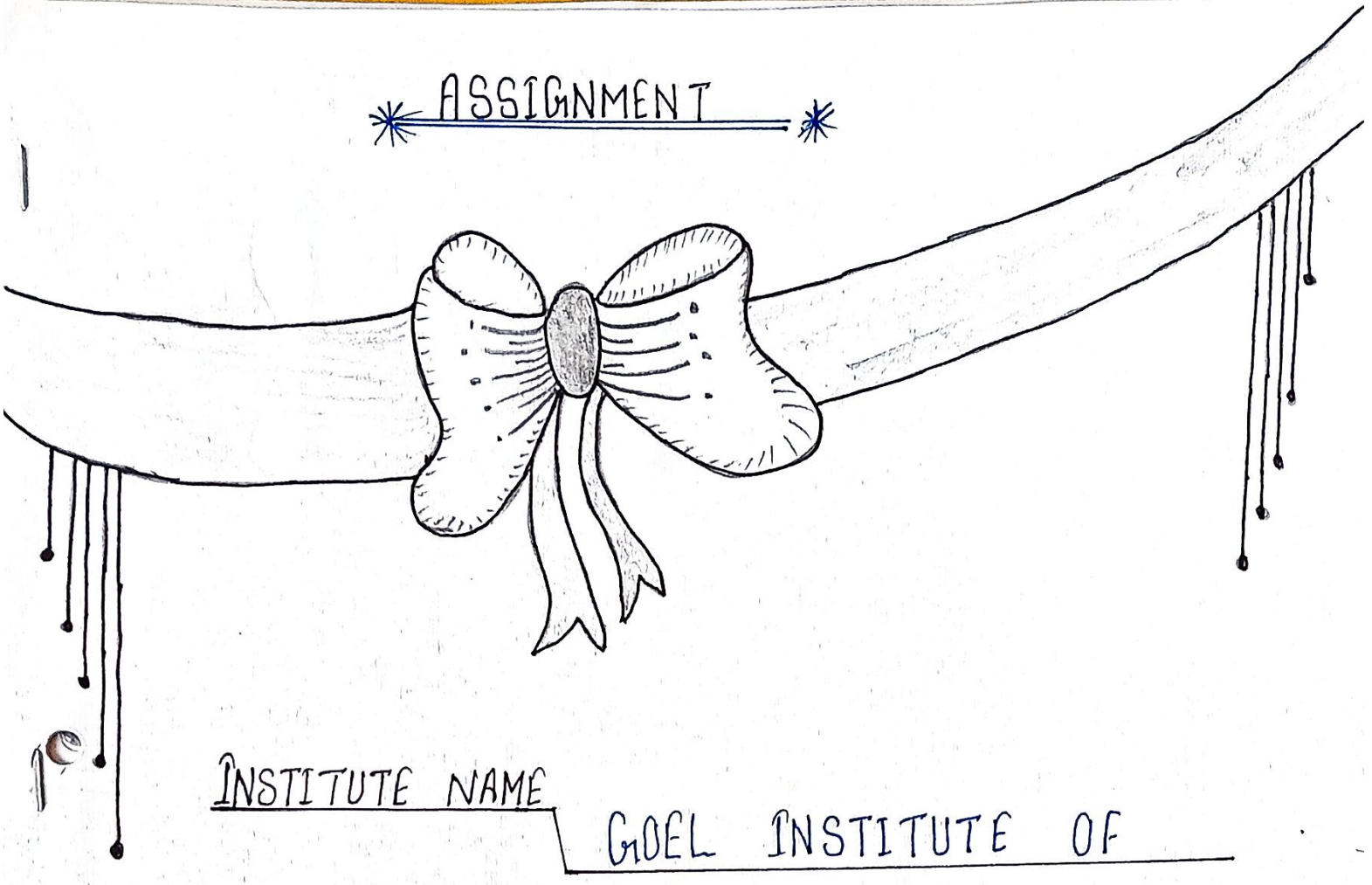
ASSIGNMENT



INSTITUTE NAME — GOEL INSTITUTE OF PHARMACY AND SCIENCES

NAME. — IRFAN ANSARI

CLASS. — B-PHARM IInd YEAR (BATCH-A)

SUBJECT. — PHARMACEUTICAL MICROBIOLOGY

TOPIC. — Isolation and Preservation method for Pure Culture

ROLL NO. — 2103920500047

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PURE CULTURES

If the bacterial species being sought comprises a suitably high proportion of the mixed population, it can be isolated in pure culture. The descendents of a single isolation in pure culture comprises a Strain. A Strain is usually made up of a succession of Cultures and is often derived from a single colony; however, the number of bacteria which gives rise to the original colony is usually Unknown If a Strain derived from a single parent cell, it is termed a Clone. Each Strain is designated by an identifying number and its history is recorded (the source from which the isolation was made, the name of the person who made the isolation, the date of the isolation, and the Culture Collection in which the Strain is maintained and from which the it can be obtained for Study.)

A variety of techniques have been developed where by isolation into pure culture can be accomplish Each technique has certain advantages and limitations and there is no one method that can be used for all bacteria.

It contains only a single species or bacteria after form culture media, put the conical flask or test tube, then pour into petri - disc then close in 'Incubator' for 2 or 3 days many bacteria are grow.

Methods of Isolating Pure Cultures

By means of a transfer loop, a portion of the mixed culture is placed on the surfac of an agar medium and streaked across the surface. This manipulation "thins out" th bacteria on the agar surface so that some individual bacteria are separated from each other.

There are three Method of Isolating Pure Cultures—

The Streak Plate Techniques

Streaking is a process of spreading the microbial culture with an inoculating needle or loop on the surface of media.

Step - 1

First we sterilize the inoculating needle by flame to make it red hot and then allow it to cool for 30 sec. or few seconds.

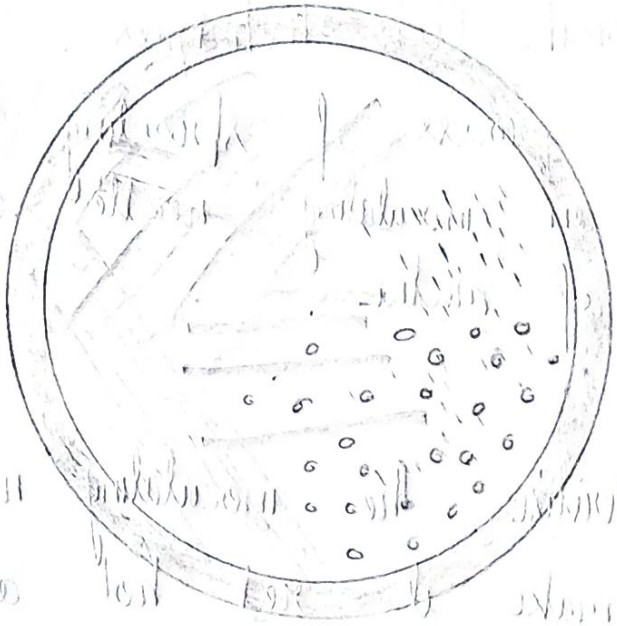
Step - 2

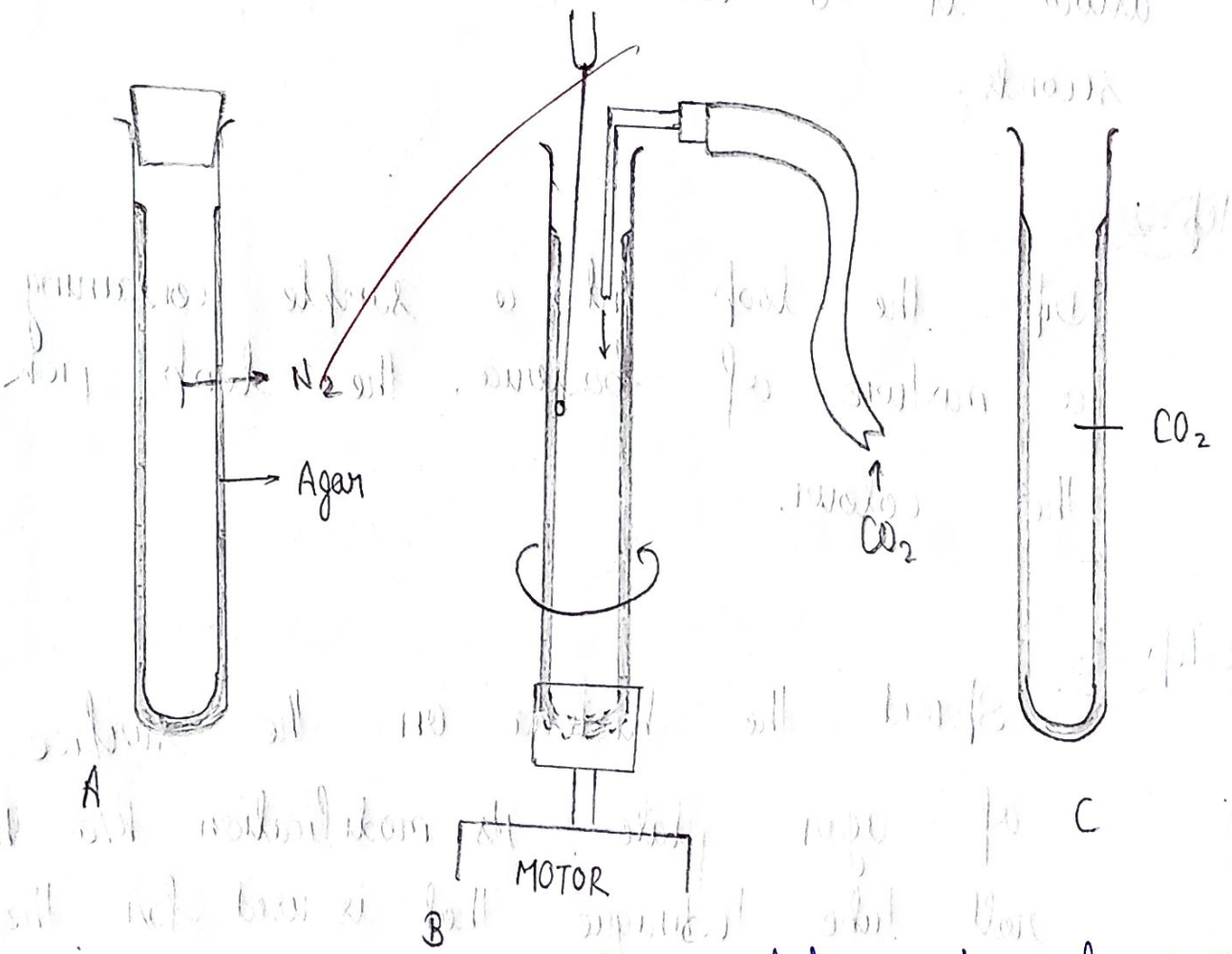
Dip the loop into a sample containing a mixture of bacteria, the loop pick the colour.

Step - 3

Spread the bacteria on the surface of agar plate. Its modification k/a the roll-tube technique that is used for the isolation of ~~s~~trigents anaerobes

- Streak plate cult showing areas of isolated colonial growth.



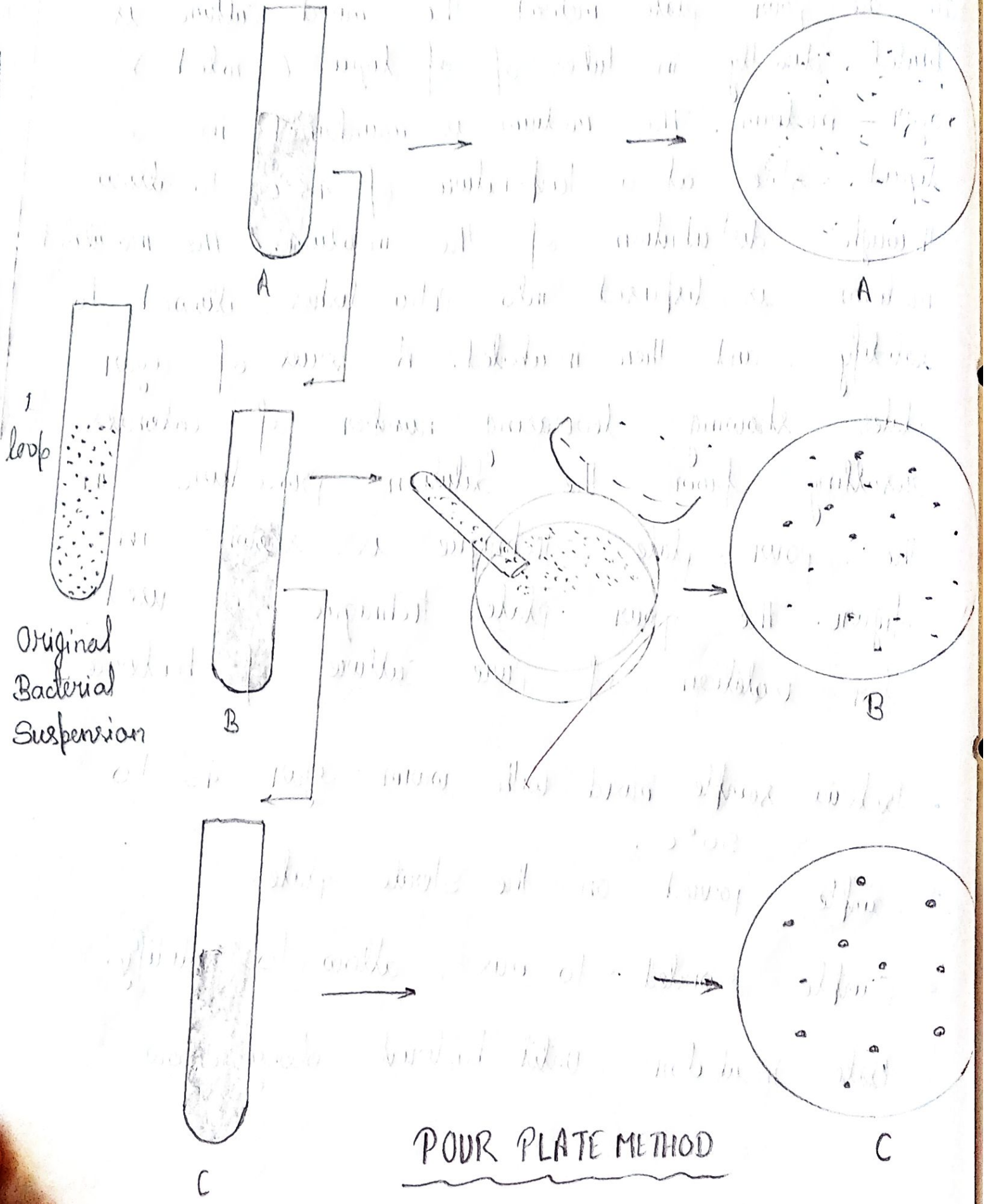


- Roll-tube method for isolating stringent anaerobes

The Pour Plate Method

In the pour - plate method the mixed culture is diluted directly in tubes of ~~of~~ liquid (cooled) agar medium. The medium is maintained in a liquid state at a temperature of 45°C to allow through distribution of the incolum. The inoculated medium is dispensed into petri dishes, allowed to solidify, and then incubated. A series of agar plates showing decreasing number of colonies resulting from the dilution procedure in the pour - plate technique is shown in figure. The pour plate technique is used for isolation of pure culture of bacteria.

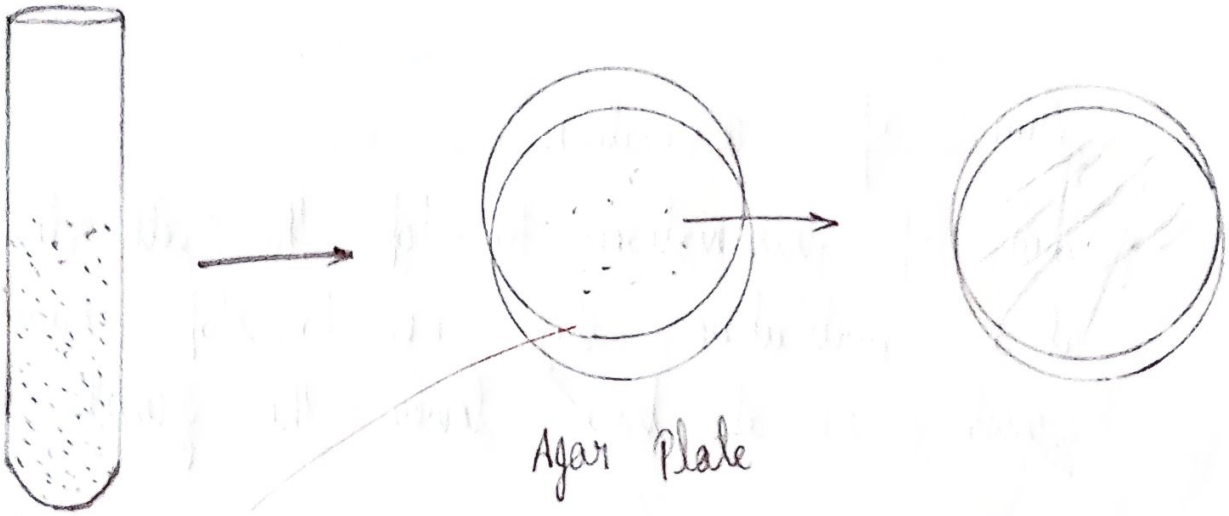
- Bacteria sample mixed with warm agar 45 to 50°C.
- Sample powred on the Sterile plate.
- Sample swirled to mix, allow to solidify.
- Plate incubator until bacterial colony grow.



POUR PLATE METHOD

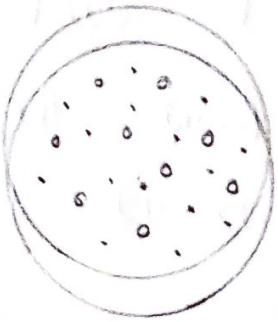
Spread Plate Method

This is the best method to isolate the pure colonies in this techniques, the Culture media is not mix with agar media. Instead it mix with normal diluted Saline.



Culture Media

After Incubation



Colonies Develop

Preservation of Pure Culture

- Preservation method either stop or slow down the metabolism and multiplication of microbes.
- To maintain the pure culture for extended periods in a vival condition, without any genetic changes is referred as a preservation.

Aim of Preservation

- Aim of preservation to stop the cell division at a particular stage i.e. to stop microbial growth or at least lower the growth rate.
- To maintain or isolate the pure culture for extended periods in a vival condition.
- To Avoid the Contamination.
- To restrict the genetic changes.

There are two method for Preservation of pure Culture are as follows:—

- Short - Term Method
- Long - Term method

Short Term Method

Periodic Transfer to Fresh Media

Culture can be maintained by periodically preparing a fresh culture from the previous stock culture.

Bacteria Can be Frozen using 15% Glycerol

The Glycerol is diluted to 30% and an equal amount of culture growth are mixed, dispensed into tubes and then frozen at -10°C. The Vivality of organism barried such as E. Coli.

Storage in Distilled Water

Most Organism grow poorly in distilled water. But some survive for prolonged Period.

Storage by Refrigerator

Culture media can be successfully stored in Refrigerator or cold room, when the temperature is maintain at 0 to 4°C.

Long - Term Method ***

① Liquid Paraffin Storage

In this method Sterile liquid paraffin is pour over the slant cultures of microbes and stored upright at room temperature.

The preservation method for bacteria from the genera Azotobacter and mycobacterium is from 1 to 10 yr, Bacillus (6 to 12 yr).

② Storage in Saline Suspension

Bacterial culture is preserve in 1% salt concentration in screw cap tubes to prevent evaporation.

③ Storage in Sterile Soil

Preservation in sterile soil of micro-organism such as Rhizopus, Penicillium, stored in sterile soil.

④ Lifolization / Freeze Drying

Culture put in low temp. then reduce the pressure so microbial cells are dehydrated and their metabolic activity are stop. After it shield & stored in dark at 4°C in refrigerater.