Bacterial cultivation and culture methods

By

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A population of bacteria grown in the laboratory is referred to as aculture. Apureculture contains only one single type; a mixedculture contains two or more different bacteria.

If a bacterial culture is left in the same media for too long, the cellsuse up the available nutrients, excrete toxic metabolites, and eventually the entirepopulation will die. Thus bacterial cultures must be periodically transferred, orsubcultured, to new media to keep the bacterial population growing.

Microbiologists use subculturing techniques to grow and maintain bacterial cul-tures, to examine cultures for purity or morphology, or to determine the numberof viable organisms. In clinical laboratories, subculturing is used to obtain a pureculture of an infectious agent, and also for studies leading to the identification ofthe pathogen. Because bacteria can live almost anywhere, subculturing steps mustbe performed aseptically, to ensure that unwanted bacterial or fungal contamina-tion is kept out of an important culture.

CULTURE METHODS

- Culture methods employed depend on the purpose for which they are intended.
- Purposes:
  - To isolate bacteria in pure cultures.
  - To demonstrate their properties.
  - To obtain sufficient growth for the preparation of antigens and for other tests.
  - For bacteriophage & bacteriocin susceptibility.
  - To determine sensitivity to antibiotics.
  - To estimate viable counts.
  - Maintain stock cultures.

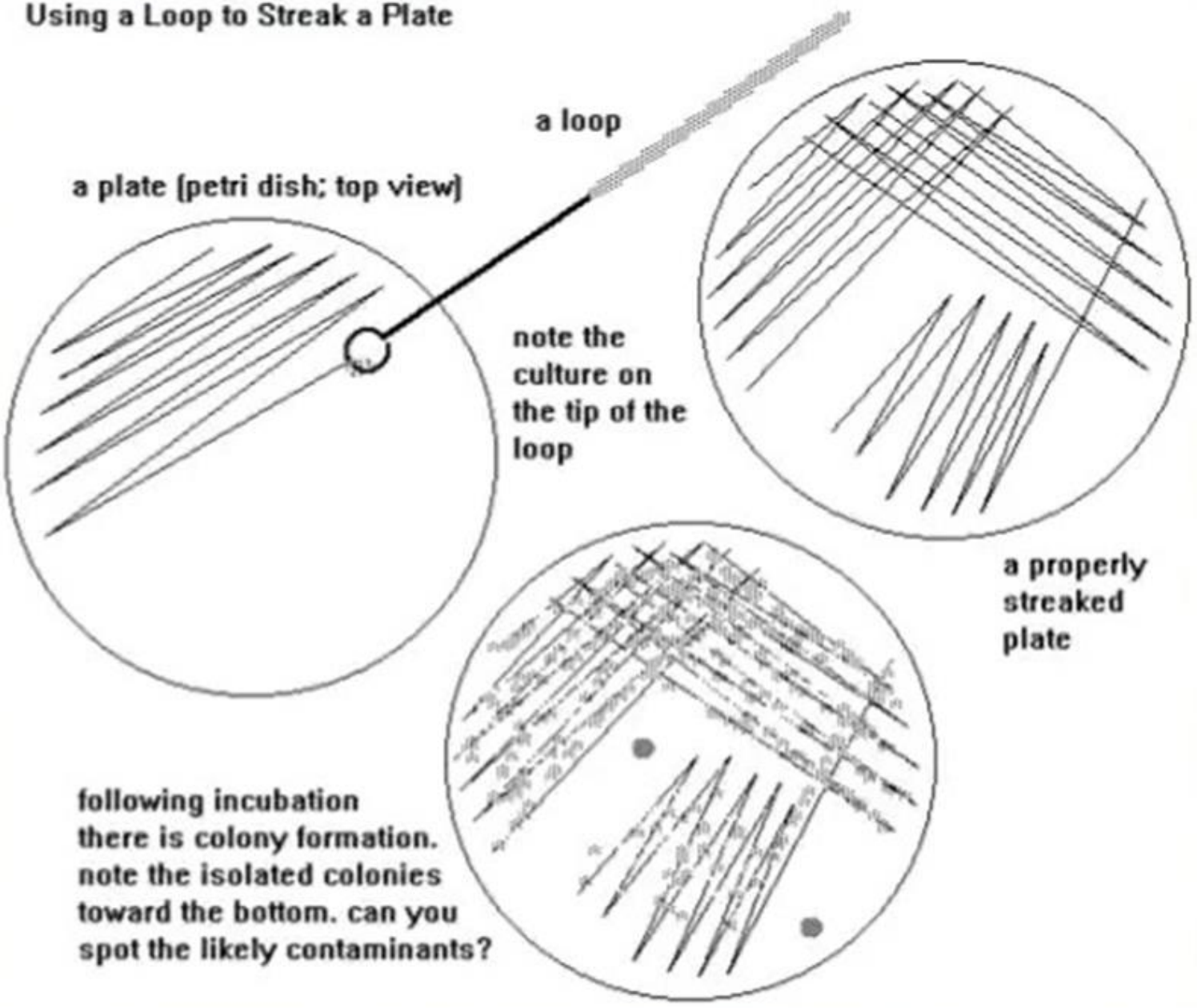
Culture methods include:

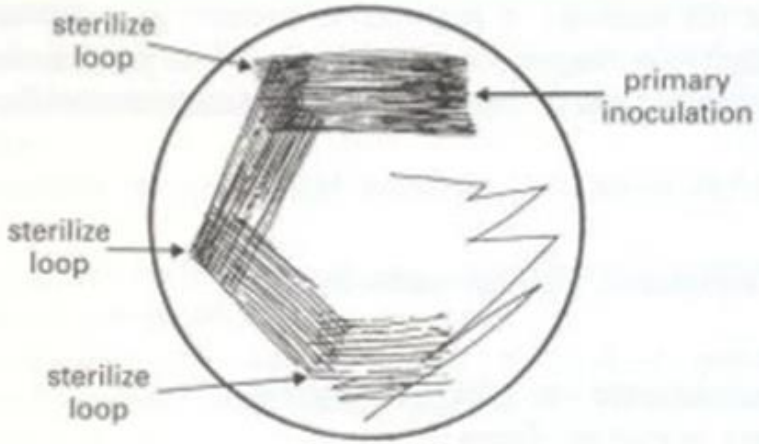
- Streak culture
- Lawn culture
- Stroke culture
- Stab culture
- Pour plate method
- Liquid culture
- Anaerobic culture methods

STREAK CULTURE

- Used for the isolation of bacteria in pure culture from clinical specimens.
- Platinum wire or Nichrome wire is used.
- One loopful of the specimen is transferred onto the surface of a well dried plate.
- Spread over a small area at the periphery.
- The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
- On incubation, separated colonies are obtained over the last series of streaks.

Using a Loop to Streak a Plate




sterilize loop
primary inoculation
sterilize loop
sterilize loop

LAWN CULTURE

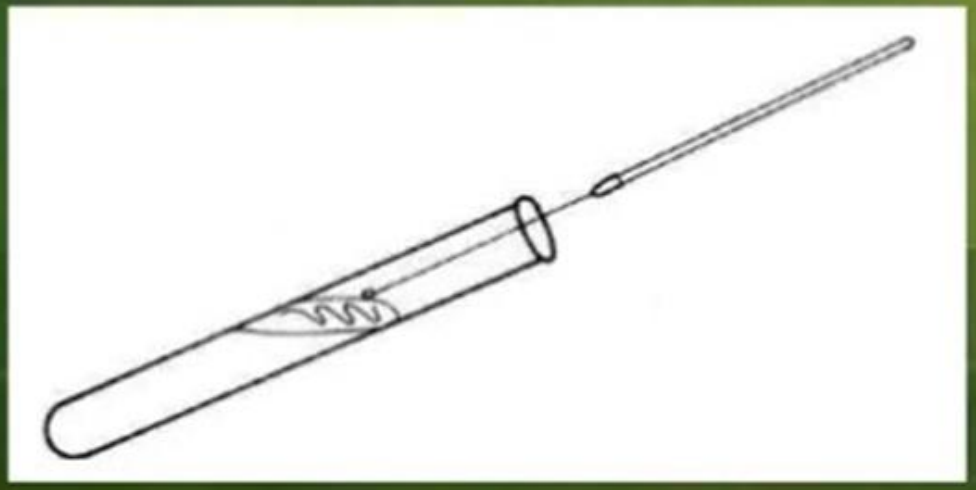
- Provides a uniform surface growth of the bacterium.
- Uses
  - For bacteriophage typing.
  - Antibiotic sensitivity testing.
  - In the preparation of bacterial antigens and vaccines.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium.

Antibiotic sensitivity testing

STROKE CULTURE

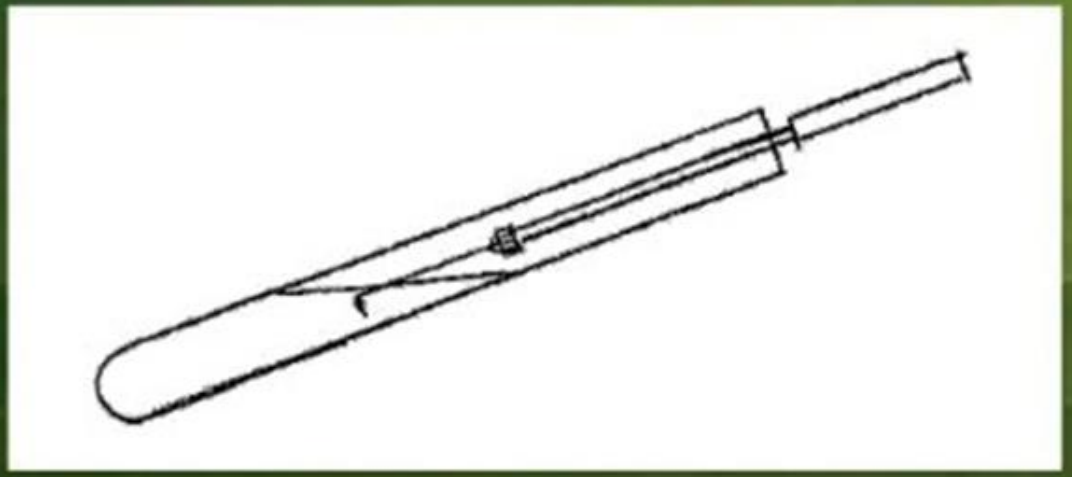
- Stroke culture is made in tubes containing agar slope / slant.

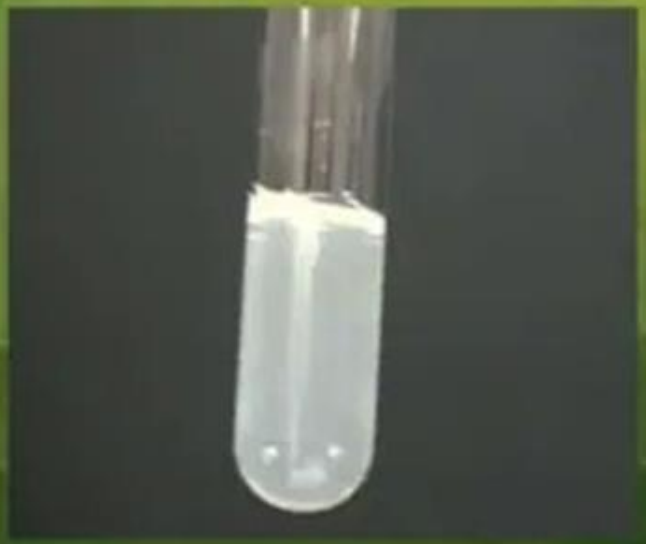
- Uses
  - Provide a pure growth of bacterium for slide agglutination and other diagnostic tests.



STAB CULTURE

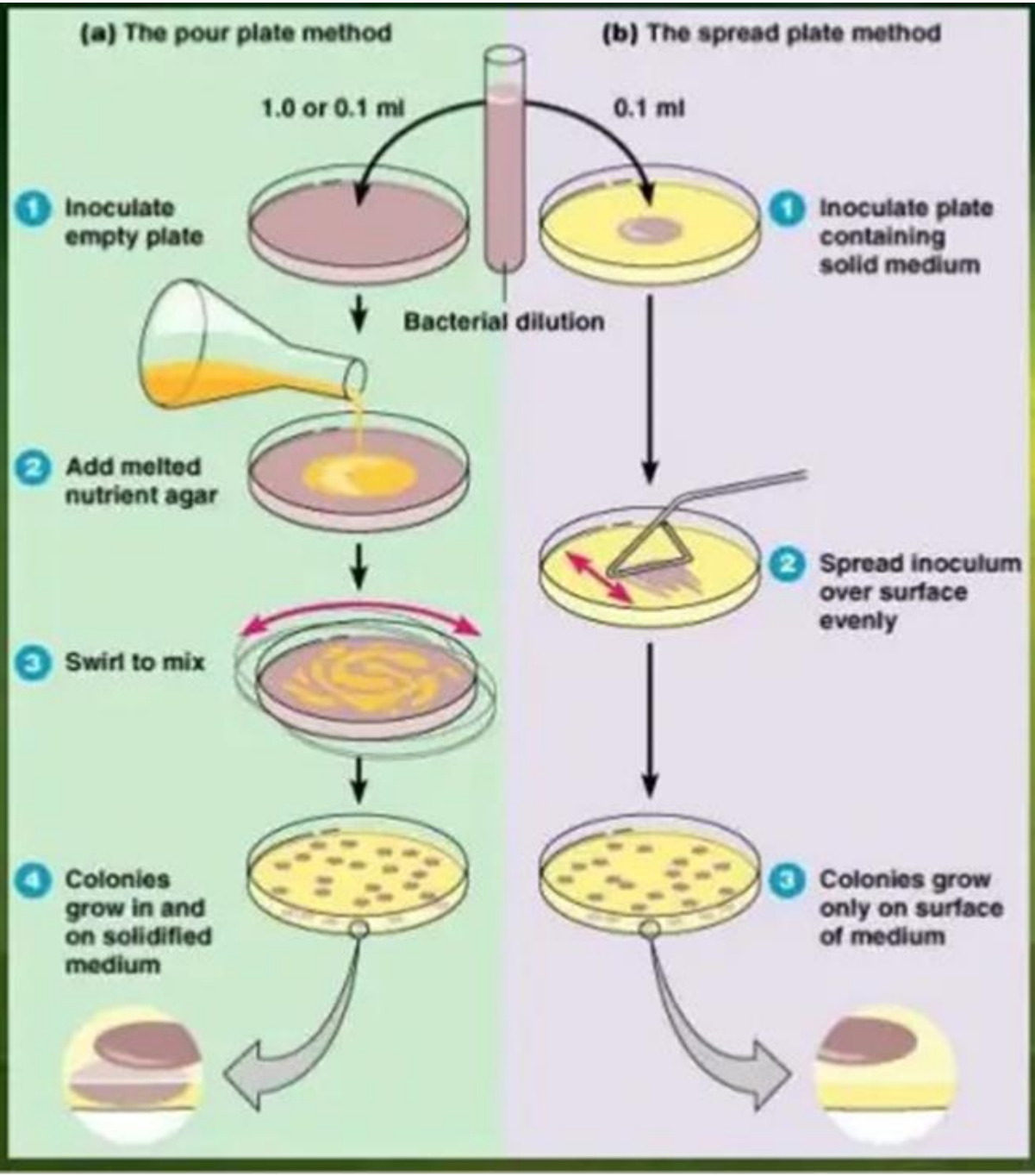
- Prepared by puncturing a suitable medium – gelatin or glucose agar with a long, straight, charged wire.
- Uses
  - Demonstration of gelatin liquefaction.
  - Oxygen requirements of the bacterium under study.
  - Maintenance of stock cultures.





POUR PLATE CULTURE

- Agar medium is melted (15 ml) and cooled to 45°C.
- 1 ml of the inoculum is added to the molten agar.
- Mix well and pour to a sterile petri dish.
- Allow it to set.
- Incubate at 37°C, colonies will be distributed throughout the depth of the medium.
- Uses
  - Gives an estimate of the viable bacterial count in a suspension.
  - For the quantitative urine cultures.


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
1 Inoculate plate containing solid medium
Bacterial dilution
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium

LIQUID CULTURES

- Liquid cultures are inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.
- Uses
  - Blood culture
  - Sterility tests
  - Continuous culture methods
- Disadvantage
  - It does not provide a pure culture from mixed inocula.

Blood culture bottles

THANK YOU