

Lab:6 Culture Methods

Indications for culture -

- Isolate bacteria in pure cultures.
- Demonstrate their properties.
- Obtain sufficient growth for preparation of antigens & for other tests.
- Typing bacterial isolates.
- Antibiotic sensitivity.
- Estimate viable counts.
- Maintain stock cultures.

1
Label an agar plate. For advice on media and incubation conditions, refer to TIB.081, Recommended Growth Requirements.

2
Heavily saturate a swab with hydrated material from a KWIK-STIK™ or LYFO DISK™. Alternatively, use a sterile loop to transfer a colony or colonies from one agar plate to another.

3
Gently inoculate one-third of the plate with the swab or sterile loop.

4
Turn plate. Streak a sterile loop through edge of inoculated area three to four times into second area as pictured.

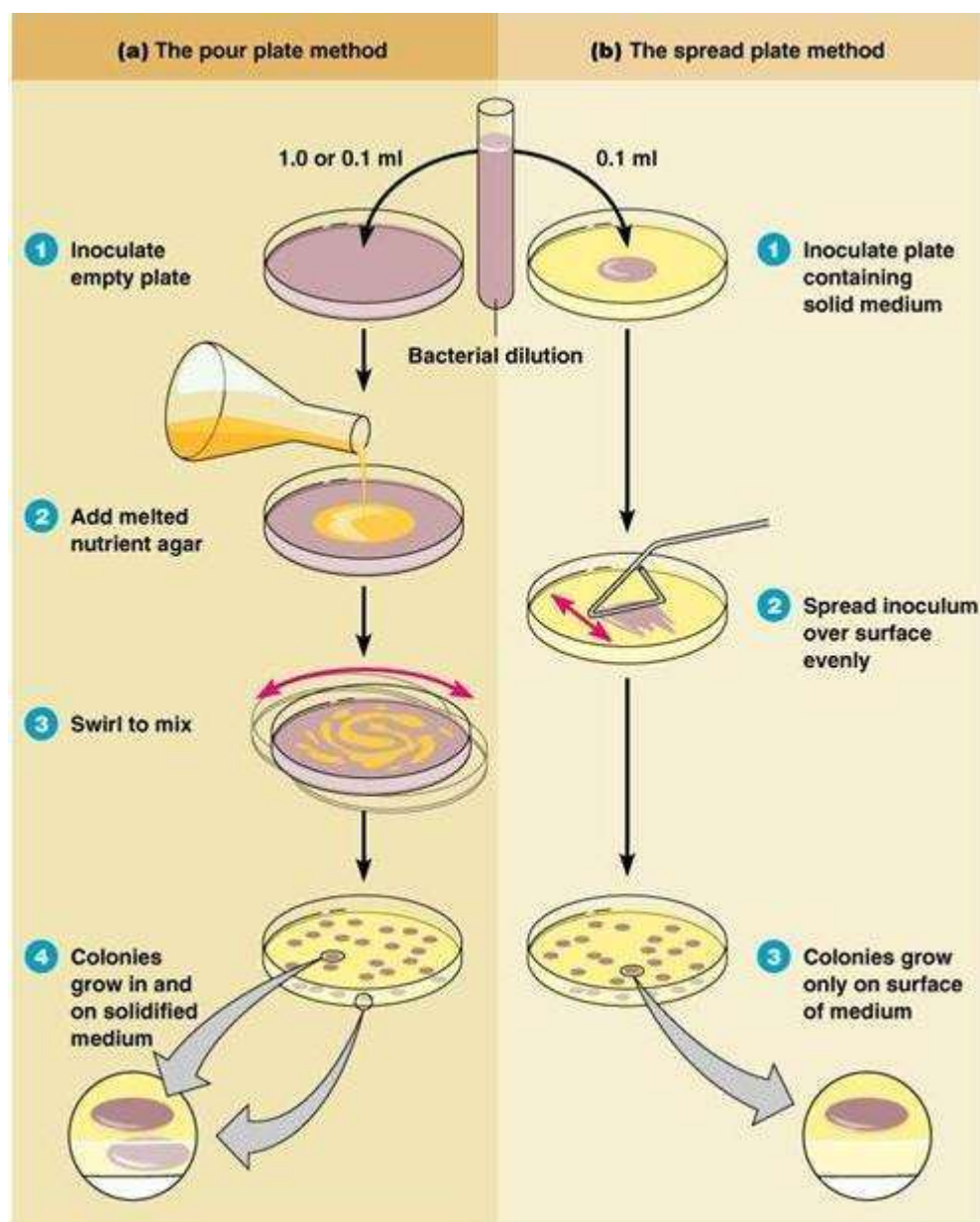
5
Turn plate. Streak loop through edge of second area 3 to 4 times into third area as pictured.

6
Turn plate. Streak loop through edge of third area three to four times into fourth area as pictured.

7
Immediately incubate the inoculated plate.

8
Note: Sterilizing loop between each area will achieve maximum isolation of colonies.

Microbiologics®

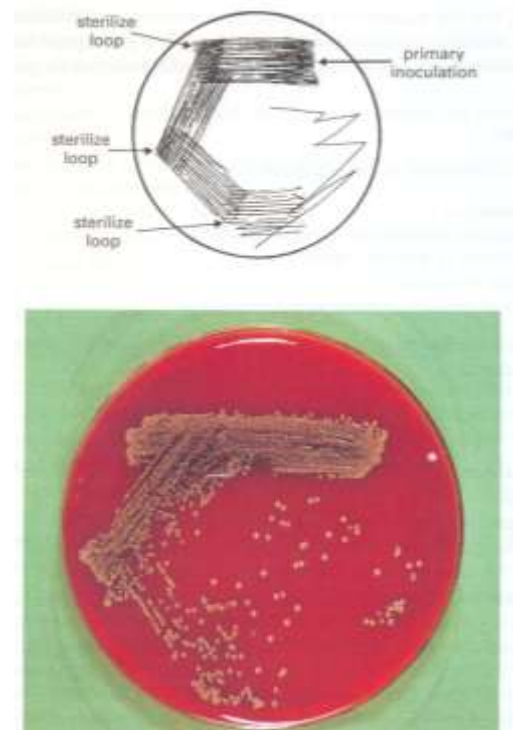
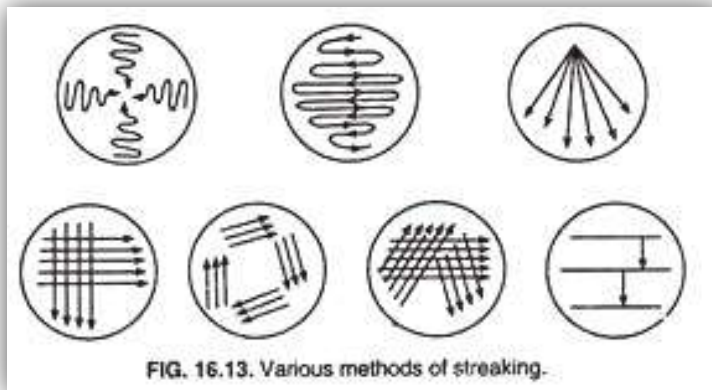


Methods of Isolation:

- Streak culture or surface plating
- Lawn or carpet culture
- Stroke culture
- Stab culture
- Pour plate method
- Anaerobic methods of culturing bacteria

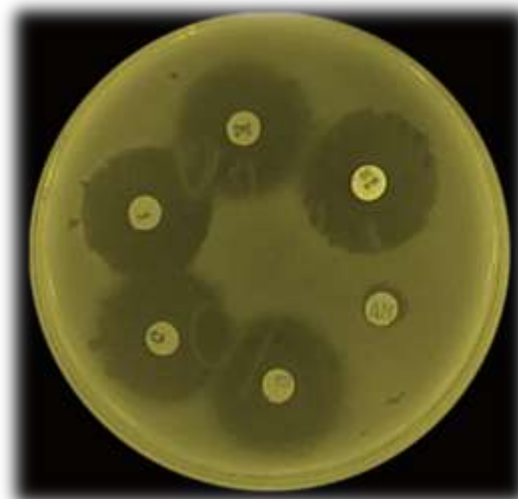
1. Streaking:

- Routinely employed for isolation
- Platinum / Nichrome loops



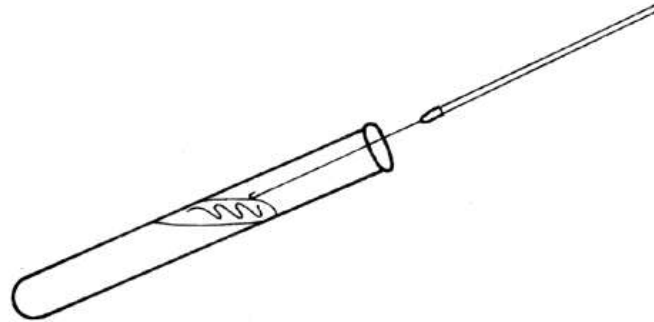
2. Lawn or Carpet Culture

- Uniform surface growth
- Bacteriophage typing
- Antibiotic sensitivity testing
- Preparation of bacterial antigens & vaccines



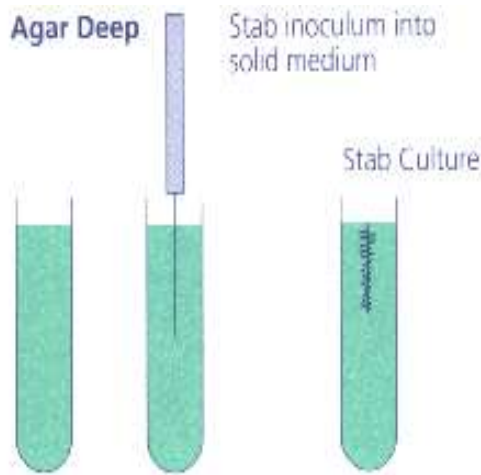
3- Stroke Culture

- Tubes containing agar slopes
- For slide agglutination & other diagnostic tests.



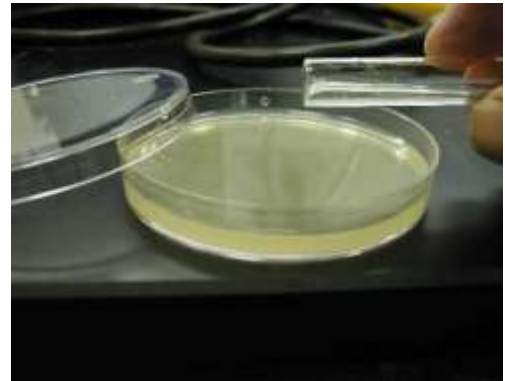
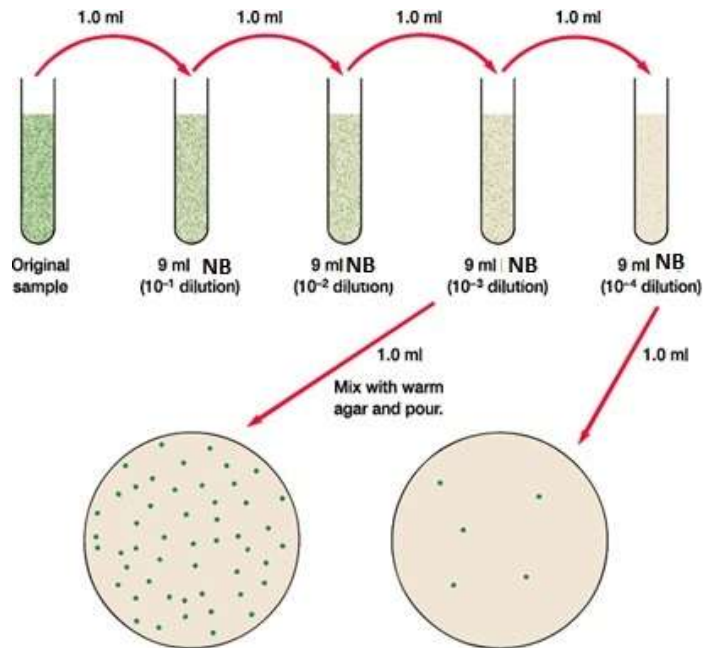
4- Stab Culture

- By puncturing a suitable medium with a long, straight charged wire.
- For gelatin liquefaction, stock cultures & motility



5- Pour Plate Method

- 1 ml of appropriately diluted inoculum is added to 15 ml of molten agar and poured on petridish.
- Colonies appear through out the depth of medium.
- Used to estimate viable count, recommended method for quantitative urine cultures.



Broth/Liquid Culture

- Inoculated by a charged loop, pipette or syringes.
- For blood cultures & sterility testing



6. Anaerobic Culture Methods

Anaerobic condition can be achieved by:

- Cultivation in vacuum
- Displacement of oxygen with other gases
- Chemical or biological methods
- By displacement and combustion of oxygen
- By reducing agents
- Anaerobic chamber

Displacement Method

- Displacement of O₂ with gases like H₂, N₂, He or CO₂.
- Rarely produces complete anaerobiosis.

e.g. Candle jar



Chemical or Biological Methods

- Alkaline pyrogallol (pyrogallic acid in NaOH) absorbs O₂
- Yellow phosphorous
- Rosenthal method - Mixture of chromium & sulphuric acid
- Gaspak

Biological Methods

Absorption of oxygen from small closed systems has been attempted by incubation along with

- Aerobic bacteria EXAMPLE:- *Pseudomonas aeruginosa*
- Anaerobiosis produced by this method is slow and ineffective.