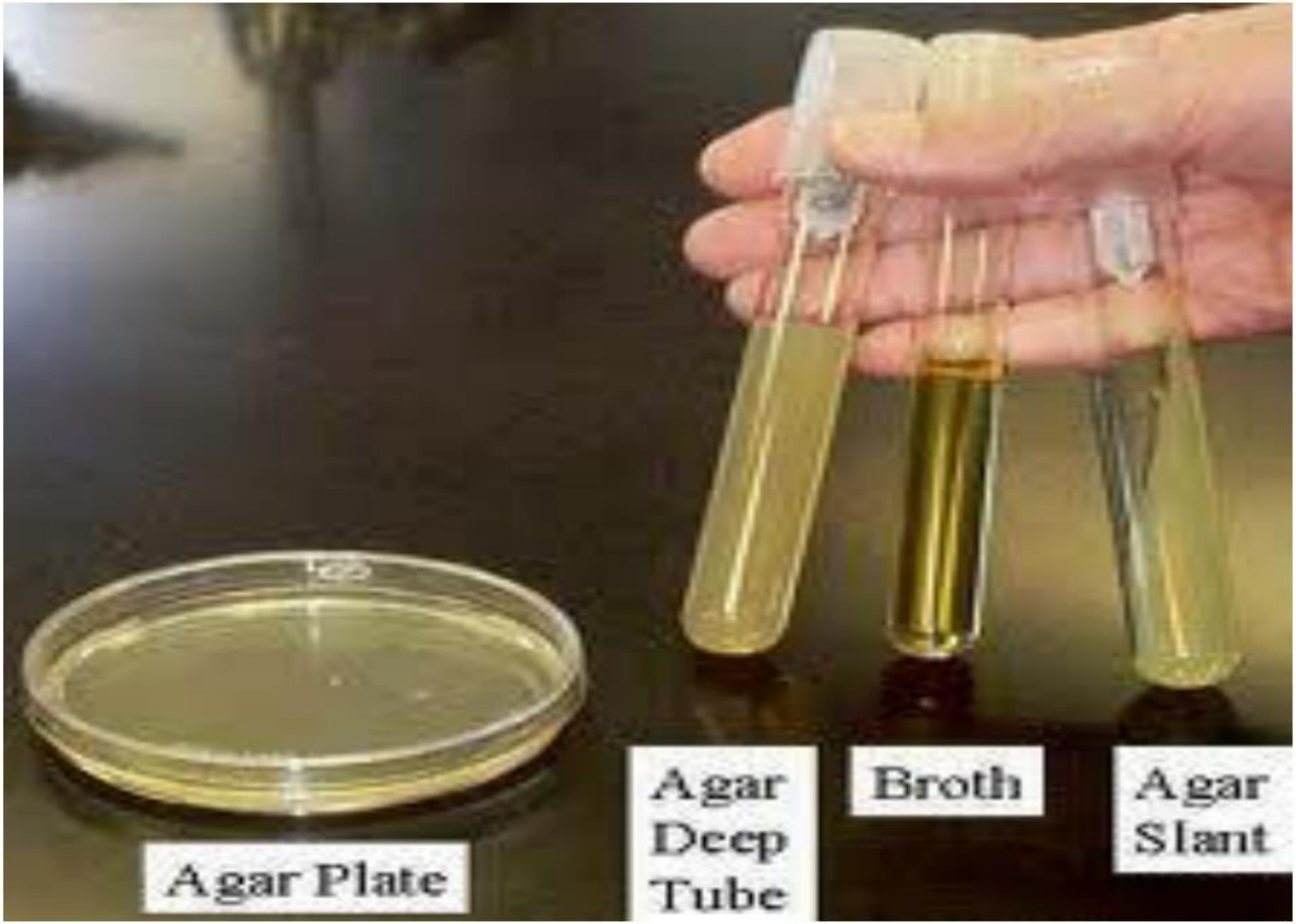
How to inoculate culture media

Inoculation

- From Latin word "Inoculare" which means to implant or to introduce.
- It means to implant or introduce microorganisms or infectious material into a culture medium for growth of microorganisms.
- **Aseptic techniques** should always be followed while inoculating;

1. Media in petri dishes.
2. Slope media (Agar Slants).
3. Inoculation of stab media (deeps).
4. Inoculation of fluid media.


Agar Plate
Agar Deep Tube
Broth
Agar Slant

Aseptic Techniques

- Aseptic technique refers to a procedure that is performed under sterile conditions to prevent any contamination **e.g. use of flame.**

Aseptic procedures can be done in a number of ways:

1. Use of Bunsen burner or spirit lamp to sterilize wire loops/inoculating needles before and after use (when loop turns red hot it become sterilized).

2. Flame the necks of specimen bottles, culture tubes after removing and before replacing caps.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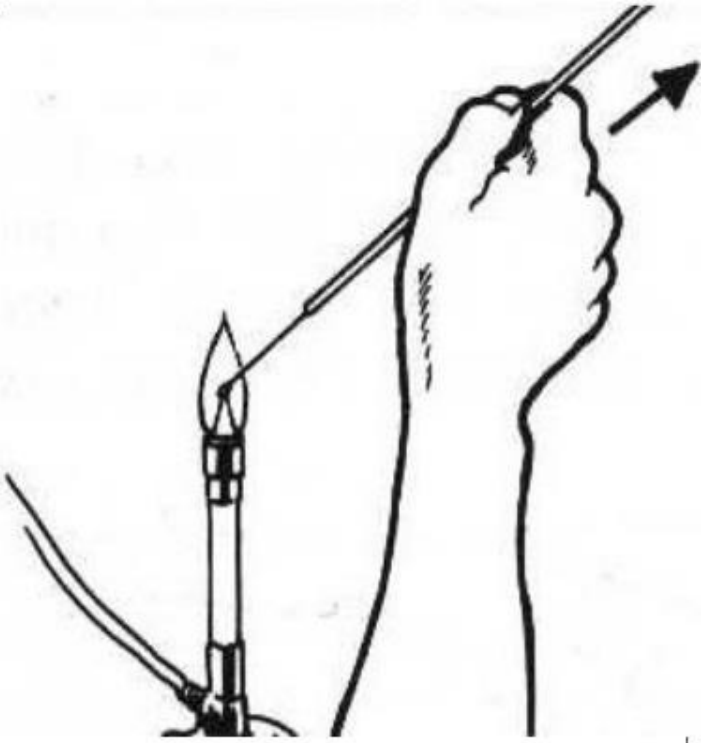


3. While inoculation, do not let the caps of tubes to touch an unsterile surface. This can be avoided by holding the cap in the hand.

4. Decontaminate the work place before starting the day's work and after finishing (by using 70% ethanol or spirit etc).

5. Use of a safety cabinet when working with hazardous pathogens.

6. Wear protective clothing, wash the hands after handling infected material and never mouth pipette any solvent, eat, drink in the laboratory.

Aseptic Removal of Microorganisms from a Broth Culture

A. Sterilize the loop.

B. Remove the cap of the broth culture.

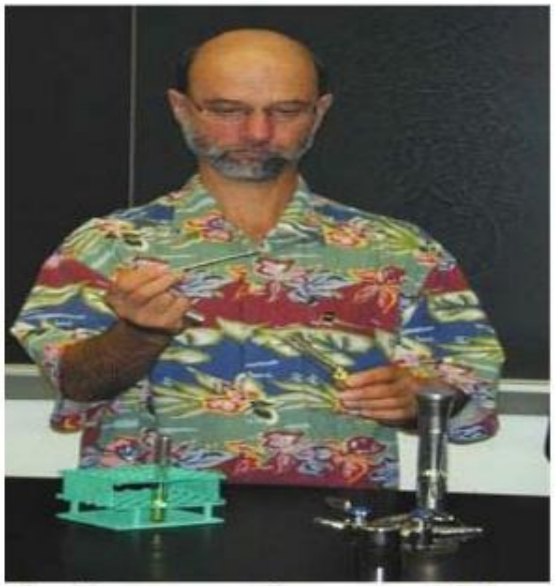
C. Pass the lip of the tube through the flame.

D. Remove the inoculum.

E. Pass the lip of the tube through the flame.

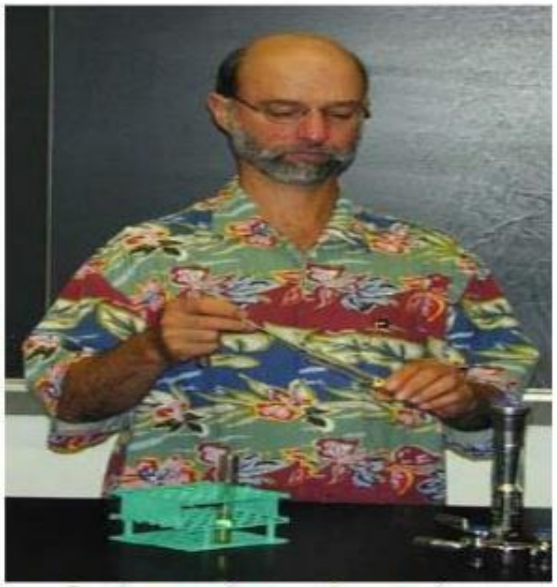
F. Replace the cap.

Transferring Microorganisms into a Broth Tube



A. Remove the cap of the broth tube.

B. Pass the lip of the tube through the flame.



C. Inoculate the tube.

D. Pass the lip of the tube through the flame.

E. Replace the cap.

F. Resterilize the loop.

Aseptic Removal of Microorganisms from a Plate Culture

A. Sterilize the loop.

B. Scrape off a small amount of the organisms and close the lid.

1. Inoculation of media in petri dishes

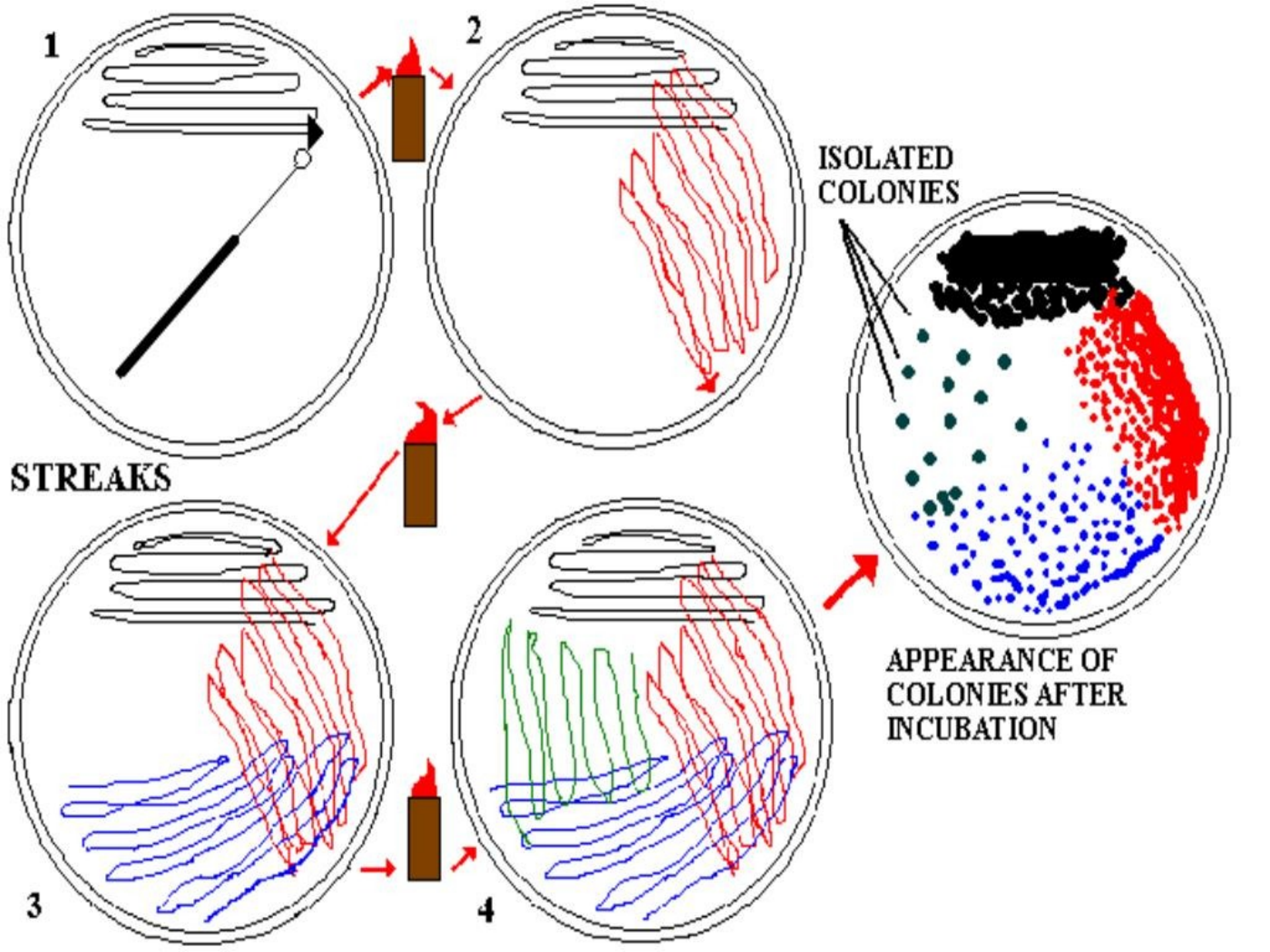
- Plating out or looping out is the term used for inoculating media in a petri dish.
- Using a sterile loop, apply the inoculum to a small area of the plate (the 'well').
- Flame sterilize the loop. When cool, spread the inoculum. ***Each time flame sterilize the loop.***

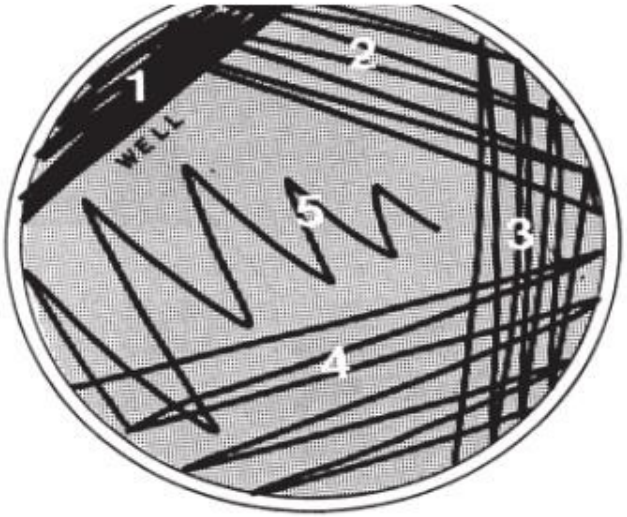
- The technique used to inoculate media in petri dishes (plates) must:

a. Provide single colonies for identification.

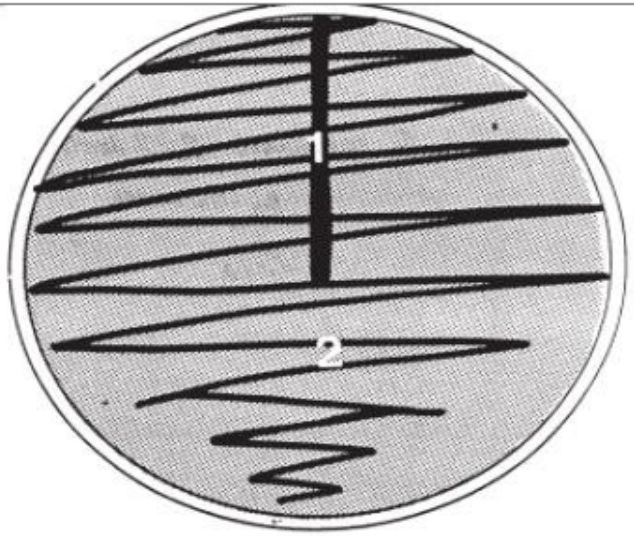
b. It must also show whether a culture is pure or mixed, i.e. consisting of a single type of organism or several different organisms.

- A pathogen must be isolated in pure culture before it can be identified and tested for antimicrobial sensitivity.

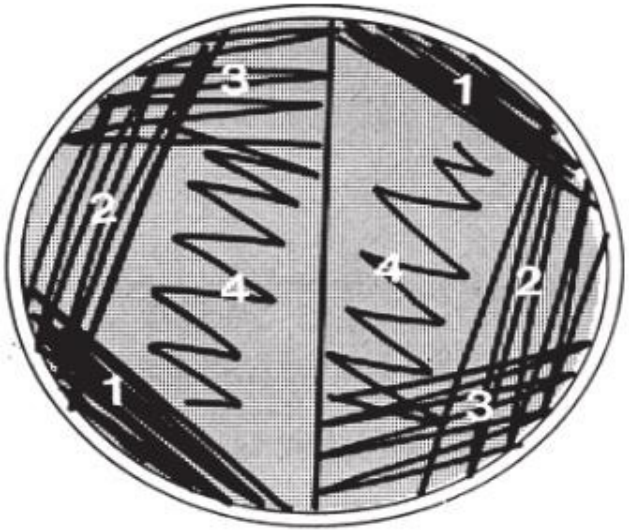

1
2
ISOLATED
COLONIES
STREAKS
3
4
APPEARANCE OF
COLONIES AFTER
INCUBATION



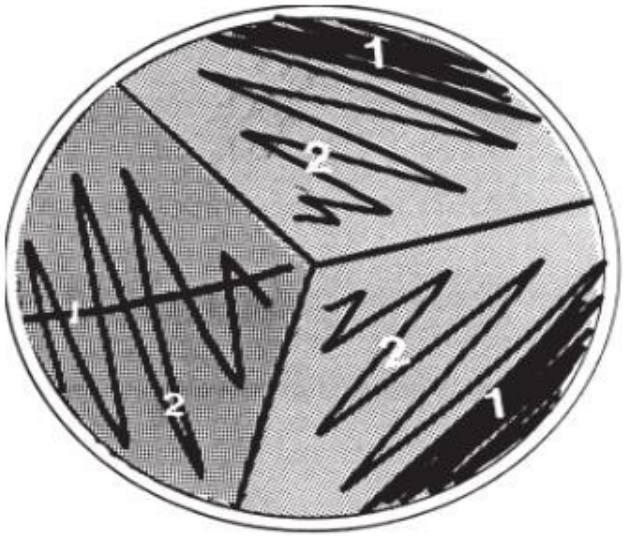
Inoculation of a plate of culture medium to give single colonies



Simplified technique of inoculating a plate of culture medium



Inoculation of half a plate of culture medium

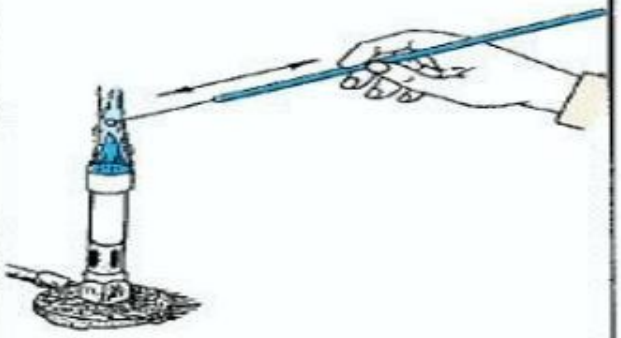


Different ways of inoculating a third of a plate of culture medium

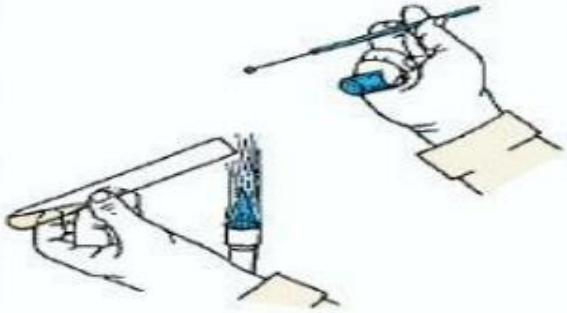
2. Inoculation of Slopes

- To inoculate slopes such as Dorset egg medium or Loeffler serum, use a sterile straight wire streak the inoculum down the centre of the slope and then pull the wire and spread the inoculum in a zigzag pattern on the slope surface.

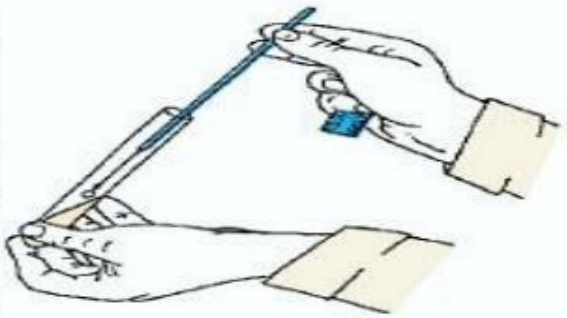
- To inoculate a slope (slant) and butt medium, such as triple sugar iron agar, use a sterile straight wire to stab into the butt first and then use the same wire to streak the slope in a zig-zag pattern.



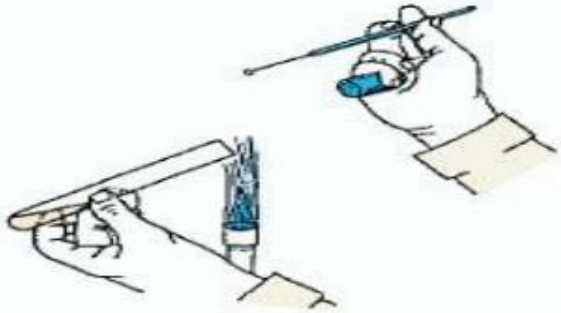
1 Inoculating loop is heated until it is red-hot.



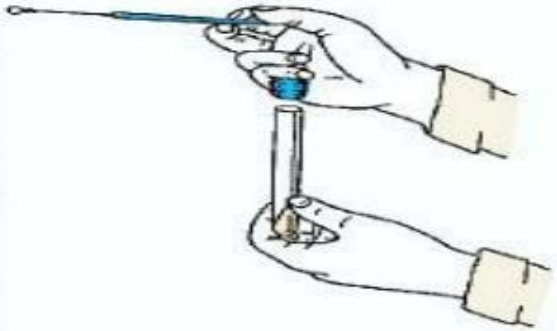
2 Cap is removed from slant culture and tube mouth is heated.



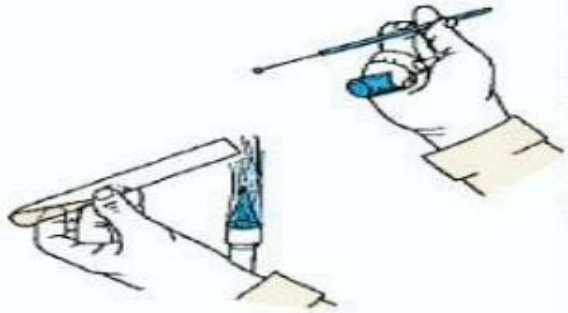
3 Organism is picked up from slant with inoculating loop.



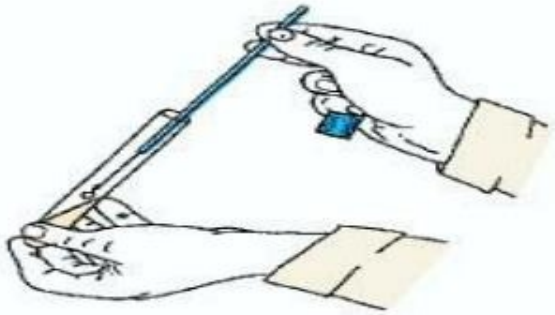
4 Mouth of tube is flamed. Inoculating loop is not flamed.



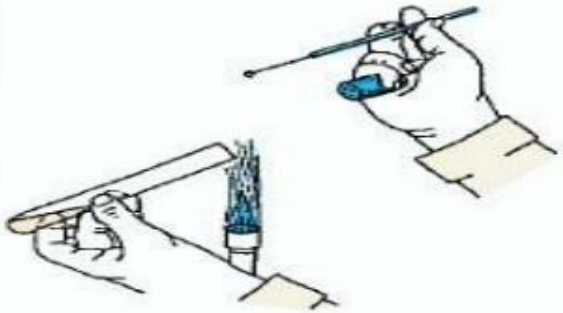
5 Slant culture is re-capped and retruned to test tube rack.



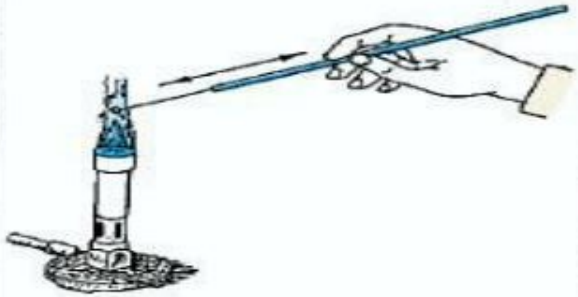
6 Tube of sterile agar slant is uncapped and mouth is flamed.



7 Slant surface is streaked with unflamed loop in serpentine manner.

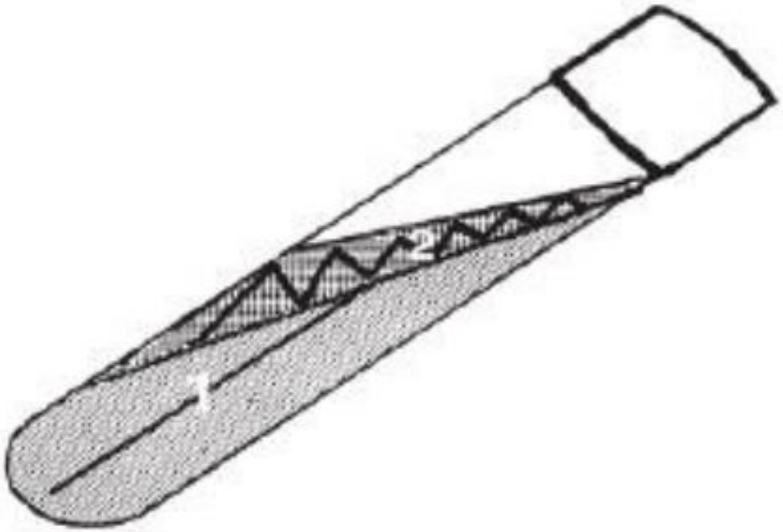


8 Tube mouth is flamed, recapped and incubated.

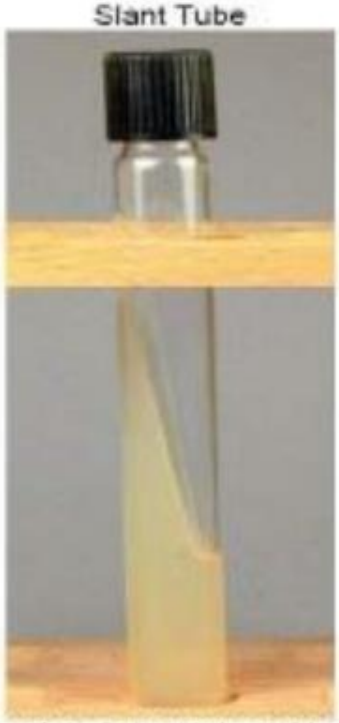


9 Loop is flamed red-hot and returned to receptacle.

Inoculation of an agar slope



Inoculation of a butt and slope. Use a straight line to inoculate the butt first

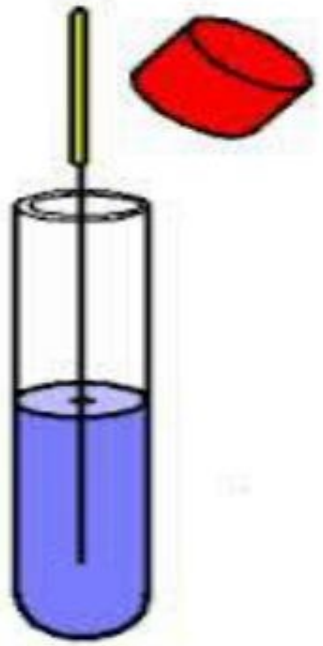


Uninoculated slant tube (side view)

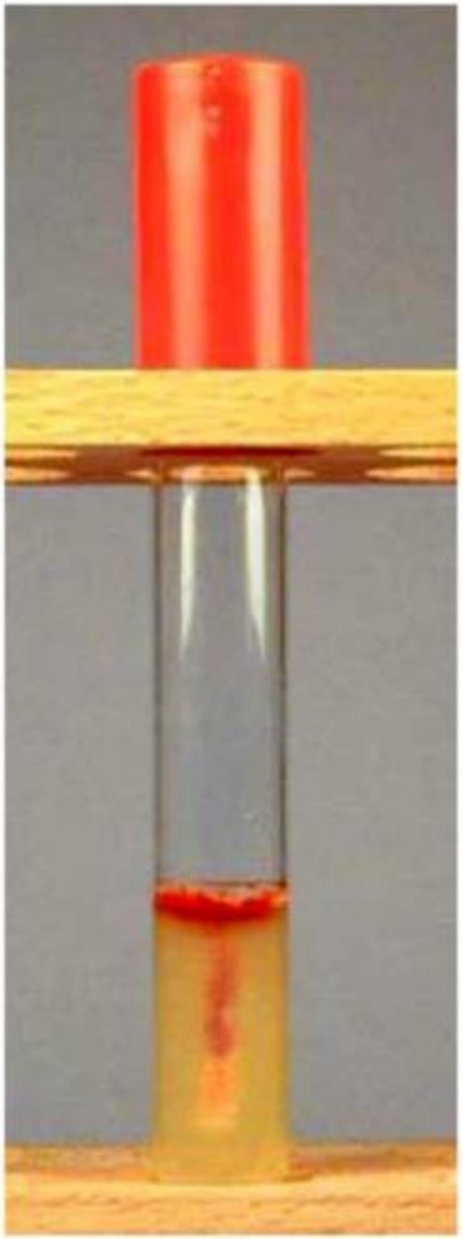
Bacterial Growth on a Slant tube

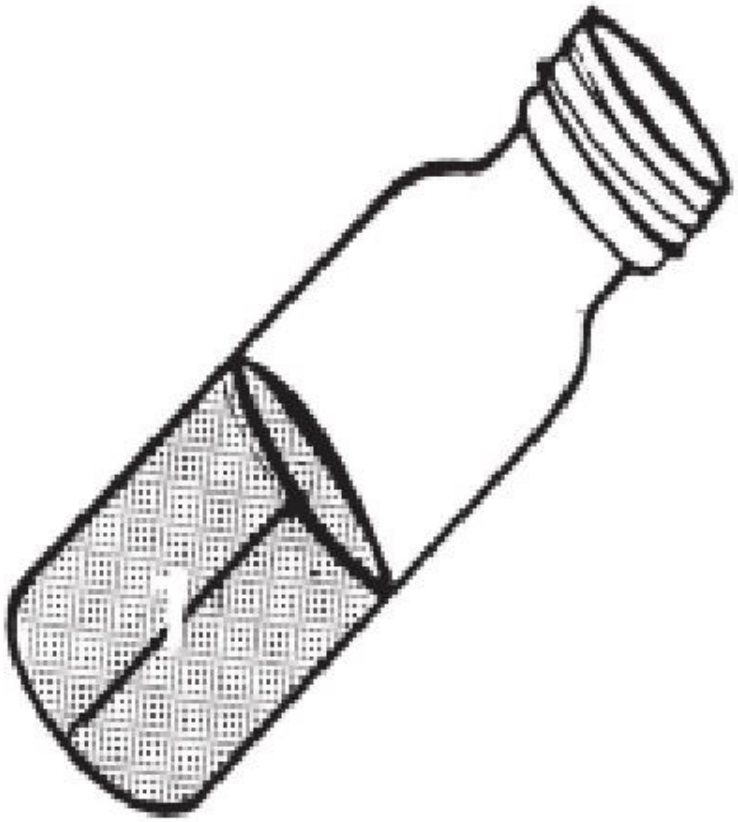
3. Inoculation of Stab media (deep tube)

- Use a sterile straight wire to inoculate a stab medium.
- Stab through the centre of the medium taking care to withdraw the wire along the line of inoculum without making further stab lines.



Bacterial Growth in a Stab Tube



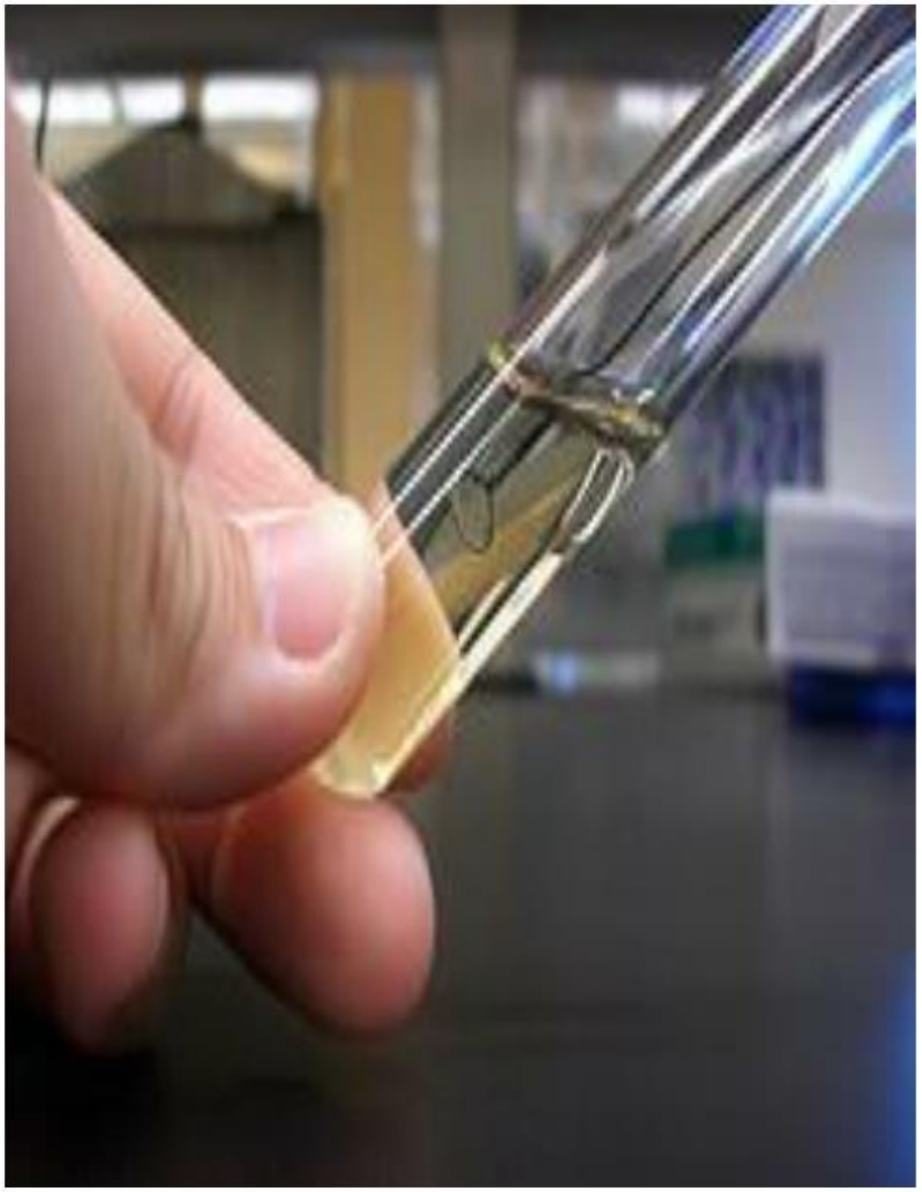


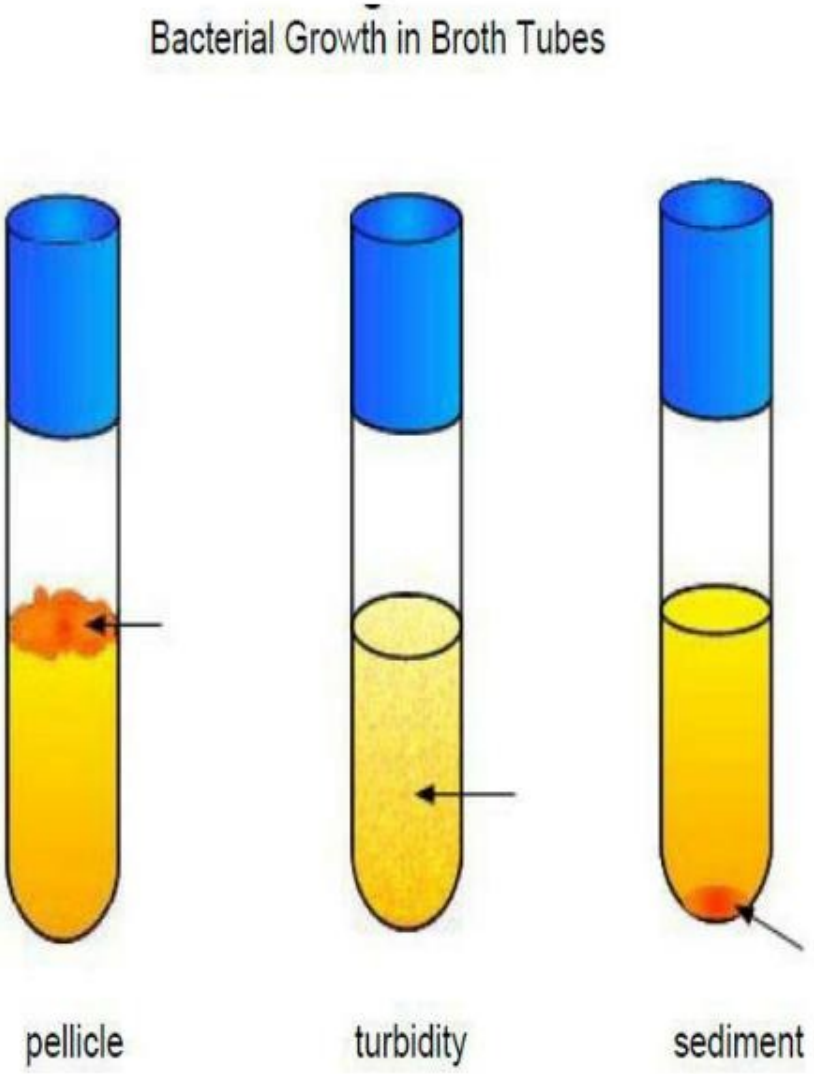
4. Inoculation of fluid media

- Using a sterile wire loop, obtain a sample of microbial culture (a small amount of bacterial colony).

- Hold the bottle or tube to be inoculated at an angle and rub the loop against the side of the container below the level of the fluid.

- Growth can be observed in the inoculated tube as turbidity or milky appearance after incubation at 37^0C for 24 hours.




Bacterial Growth in Broth Tubes
pellicle
turbidity
sediment

Inoculated Broth (Turbid)

Un-inoculated Broth (No Growth)