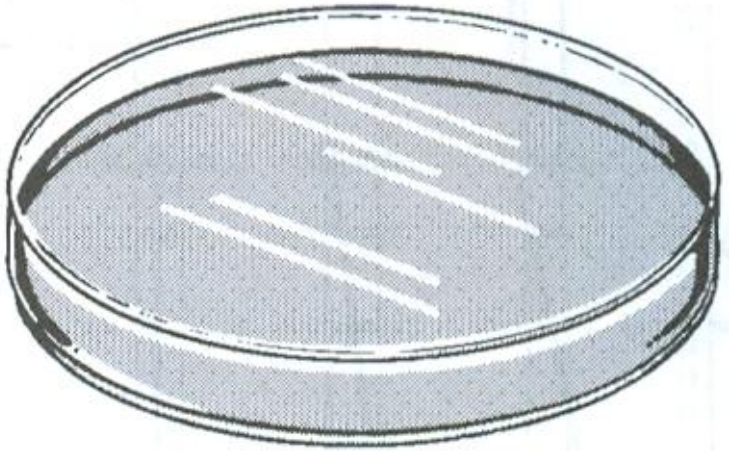


Slant agar

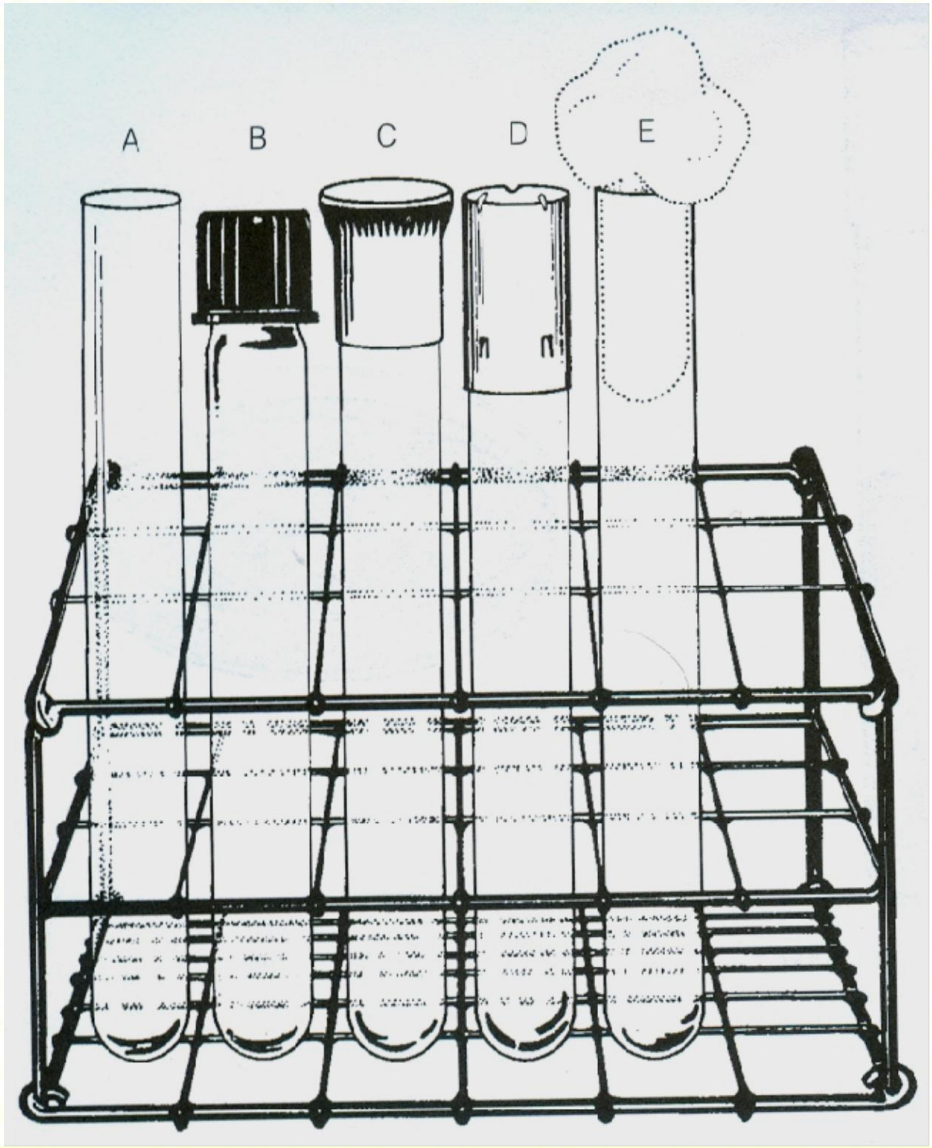


Agar deep tube

Agar plate

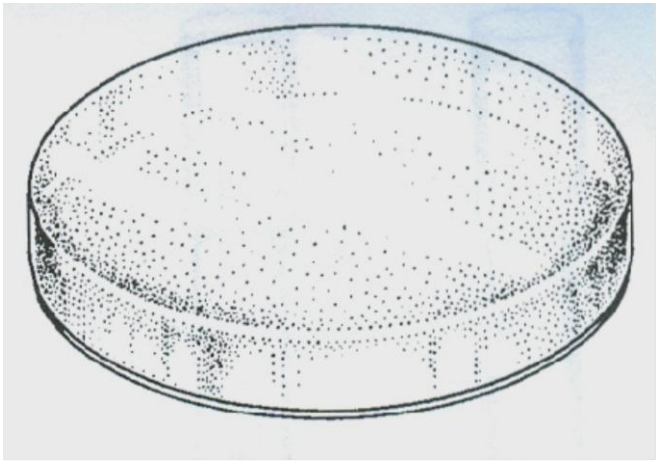


Culture vessels and plate

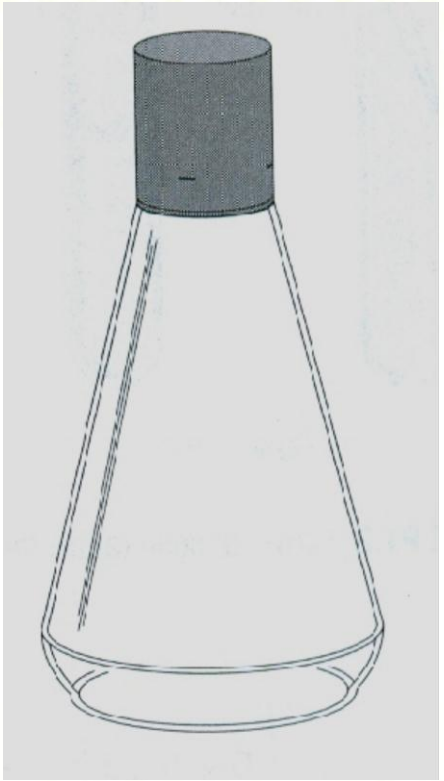


A Bacteriological tube
B Screw cap
C Plastic closure
D Metal closure
E Nonabsorbent cotton

Culture vessels and plate



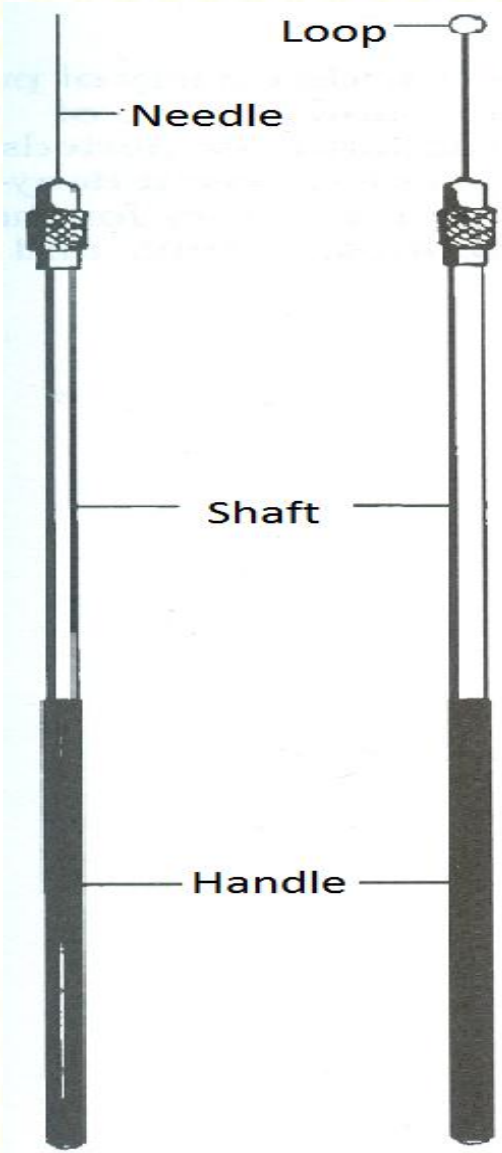
Petri dish



Conical flask

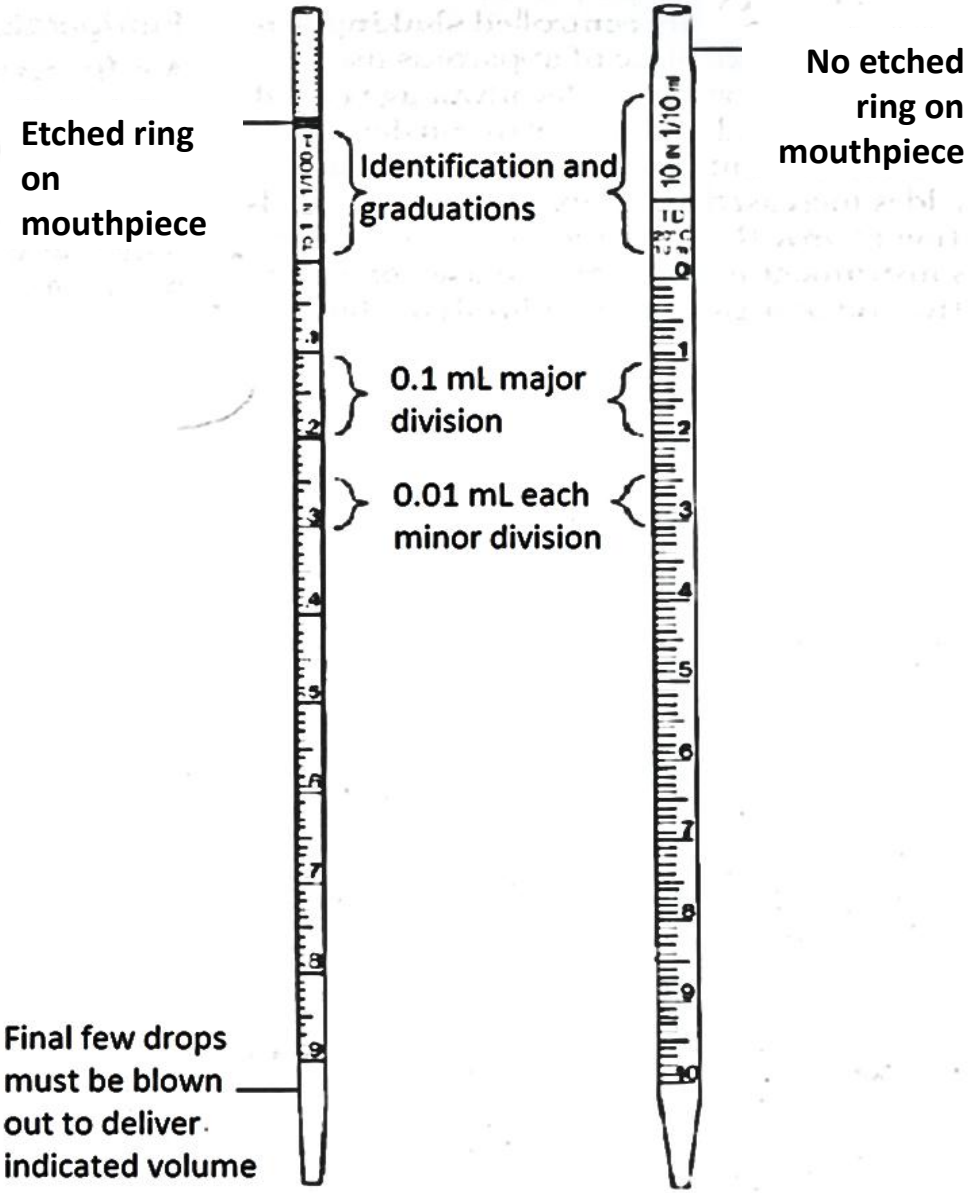
Transfer instruments

Transfer needle

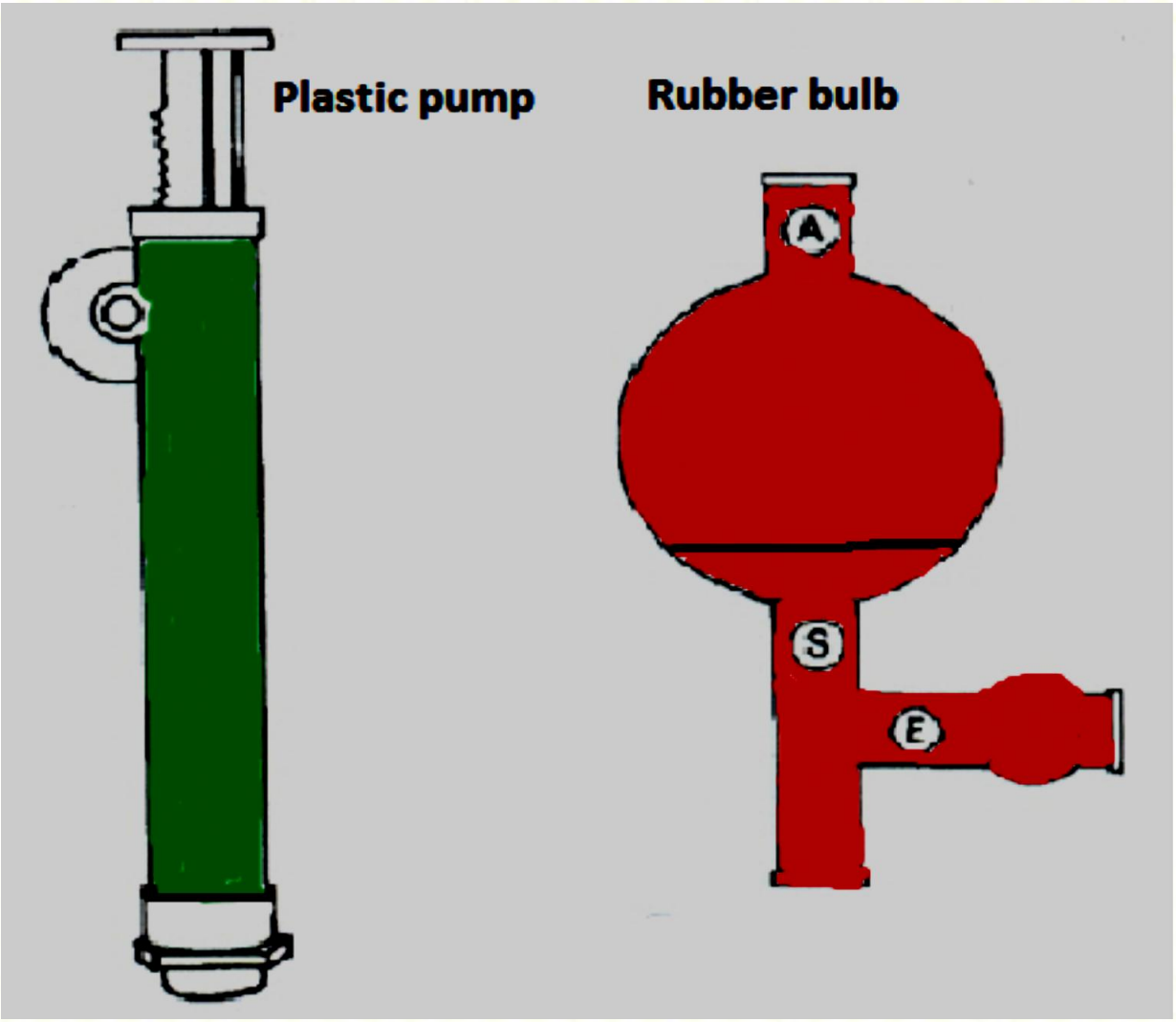


Transfer loop

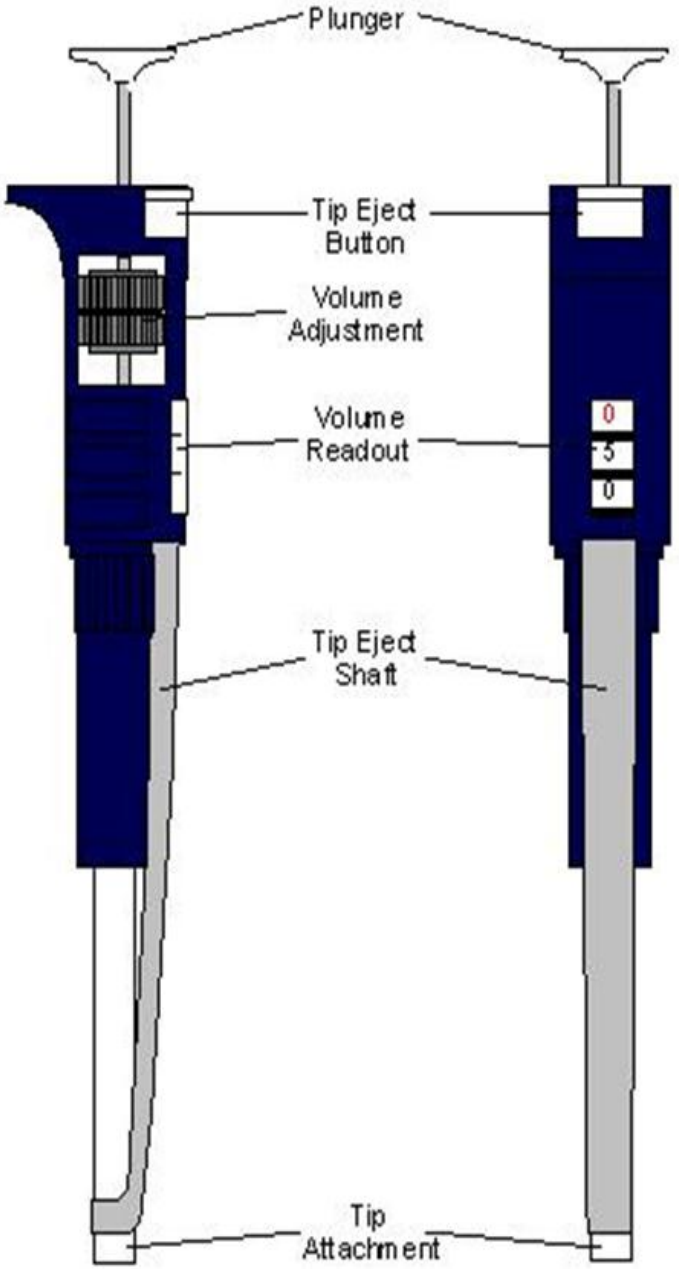
Transfer instruments



Transfer instruments



Mechanical pipette aspirator

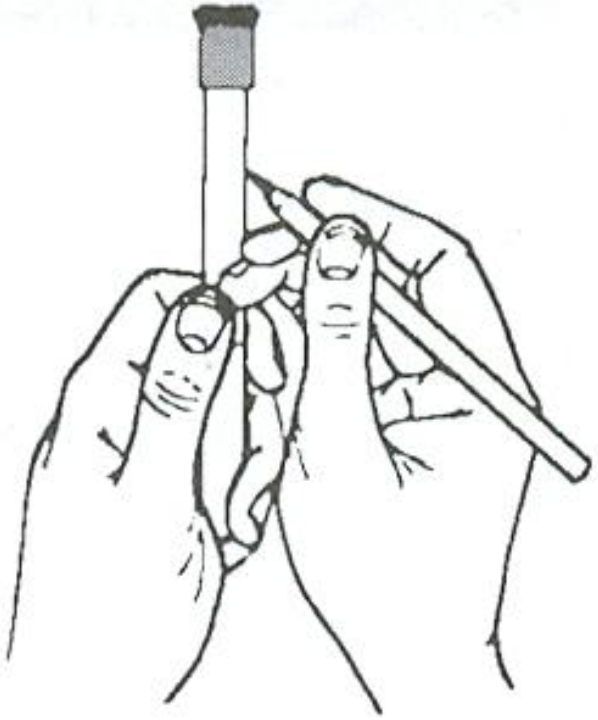


Side view Front view

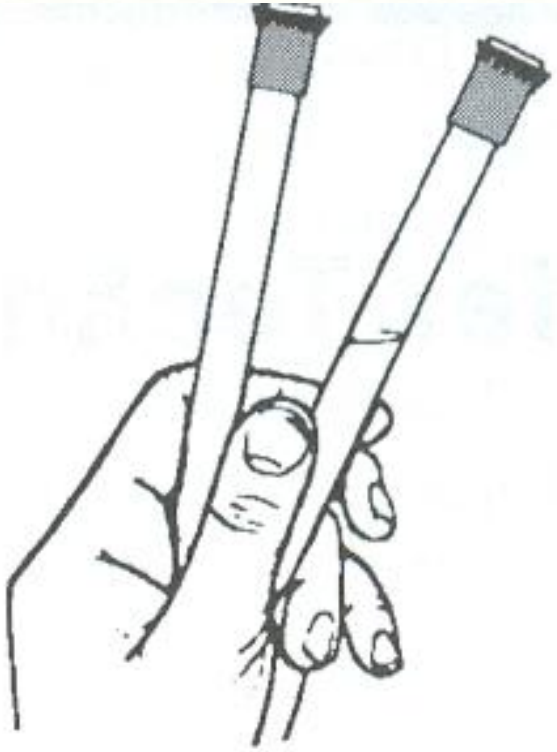
Micropipette

1. Micropipettes have 3 positions:
   - Rest position
   - First stop
   - Second stop
2. Fit the tip to the end of the shaft. Press down and twist slightly to ensure an airtight seal.
3. Hold the pipette in a vertical position. Depress the plunger to the first stop. Air equal to the volume of the setting (e.g. 100 mL) is displaced.
4. Immerse the tip into the liquid. Release the plunger back to the rest position. Wait a second for liquid to be sucked up into the tip. The volume of liquid in the tip will equal the volume of the setting of the micropipette.
5. Place the tip at an angle (~ 45°) against the wall of the vessel receiving the liquid. Depress the plunger to the first stop, wait one second, press the plunger to the second stop to expel all the liquid
6. Move the end of the tip away from the liquid. Release the plunger to the rest position.

Aseptic transfer of microbe



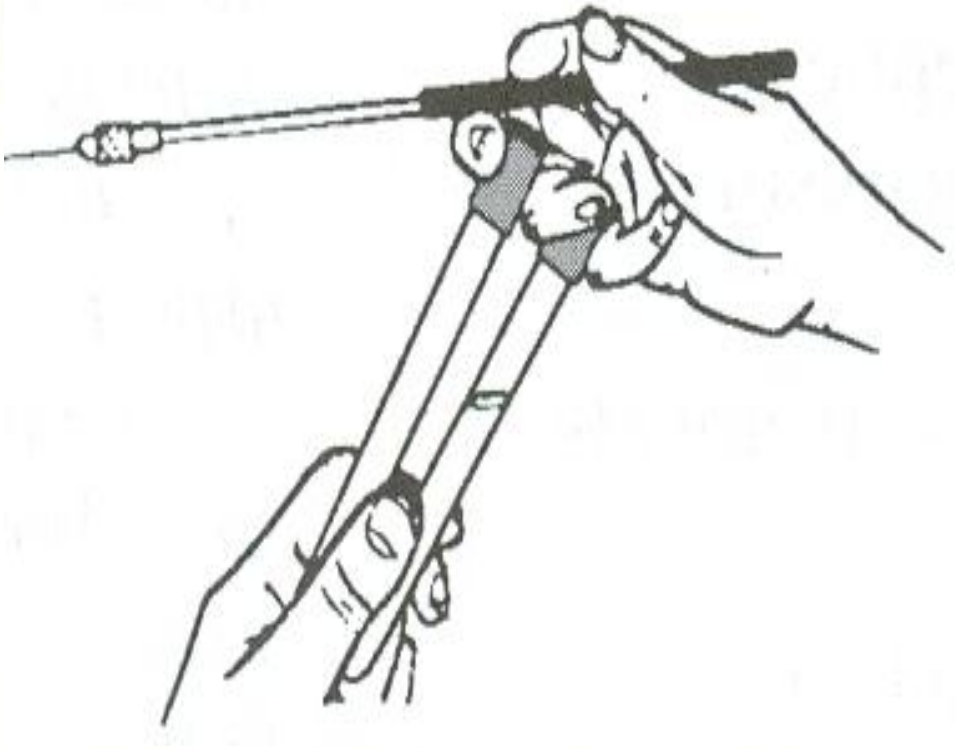
1. Label the tube to be inoculated with the name of the organism and your initial



2. Place the tubes in your palm, secure with your thumb and separate to form a ‘V’ shape

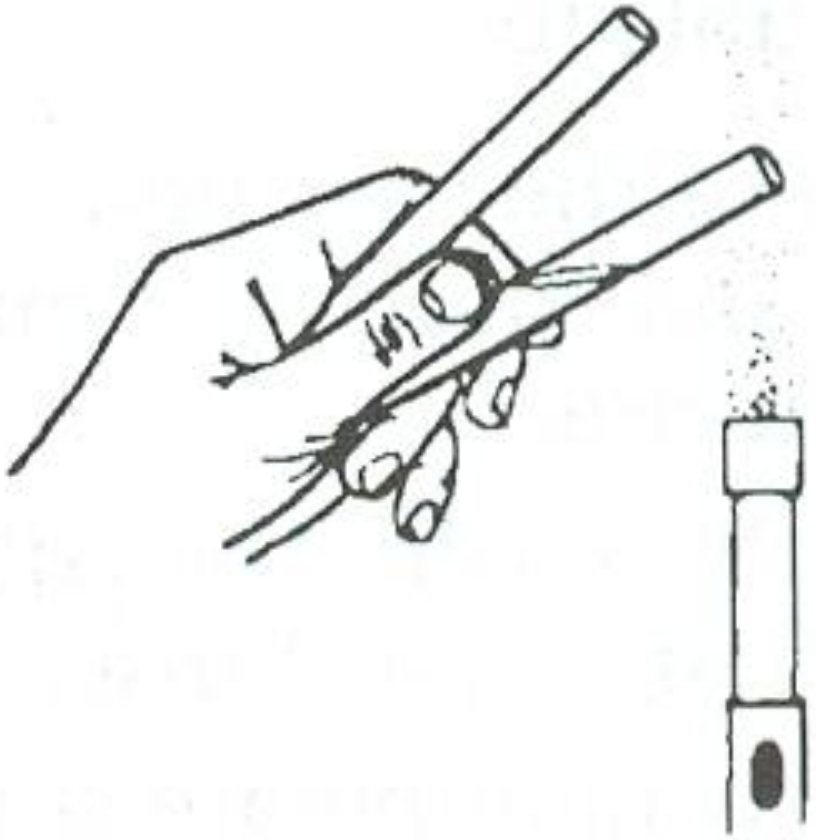
Aseptic transfer of microbe

3. Flame the needle or loop until the entire wire is red-hot



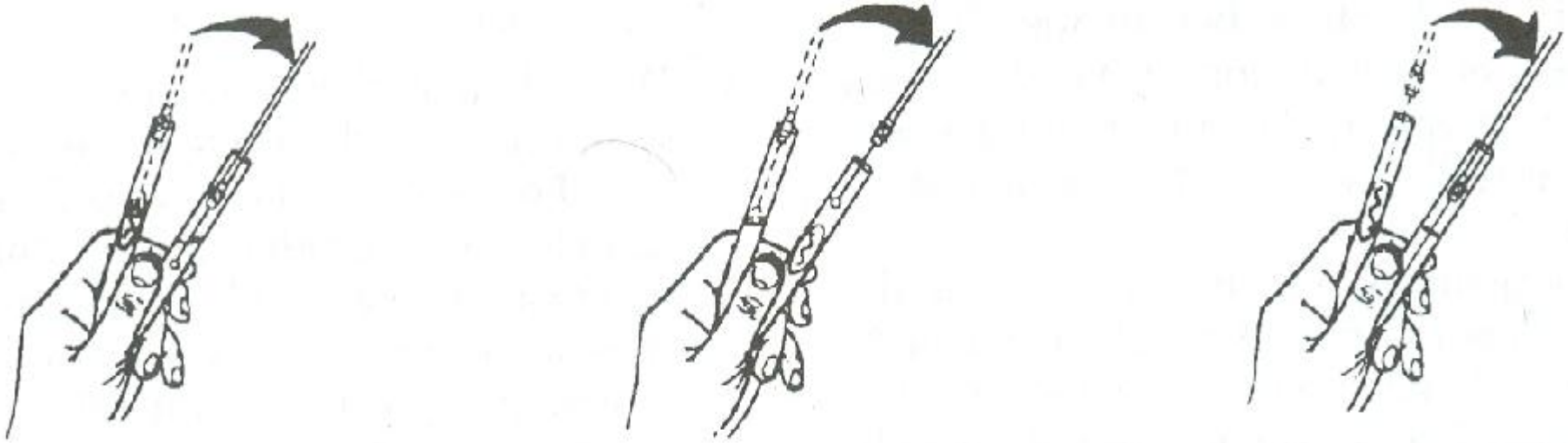
4. With the sterile loop or needle still in one hand, use another hand to uncap the tubes

Aseptic transfer of microbe



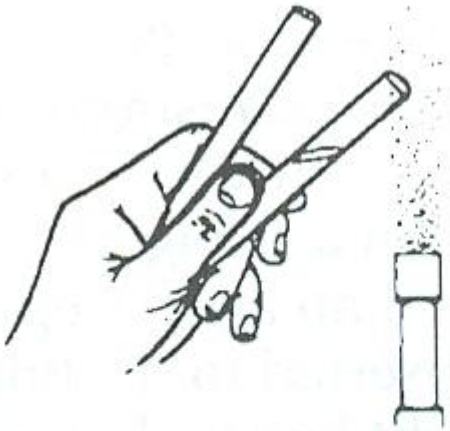
5. Flame the necks of the tubes by rapidly passing them through the flame once or twice.

Aseptic transfer of microbe

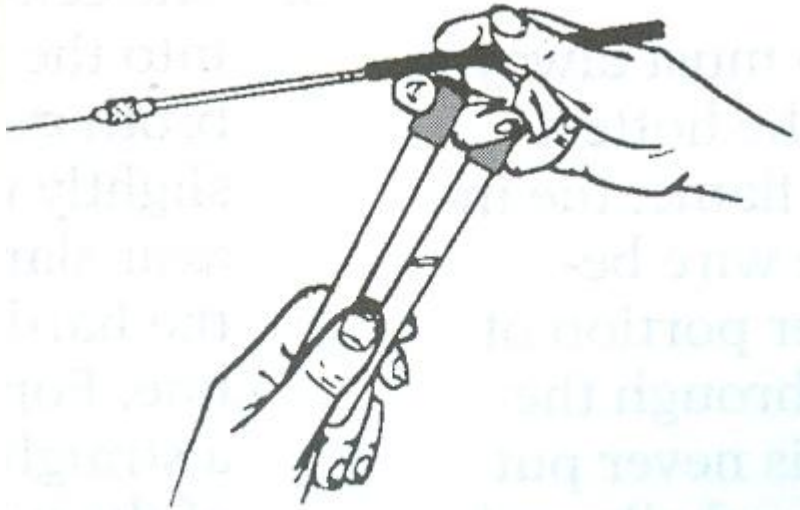


Slant-to-broth transfer: Dislodge inoculum by slight agitation. Broth-to-slant transfer: Following insertion to base of slant, withdraw to loop in a zigzag motion. Slant-to-agar deep transfer: Insert the needle to the bottom of the tube and withdraw along the line of insertion

Aseptic transfer of microbe

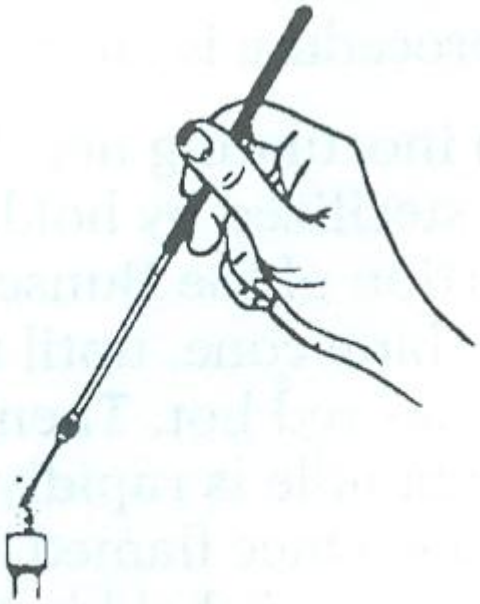


(g) Flame the necks of the tubes by rapidly passing them through the flame.

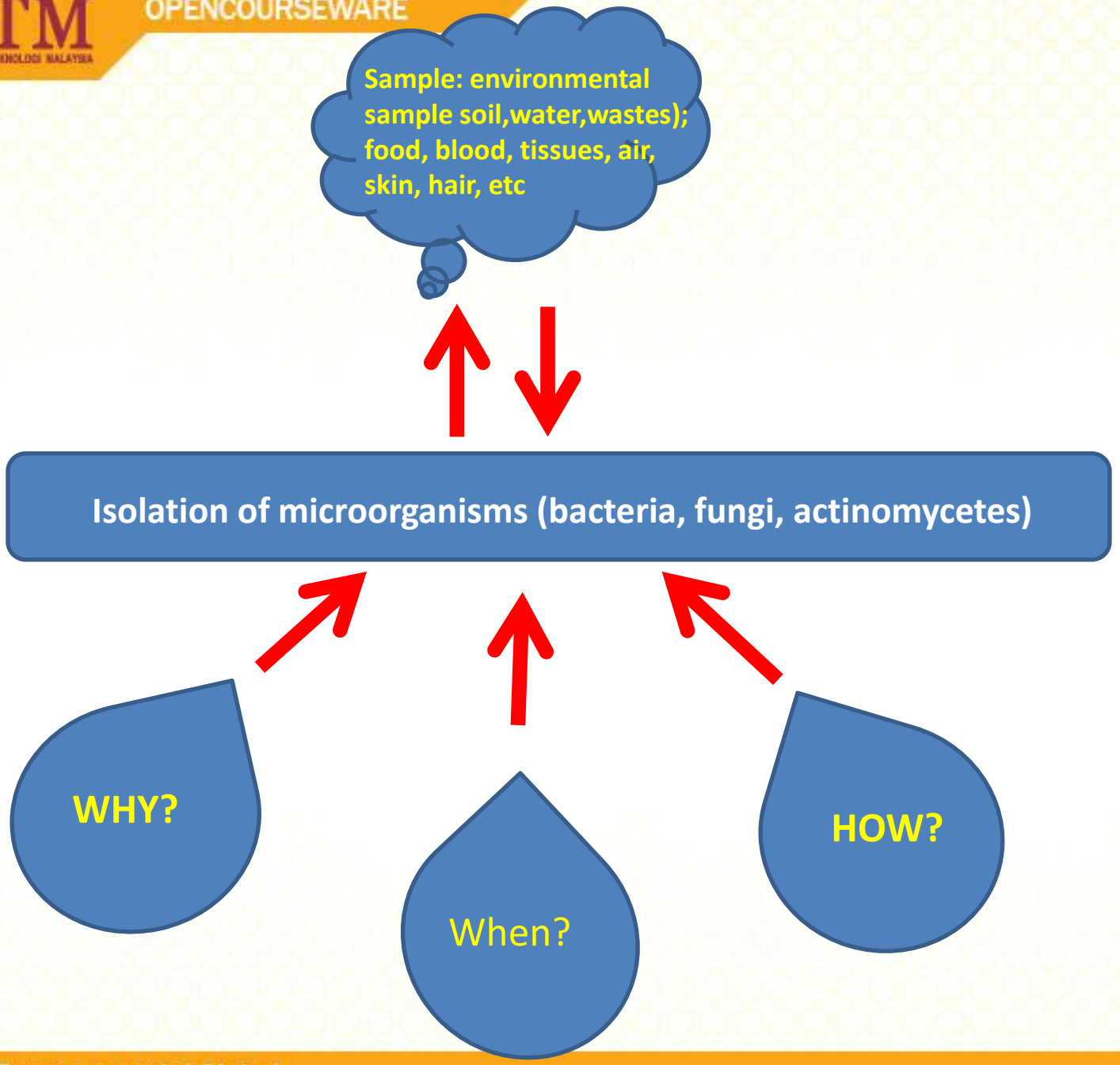


(h) Recap the tubes.

Aseptic transfer of microbe



(i) Reflame the loop or needle.


Sample: environmental sample soil,water,wastes); food, blood, tissues, air, skin, hair, etc
Isolation of microorganisms (bacteria, fungi, actinomycetes)
WHY?
When?
HOW?