



Inoculation, incubation and pure culture techniques

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Objectives:-

1-Techniques of culturing microorganism.

a-inoculation

b-incubation

c-isolation and identification

2-Types of instruments used for inoculation

3-Types of inoculation

4-Pure culture techniques(streak plate method)

Techniques of culturing microorganisms

A. Inoculation

B. Incubation

C. Isolation and Identification

Inoculation of bacteria

Spreading the sample on the surface of a solid medium -

The purpose is to: -

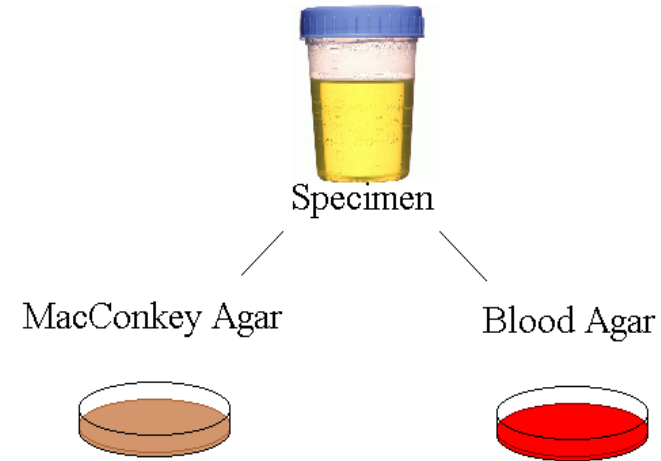
Transfer bacteria from the sample to medium -

Isolate a pure colony of bacteria -



Inoculation done from:-

1-**clinical specimens** (Blood , fluid, urine...)
For isolating microorganisms that cause diseases (primary culture).



2- **A culture** that contain certain microorganisms to isolate for learning and study (subculture).



Incubation of cultures

The cultures are incubated to achieve required -
temperature and growth environment for a length
of time

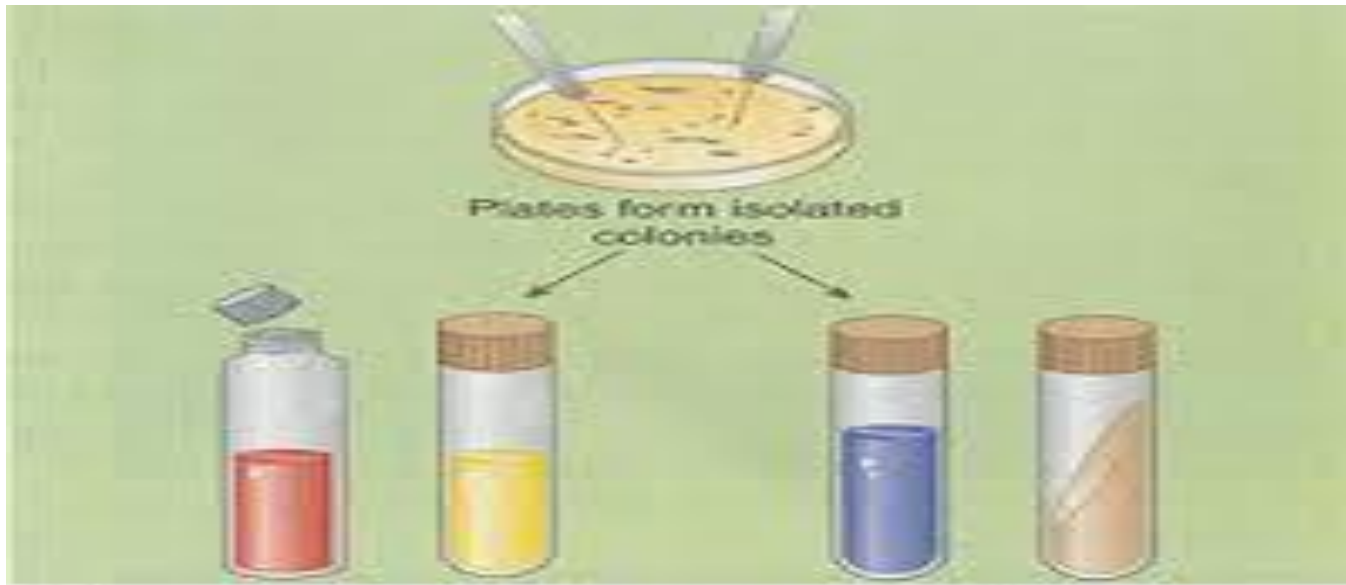
Incubator (O₂ and CO₂) -

Anaerobic Jar -



Isolation & Identification

- Isolation is done to obtain pure bacterial cultures.
- Identification is a careful process that uses many different techniques to narrow down the types of bacteria



Colony:-

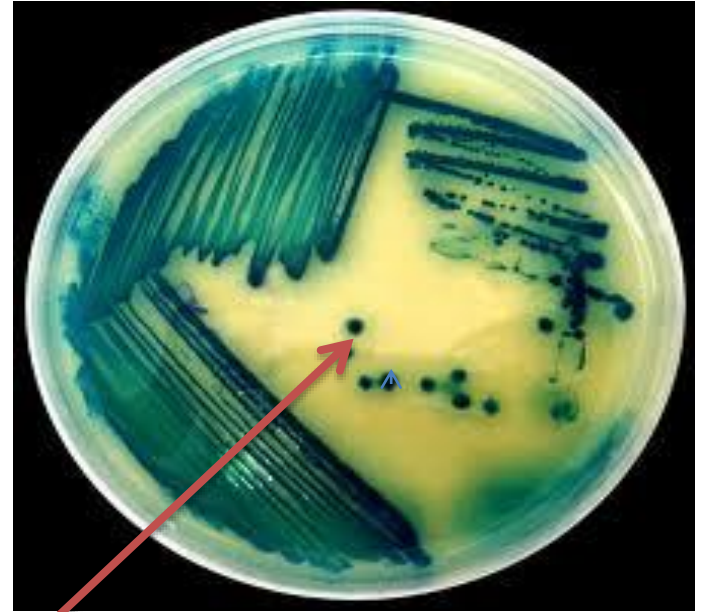
Is defined as a visible cluster of microorganisms growing on the surface of or within a solid medium, presumably cultured from a single cell.

Pure culture:-

A laboratory culture containing a single species of organism.

Mixed culture:-

presence of two or more types of organisms.

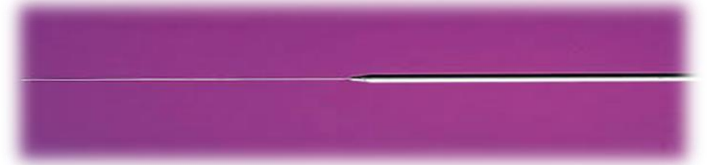


Types of instruments used for inoculation

❖ **Inoculation loop:** Nichrome wire loop, Platinum wire loop, Sterile loops



❖ **Straight wire**



❖ **Pasteur pipette**



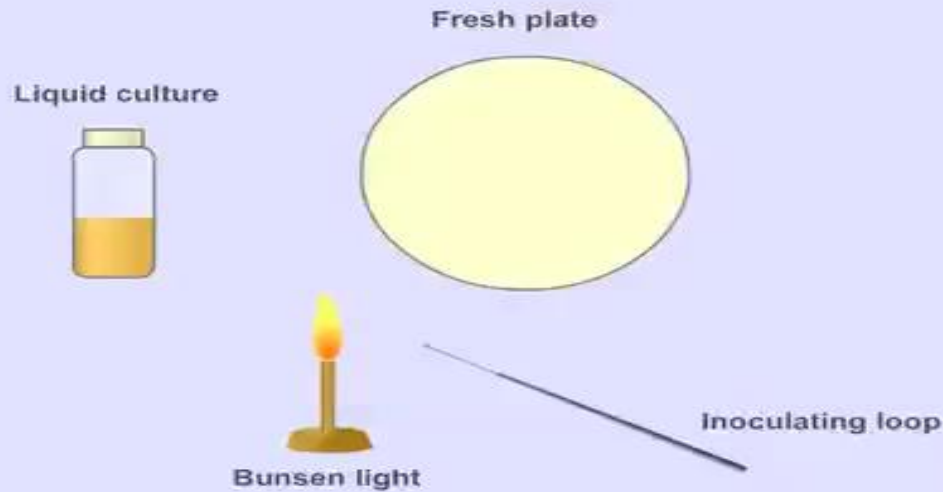
❖ **Sterile swab :** when taking samples from people or surfaces, a sterile swab is used to inoculate the petri dish.



Types of inoculation

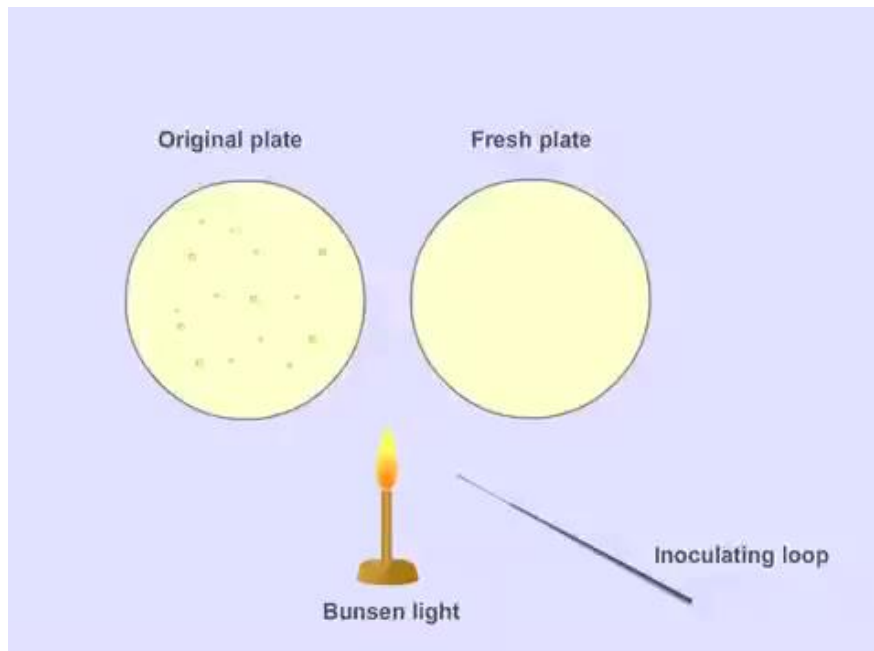
1-Inoculation of fluid media

Broth media are inoculated using sterile wire loop, or pasture pipette depending on whether the inoculum is colonial growth or a fluid specimen



2-Inoculation of solid media

Inoculation of solid media in Petri dishes (streak culture). This help for the isolation of bacteria in pure culture from clinical specimen.



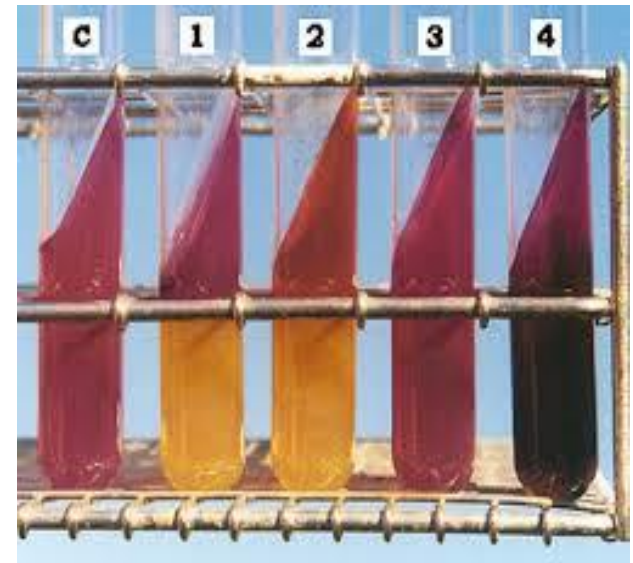
3-Inoculation of stab media (deep)

To maintain stock culture. A culture made by inserting an inoculating needle down the center of a solid medium contained in a test tube.



➤ 4-Inoculation of slopes (slant) media

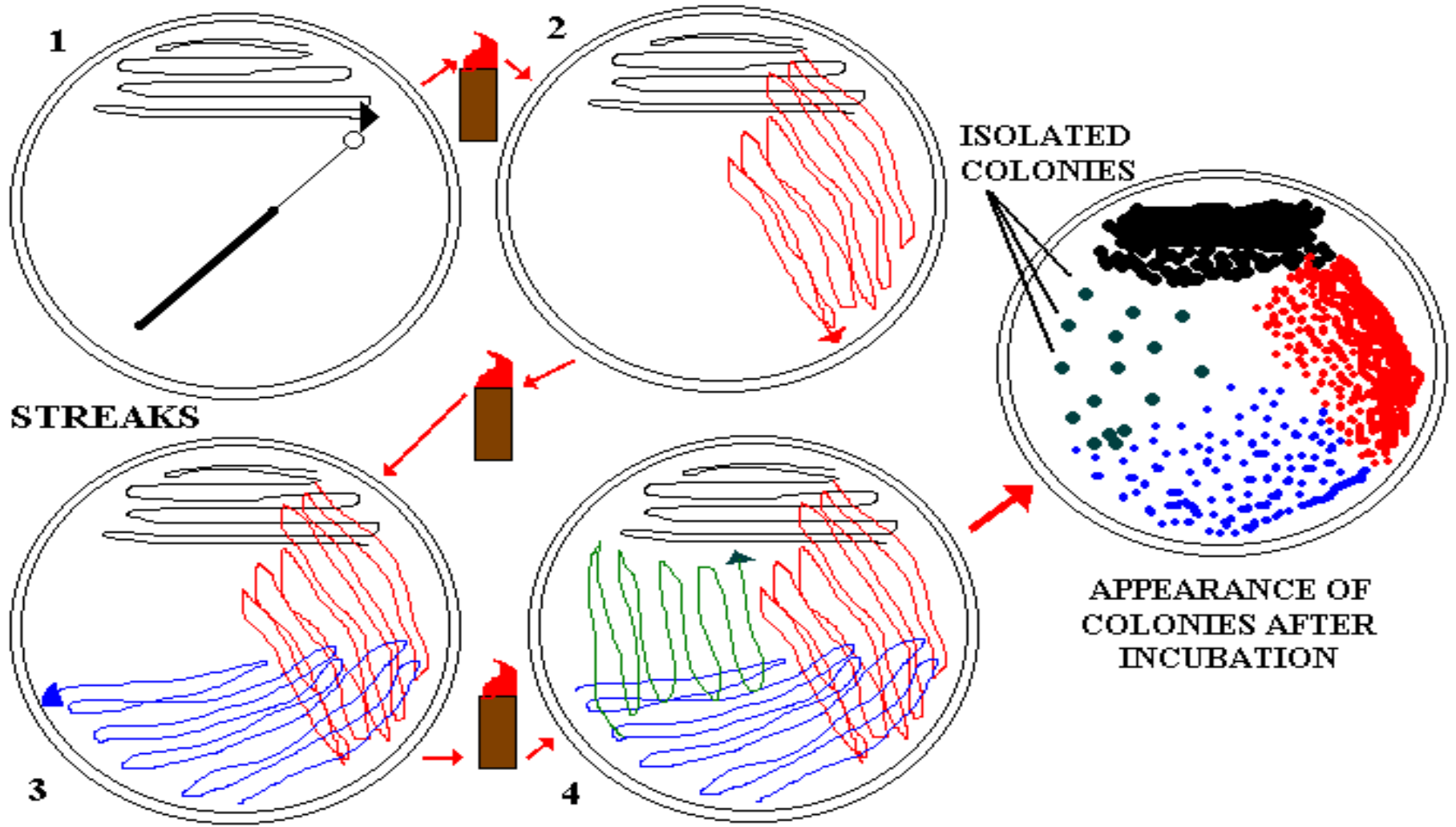
Is useful for storage because of the small surface area is not as easily contaminated and the tube do not dry out as fast as plates.



Pure culture techniques (streak plate method)

Streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. The technique of streak plate method consists of these steps:

- Sterile the **loop** and remove a **single colony** from a plate. Streak the inoculating loop over a quarter of the plate (**area 1**)
- Sterile loop again and going back to the edge of the area 1 and extend the streaks in to the **second quarter** of the plate (**area 2**) .
- Sterile loop again and going back to the edge of the area 2 and extend the streaks in to the **third quarter** of the plate (**area 3**) .
- Sterile loop again and going back to the edge of the area 3 and extend the streaks in to the **center fourth** of the plate (**area 4**).

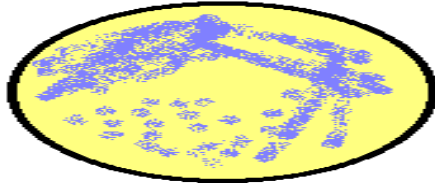


Developing Pure Cultures

Subculture From Isolation Streak to Obtain Pure Culture

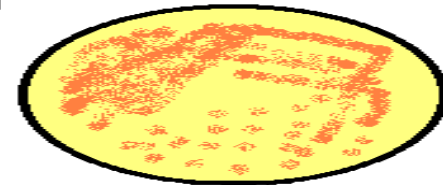
Harvest single colony type

(loop)



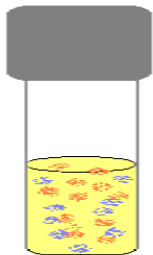
Harvest single colony type

(loop)



Pure culture
of each type

Isolation Streak

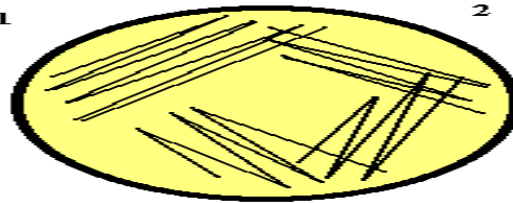


Mixed Broth
Culture
(2 types of
bacteria)

1

Loop

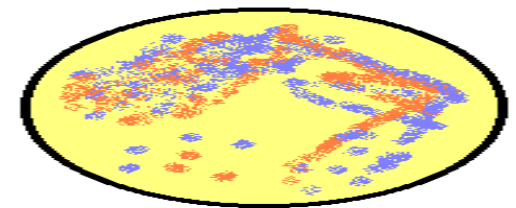
2



3

4

Isolation Streak
Showing path of loop



After 24 hours Growth
Mixed Culture is
separated into 2 colony
types

Supplies and materials Needed

- 1. Mixed culture of bacteria**
- 2. Nutrient agar plates**
- 3. Inoculating loop**
- 4. Bunsen burner**
- 5. Marking pen.**

References

- Gary W. Procop, Deirdre L. Church , et al. **2017. Koneman's Color Atlas and Textbook of Diagnostic Microbiology.** 7th Edition. Jones & Bartlett Learning
- Luis M. de la Maza, Marie T. Pezzlo, Cassiana E. Bittencourt, Ellena M. Peterson. **2020. Color Atlas of Medical Bacteriology.** (2020, Wiley) - libgen.lc.