Cultivation of bacteria

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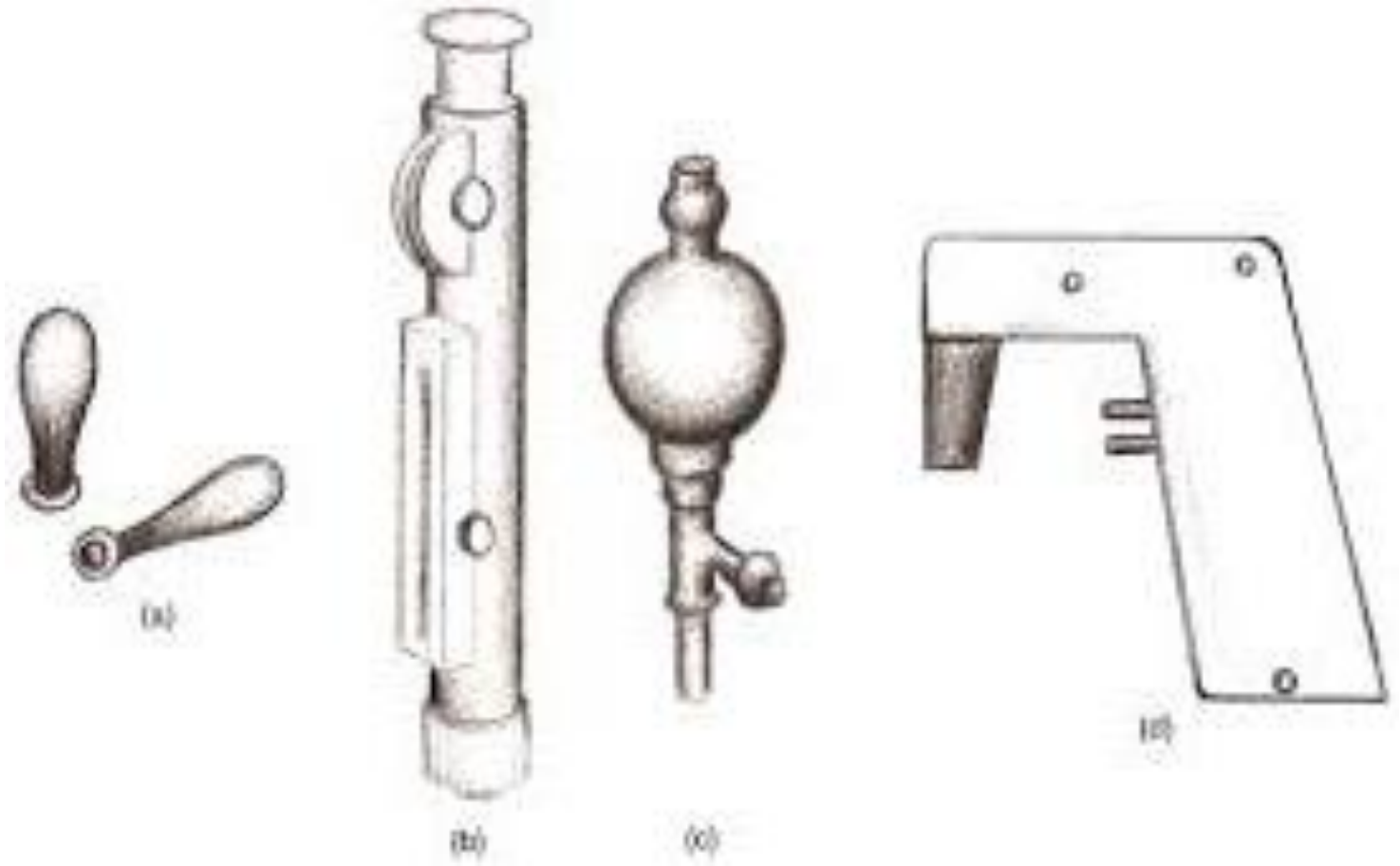
Molecular Biology and Biotechnology

By the end of the session, you should be able to

- Describe how bacterial cultures grow and maintain.
- Correctly use an inoculating loop and needle
- Correctly use a micro-pipette
- Use aseptic technique to transfer bacteria for sub-culturing
- Understand the reasoning behind pure culture
- How to prepare a stock culture

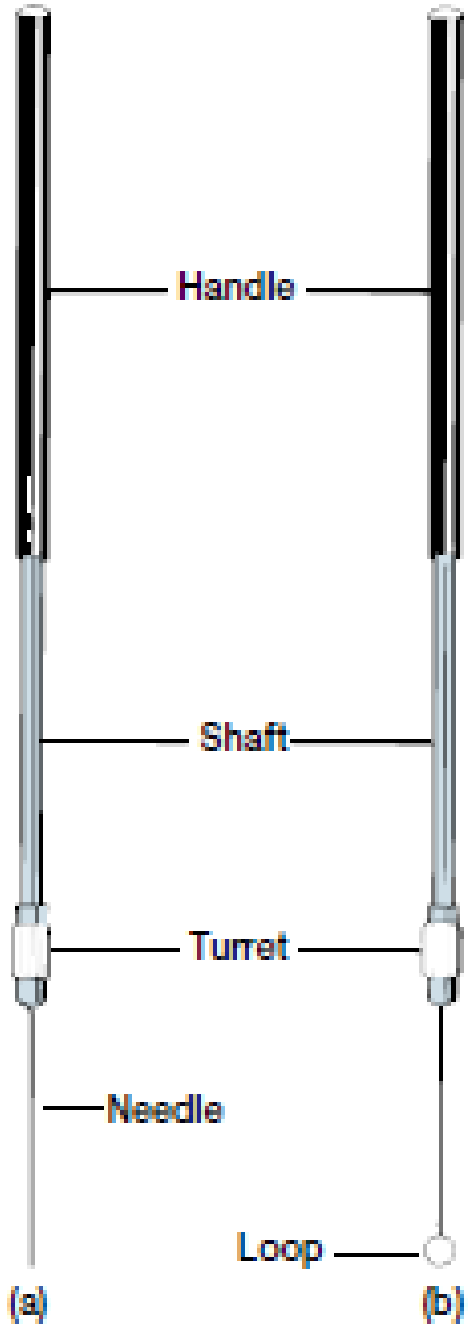
Pipettes

- A) Bulb pipette
- B) A pipette pump that can be used to take up and expel liquid
- C) Triple valve bulb
- D) Electronic pipette
- E) Micropipette



Inoculation loop and needle

- The **inoculation loop** and the **needle (straight wire)** consist of a handle, a shaft, and a turret, which holds a nickel chromium or platinum wire forming either loop or straight wire.



Culturing or cultivation of bacteria

- **Microbial culturing** is a method of multiplying microorganism by letting them reproduce in pre determined culture media under controlled laboratory conditions.

Bacterial cultivation purposes

- Isolation of a pure strain
- Culturing bacteria is the initial step for studying it’s morphology and identification
- Testing the antibiotic sensitivity
- Genetic and molecular study (DNA extraction)
- Maintenance and storage of stock culture
- etc

Step by step bacterial cultivation and maintenance

- Inoculation
- Incubation
- Isolation
- Inspection
- Identification
- Storage
- Reactivation

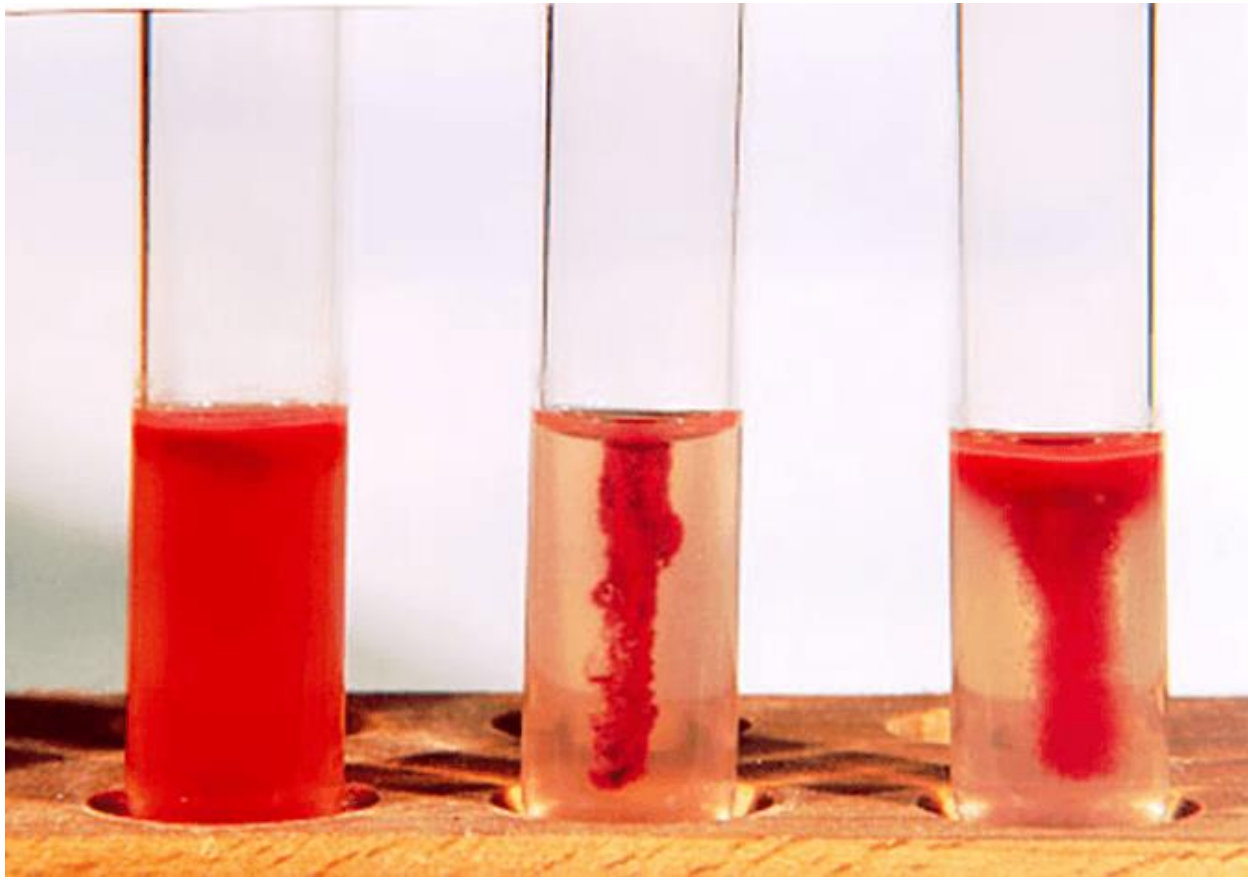
Inoculation of liquid medium

- Broth cultures are used where individual colonies of bacteria or fungi, growing on a solid surface, are **NOT** required

- **Methods for inoculation of a liquid medium**
- Using an inoculation loop to transfer a colony (picking) to a sterile broth.
- Adding one loopful of liquid sample to a new sterile broth **(inoculation loop**).
- Adding one drop to a sterile broth using a **Pasteur pipette**
- Adding a measured volume of broth (e.g. 100 µL) to a sterile broth (**micropipette**)

Inoculation of a semi-solid medium

- **Inoculation needle (Stab inoculation)** uses for inoculating the semi solid medium



Inoculation of a solid medium

- Agar is used where individual colonies of bacteria or fungi, growing on a solid surface, are required.

➢Agar inoculation methods

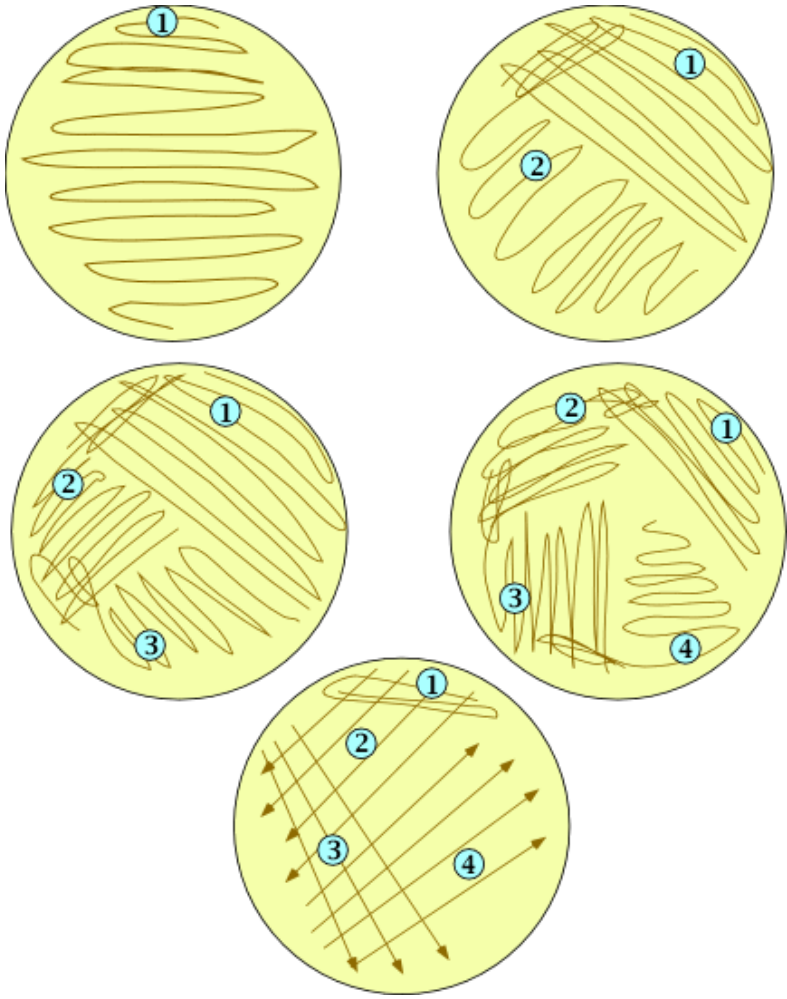
➢**Streak plate technique:** This is where a **loop** is used to inoculate the agar plate, so that well isolated individual colonies can be detected in some parts of the plate.

- **Spread technique:** This is where an aliquot of liquid sample is spread across the surface of the agar plate to make a large number of small bacterial colonies all growing very close to each other. This technique is often used for antibiotic sensitivity test.

- **Pour plate technique:** This is where a liquid sample is mixed in with molten agar as the agar is poured into the plate.

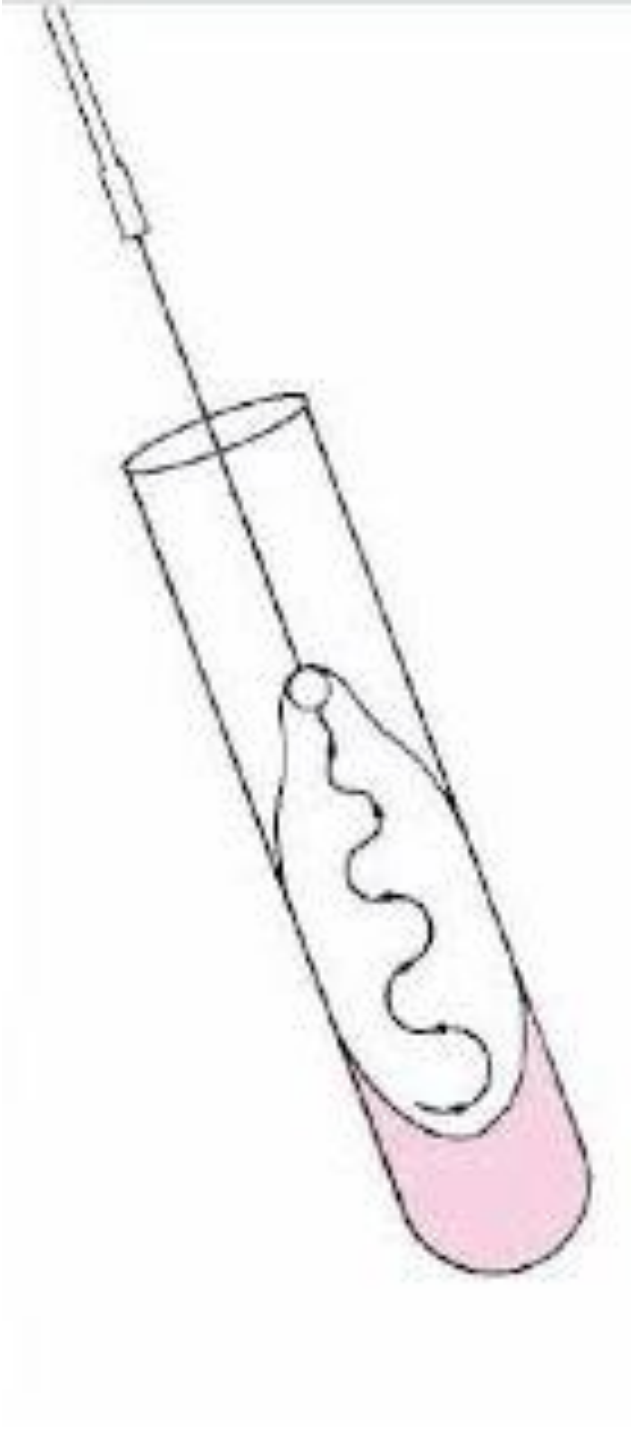
Streak plate method

- Streaks are made across the surface of the agar with a loop full (inoculation loop) of mixed culture.
- There are several different methods for streaking.
- The loop is sterilised between each set of streaks so the amount of material is progressively reduced.
- The last streaks should leave individual bacteria
- A single colony, then can be transferred to a sterile medium.



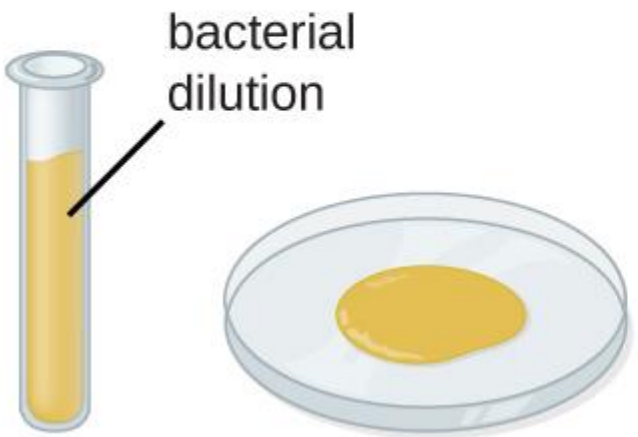
Streak slant method

- A sterile loop use for inoculation solid medium (agar) in a tube (slant).
- Slants are inoculated across the top with a 'wiggly' line up the surface of the slant.
- Maintenance and preservation of pure cultures for sub culture purposes.



Spread plate method

1 Sample (0.1 mL) poured onto solid medium



2 Spread sample evenly over the surface

3 Plate incubated until bacterial colonies grow on the surface of the medium

Incubation

- Bacterial incubation must be done under laboratory conditions, includes.
- Time
- Temperature
- Providing gases (optional) e.g. CO2
- Agitation

- **Aerobic / Anaerobic**

Maintenance/ storage

- Bacterial culture can not be stored at room temperature.

- **Short term storage**: bacterial cells can be stored at 4°C on plate for an approximately four weeks by using the refrigerator.
- **Long term storage**: bacterial liquid culture can be stored at -80°C in **15-20% glycerol** for several years by using an ultra deep freeze.
- Glycerol allows to reduce the harmful effect of ice crystals of bacteria which can damage cells.
- Lyophilising (Freeze drier).