ISOLATION & PRESERVATION METHODS FOR PURE CULTURE

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ISOLATION & PRESERVATION METHODS FOR PURE CULTURE

PRESENTED BY- MS. K. H. PARDESHI
(ASST. PROF. PHARMACEUTICS)

Pure Culture Technique

- **Culture :** Act of cultivating microorganisms or the microorganisms that are cultivated

Mixed culture : more than one microorganism

Pure culture : containing a single species of organism.

- A pure culture is usually derived from a mixed culture (one containing many species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample many times before inoculating the new medium.

Why important ?

Pure cultures are important in microbiology for the followingreasons-

1. Once purified, the isolated species can then be cultivated with the knowledge that only the desired microorganism is being grown.
2. A pure culture can be correctly identified for accurate studying and testing, and diagnosis in a clinical environment.
3. Testing/experimenting with a pure culture ensures that the same results can be achieved regardless of how many time the test is repeated.

- Pure culture spontaneous mutation rate is low
- Pure culture clone is 99.999% identical

ISOLATION TECHNIQUE OF PURE CULTURE

- Cultures composed of cells arising from a single progenitor
- Progenitor is termed a CFU
- Aseptic technique prevents contamination of sterile substances or objects
- Common isolation techniques
  - -Streak plate method
  - -Pour plate method
  - -Spread plate method
  - -Roll tube method

Special methods of isolation on of pure culture

Single cell isolation methods

Capillary pipette method

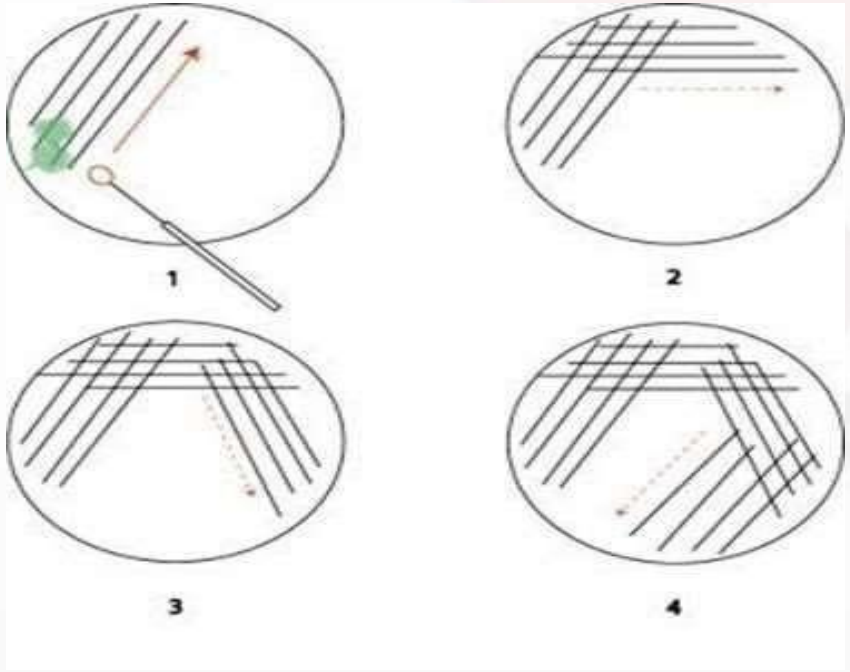
Micromanipulator method

Enrichment culture method

The enrichment culture strategy provides a specially designed cultural environment by incorporating a specific nutrient in the medium and by modifying the physical conditions of the incubation.

1.Streakplatemethod

- Streakingis the processof spreading the needle on the microbial culture with an inoculating surface of themedia.
- Sterilize the inoculating needleby flameto make red hot andallow it to cool for 30 seconds.
- Thesampleisstreakedinsuchawaytoprovideseriesofdilution.
- purpose-thinoutinnoculumtogetsepratecolonies.
- subculturingcanbedonebystreakingwel isolatedcoloniesfromstreakplatetonewplate.



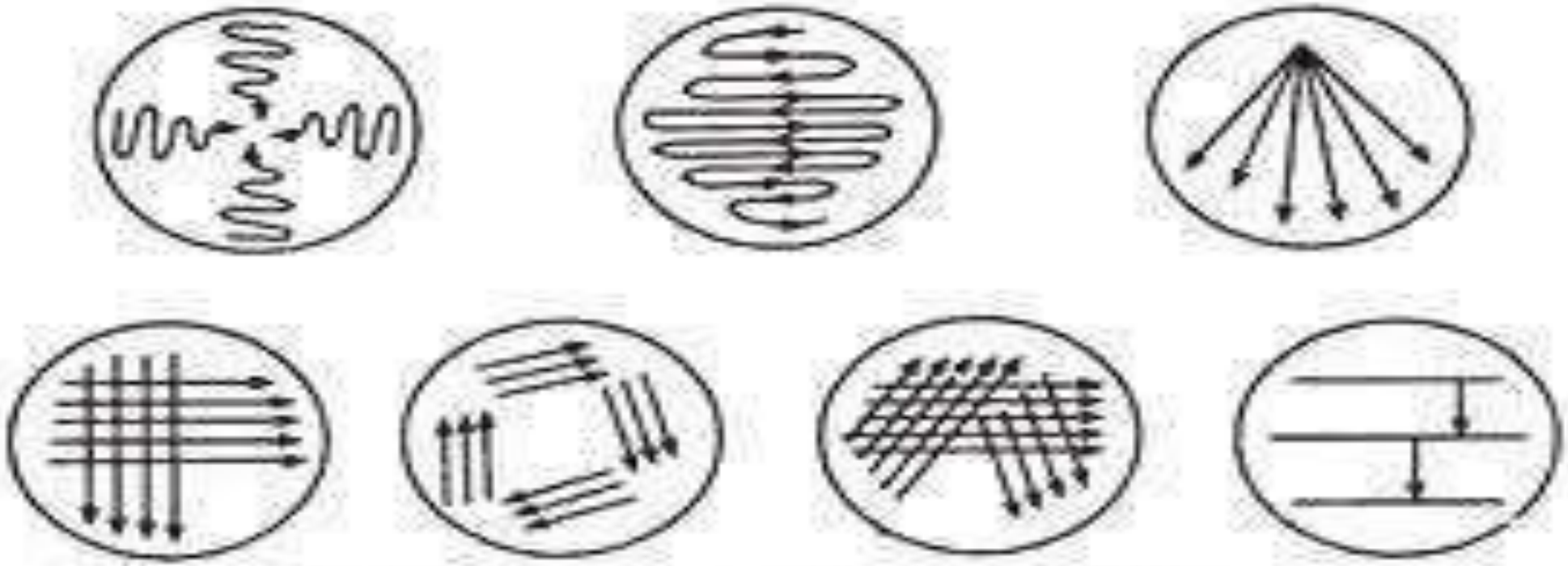


FIG. 16.13. Various methods of streaking.

2. Pour plate method

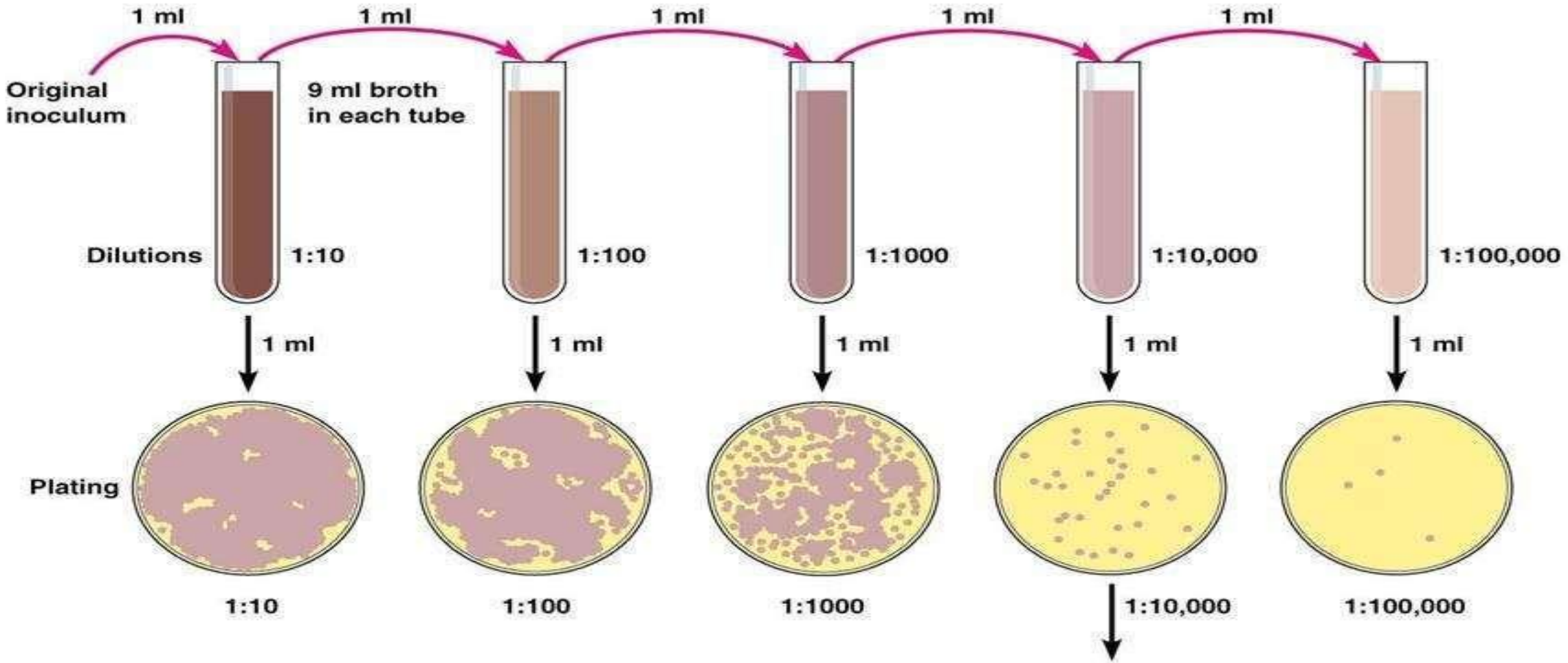
- Thebacterial culture andliquidagarmedium aremixedtogether.
- After mixing the medium, the medium containing the culturepoured into sterilized petridishes (petriplates), allowed solidifying and thenincubated.
- After incubation coloniesappear on the surface.

DISADVANTAGES-

1. Microorganism trapped beneath the surface of medium hence surface as wel as subsurface coloniesaredevelopedwhich makesthedifficuktiesincountingthebacterialcolony.

2. Tidious and time consuming method, microbesare subjected to heat shock becauseliquid mediummaintainedat45℃.

3. Unsuitable-*Psychrophile*



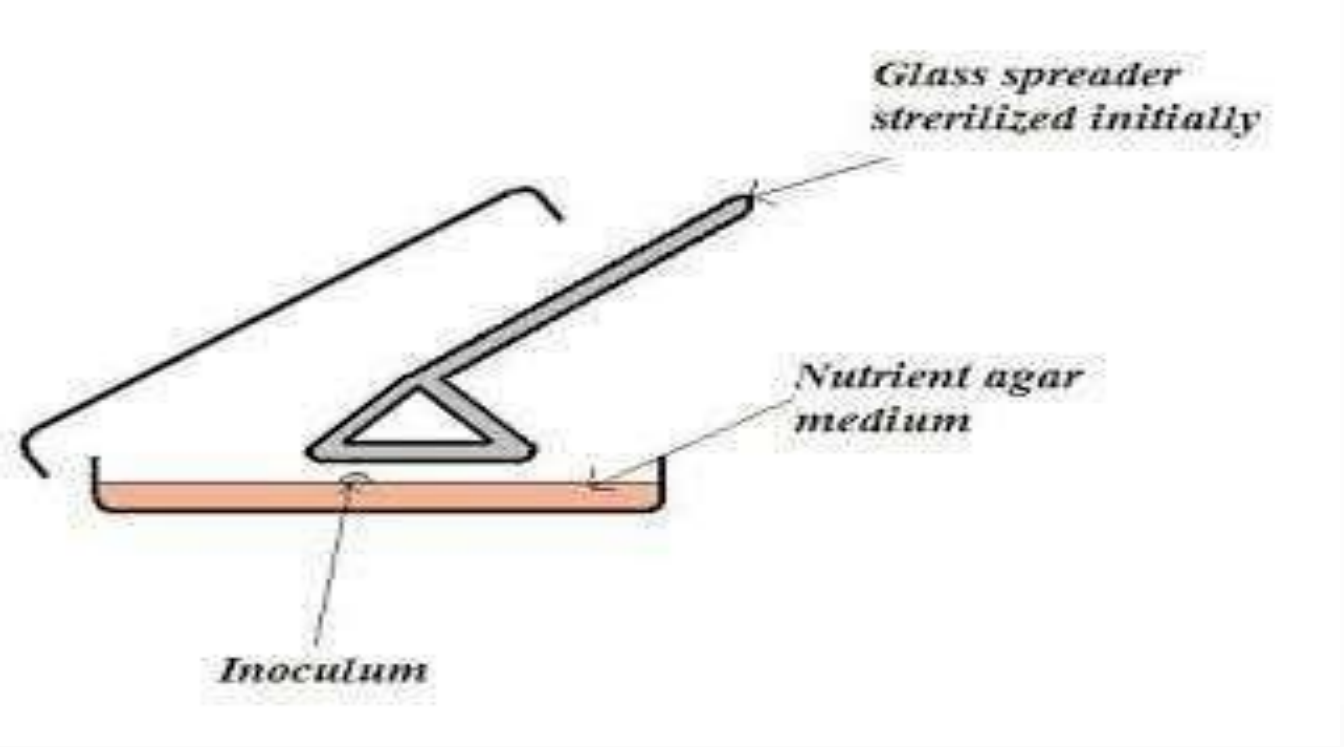
Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

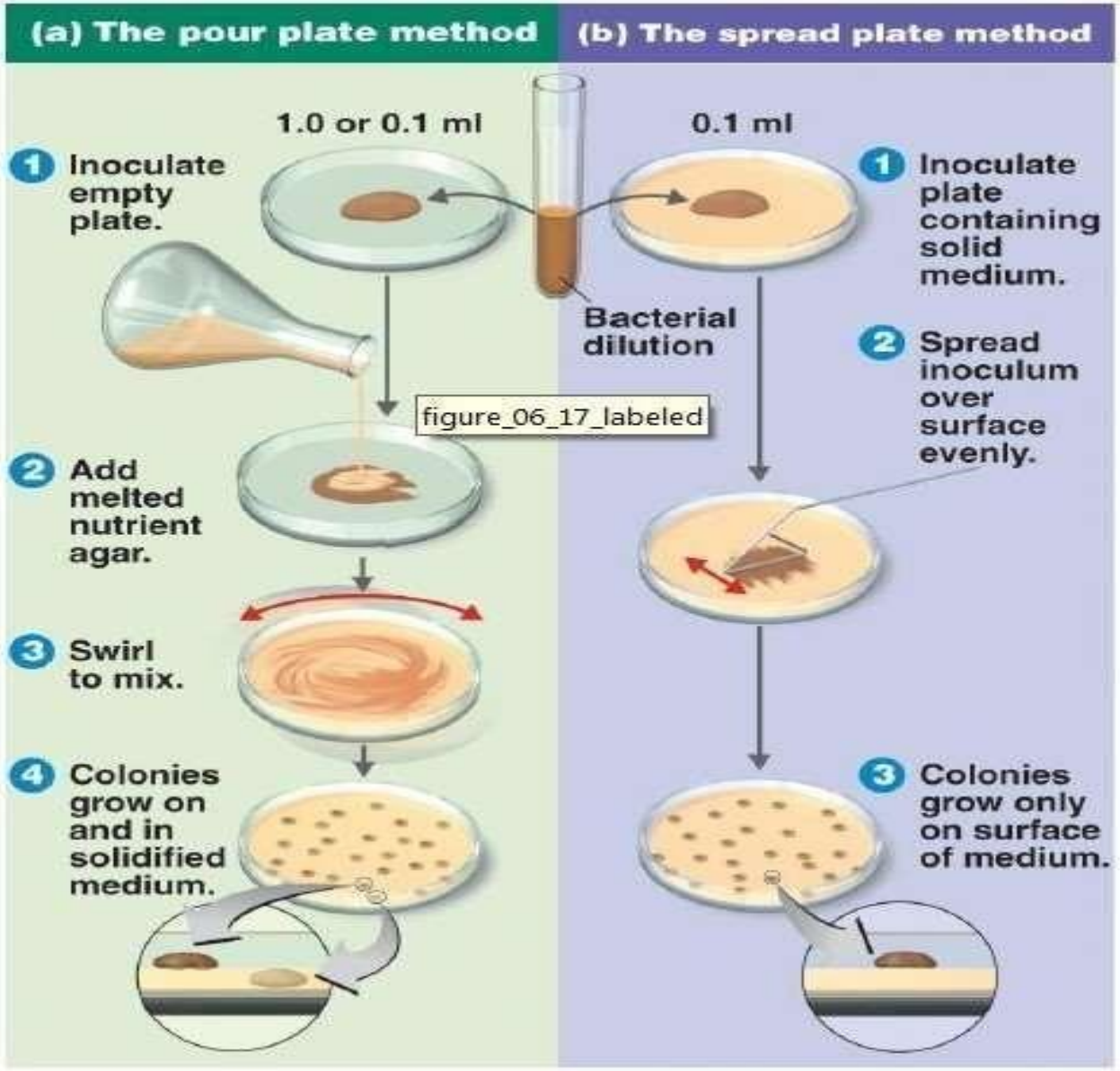
3. Spread plate method

- Thisisthe bestmethod to isolatethepurecolonies.
- In this technique, the culture is not mixedwith the agarmedium. Insteadit is mixed with normal saline andserialy diluted.
- 0.1 ml of sampletaken from diluted mixture, which is placedon the surfaceof the agarplate and spreadevenlyover the surfacebyusingLshaped glassrodcaledspreader.
- Incubate theplates
- After incubation, coloniesareobservedon the agar surface.

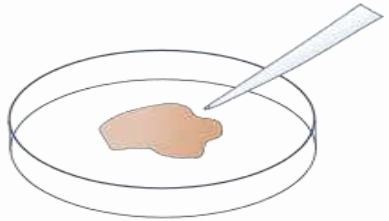
ADVANTAGES

1. It isasimplemethod.
2. In this method only surfacecoloniesare formed.
3. Micro-organismsarenot exposedto higher temperature.



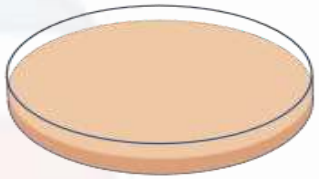

(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate.
Bacterial dilution
1 Inoculate plate containing solid medium.
2 Spread inoculum over surface evenly.
figure_06_17_labeled
2 Add melted nutrient agar.
3 Swirl to mix.
4 Colonies grow on and in solidified medium.
3 Colonies grow only on surface of medium.

Pour plate method

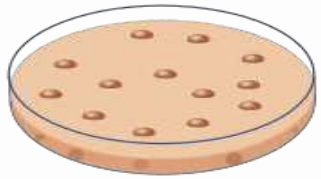


pipette inoculum onto sterile plate

add sterile medium

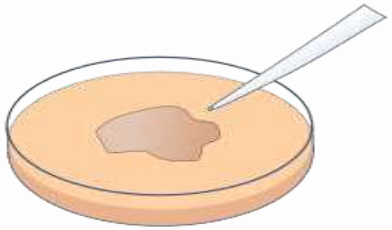


swirl to mix and incubate

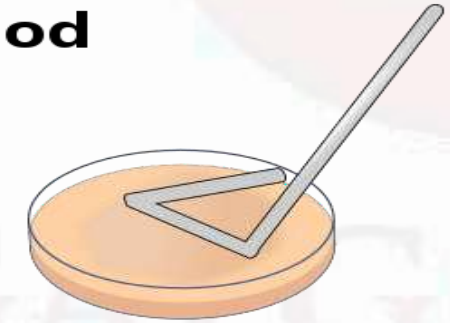


colonies grow
in and on medium

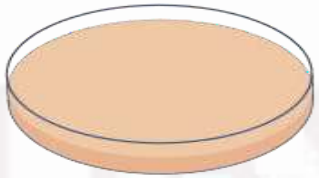
Spread plate method



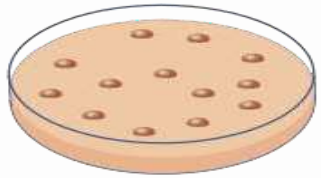
pipette inoculum onto the surface
of agar plate



spread evenly over the agar surface

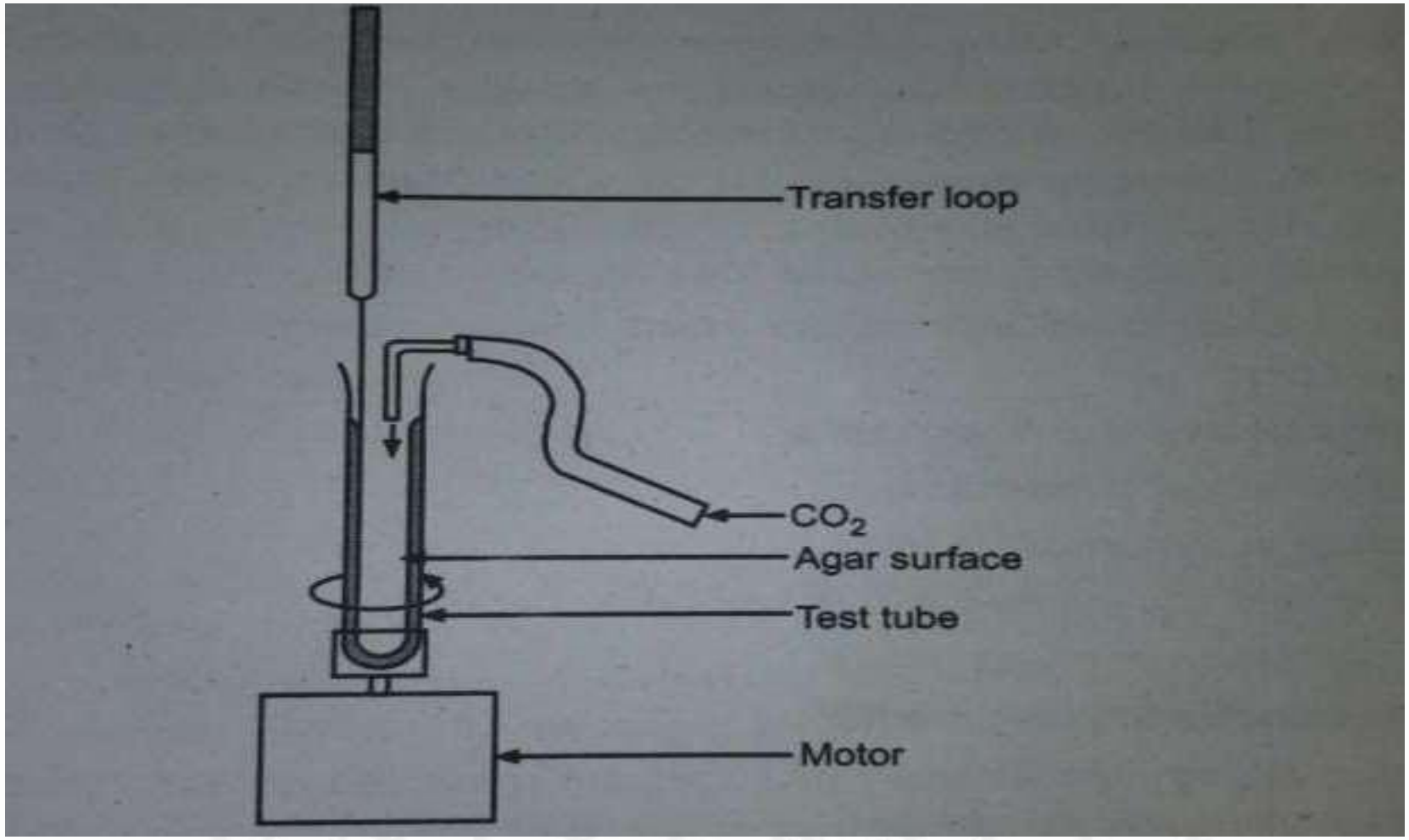


incubate



colonies grow only
on the surface of medium

4. Roll tube method.



5. Micromanipulator method

Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture. This instrument is used in

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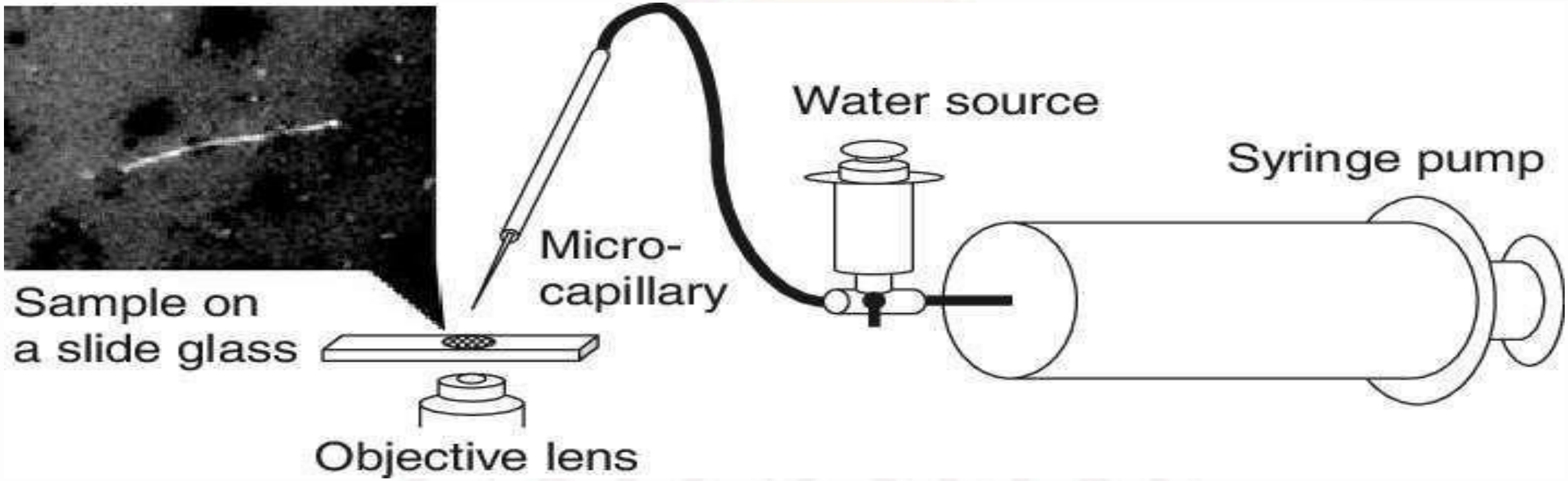
pette and transfer to large amou

R METHOD-

e reasonably sure from a single

equipment is expensive its



PRESERVATION OF PURE CULTURE

To maintain pure culture for extended periods in viable condition without any genetic change is referred as Preservation.

During preservation most important factor is to stop microbial growth or at least lower the growth rate.

Due to this toxic chemicals are not accumulated and hence viability of microorganism is not affected.

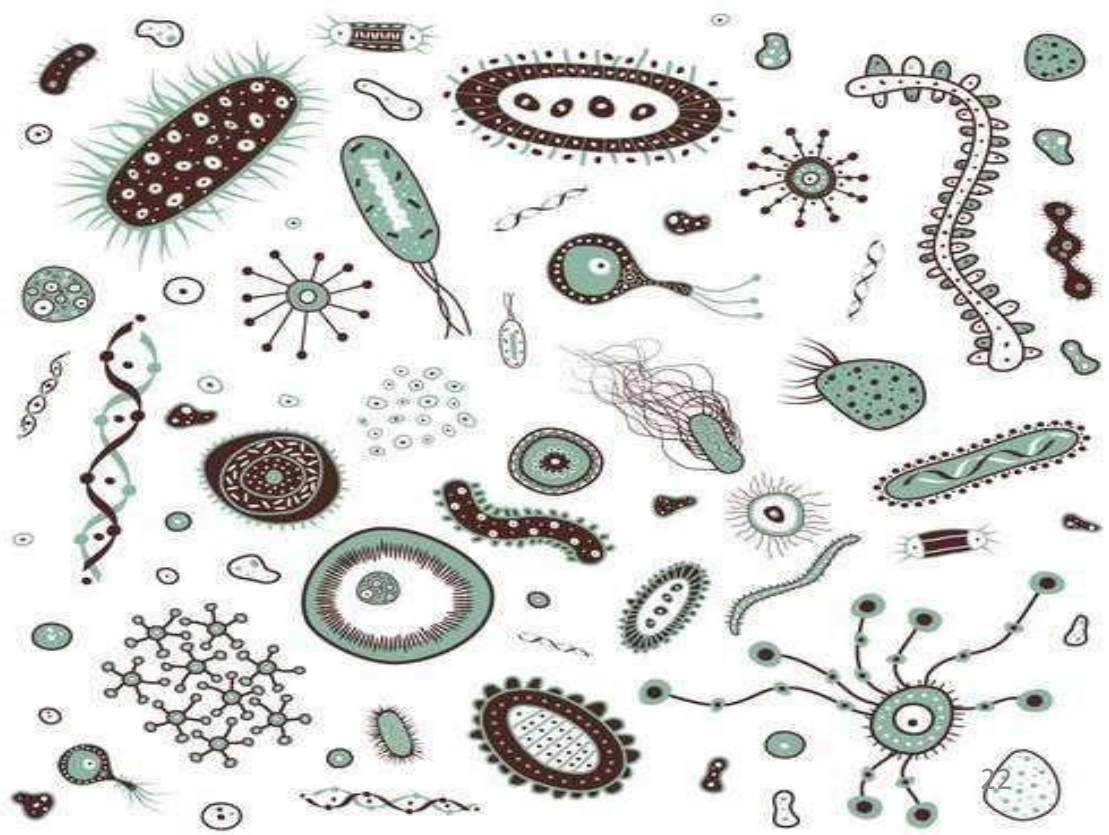
Objectives of preservation

1. To maintain isolated pure culture for extended periods in a viable conditions.
2. To avoid contamination
3. To restrict Genetic Mutation

Why to Preserve Bacteria?

- In nature there are only 1% bacteria which is pathogenic and harmful to Animalia and Plantae.
- 99% of bacterial populations are of economic importance for human beings and plants.
- In soil for nutrient uptake in food industry, in sewage treatment, in medical industry.
- So the preservation of bacteria is one of the most profitable practice economically as well as environmentally.

1. Academic purpose
2. Reserch Purpose
3. Biotechnology field
4. Fermentation Industry



Preservation methods of Bacteria

1. Periodic trnsfer to fresh medium
2. Storage at low temprature
3. Storage in sterile soil
4. Preservation by overlaying culture with mineral oil
5. Lyophillization or freeze drying

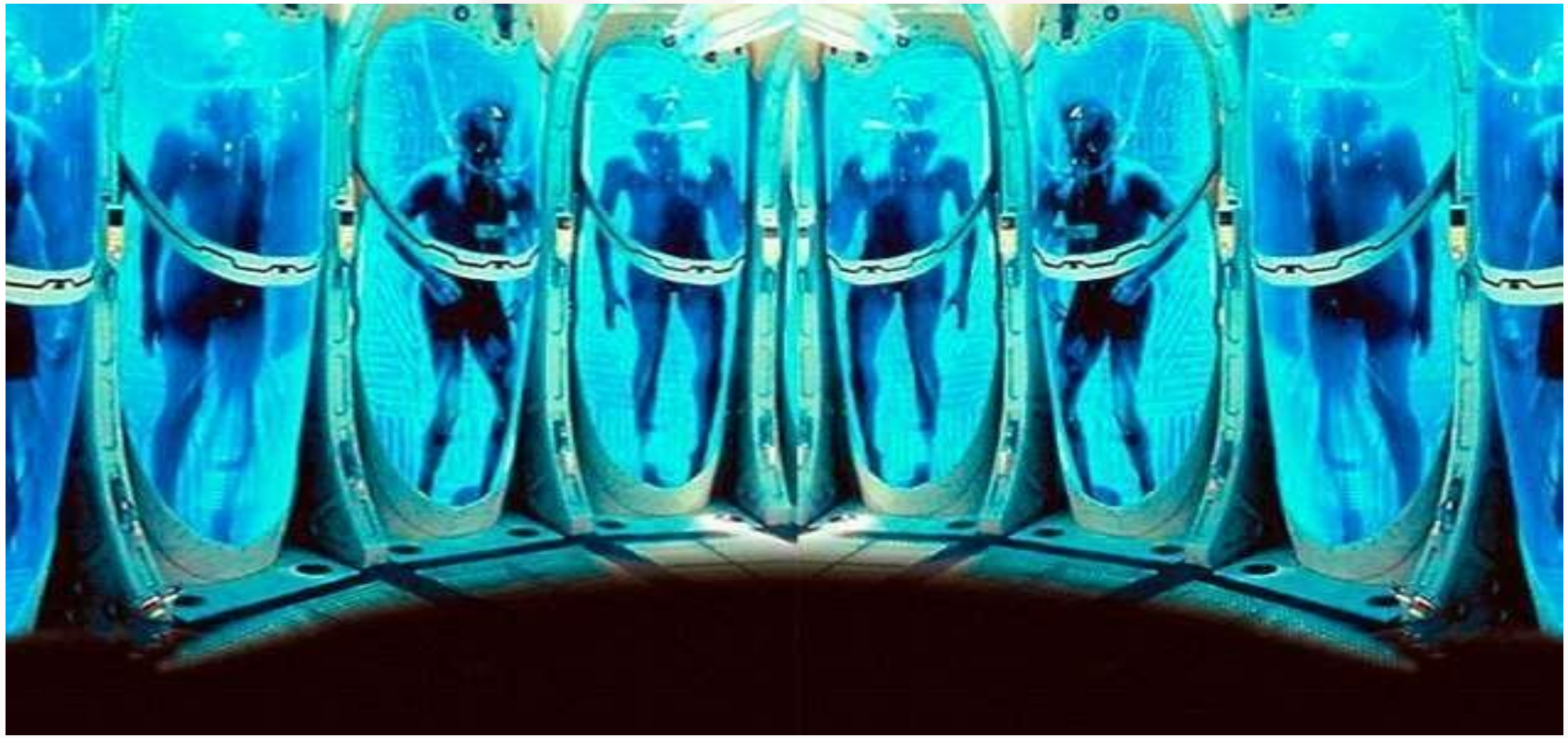
1. Periodic transfer to fresh medium

- Strains can be maintained by periodically preparing a fresh culture from the previous stock culture.
- The culture medium, the storage temperature, and the time transfers are made vary interval at which the with the species.
  - The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible.
  - Many of the more common heterotrophs remain viable for several weeks or months on a medium like Nutrient Agar.
  - The transfer method has the disadvantage of failing to prevent changes in the
  - characteristics of a strain due to the development of variants and mutants.

2. Storage at low temprature

1. REFRIGERATION

2. CRYOPRESERVATION



1. REFRIGERATION

- Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms.
- This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped.
- Thus their growth continue slowly, nutrients are utilized and waste products released in medium.
- This results in finally the death of the micro

2. CRYOPRESERVATION

- Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times.
- In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at - 196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival.
- This liquid nitrogen method has been successful with many species that cannot be preserved by lyophilization and most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics, however this method is expensive.

3. Storage in sterile soil

Storing organisms in soil fall into two groups;

1 sterile soil infested with small amount of inoculum,immediately dried and stored in refrigerator.

2 Soil infested with the organism,than incubatedallowing

The organism to grow;thus the mycelium and propagative unitof second generation are preserved.

The soil preservation method is useful for fungi,and by this method actinomycetes are maintained in soil for 4 to 5 years,and there are several bacterial spp which are also maintained in soi for several years.

4.Preservation by overlaying culture with mineral oil

1. This is a simple and most economical method of maintaining pure cultures.
2. In this method, sterile liquid paraffin is poured over the slant (slope) of culture and stored upright at room temperature. The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium.
3. This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture can be preserved form months to years (varies with species).

ADVANTAGES

1. We can remove some of the growth under the oil with a transfer needle, inoculate a fresh medium, and still preserve the original culture.
2. The simplicity of the method makes it attractive, but changes in the characteristics of a strain can still occur.

Paraffin Method/ Preservation by Overlaying Cultures with Mineral Oil

- Simple, most economical method.
- Agar slants are inoculated & incubated.
- Then, covered with sterile mineral oil to a depth of 1cm above the tip of slant surface.
- Transfers are made by removing a loop full of growth- touching the tip to the glass surface to drain off excess oil- inoculating a fresh medium- preserving the initial stock culture.
- Functions- providing an aerobic condition, prevents the dehydration of the medium and decreases the metabolic rate of the organisms.

5. Lyophillization or freeze drying

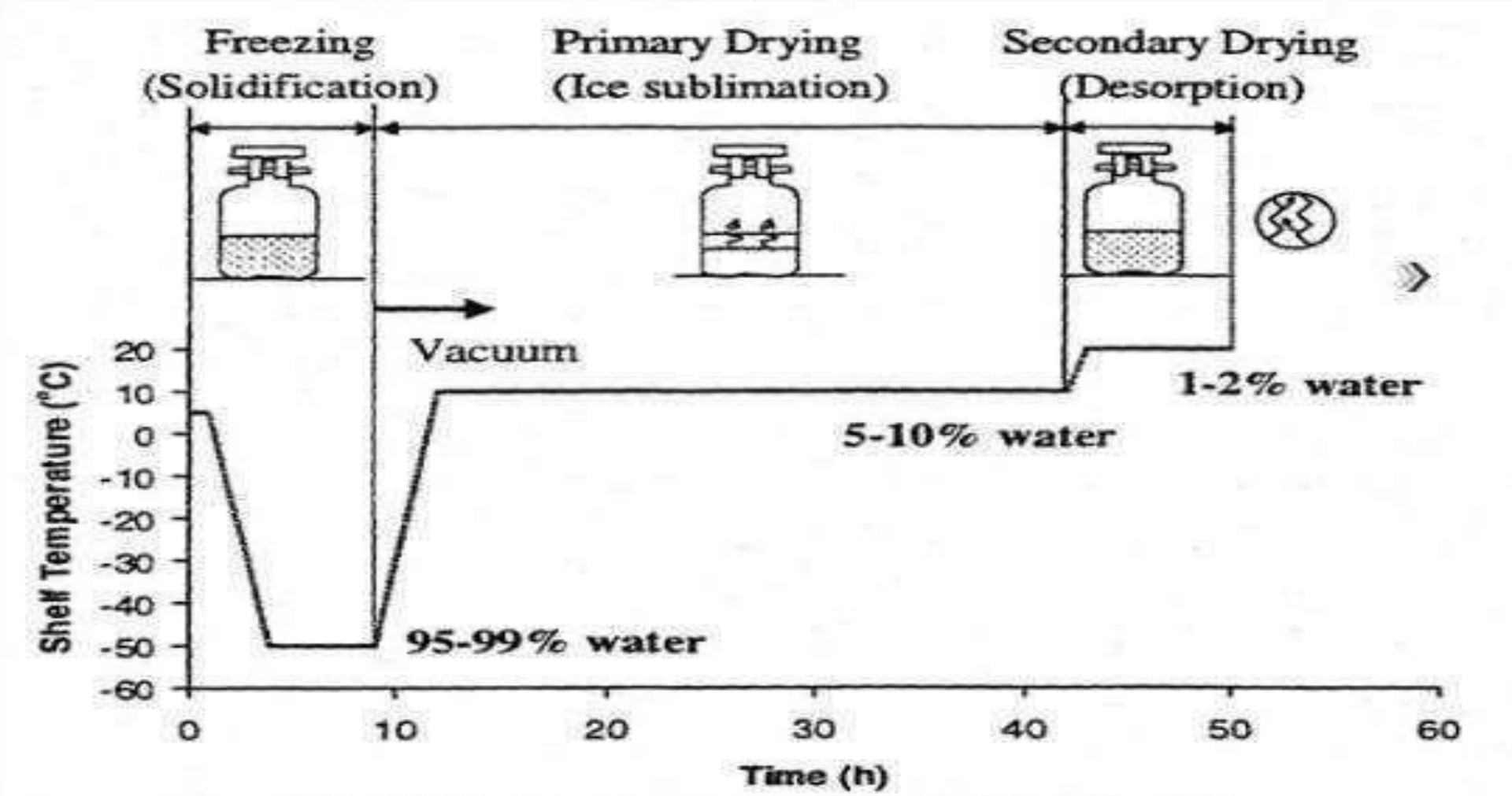
- Freeze drying is a stabilizing process in which a substance is first frozen and then the quantity of the solvent is reduced, first by sublimation (primary drying stage) and then desorption (secondary drying stage)
- Better preservation occurs with freeze-drying than with other methods because freeze-drying reduces the risk of intracellular ice crystallization that compromises viability
- Removal of water from the specimen effectively prevents this damage
- Lyophilization is greatest with gram-positivebacteria (spore formers) and decrese with gram -negative bactetia but viability can be maintained as long as 30 years.

- Large numbers of vials of dried microorganisms can be stored with limited space, and organisms can be easily transported long distances atroomtemperature

- The process combines freezing and dehydration~ Organisms are initially frozen and then dried by lowering the atmospheric pressure with a vacuumapparatus

- Specimens can be connected individually to the condenser (manifold method) or can be placed (in a chamber) where they are dehydrated in one larger airspace

Lyophillization Process



StorageVials

1. Glassvials are used for allfreeze-driedspecimens
2. When freeze-drying is performed in a chamber, double glass vials are used

outer soft gla

e dehydratedspe imen

placed in th bot om vial before

otton

used

d he dry

CryoprotectiveAgents

- Skim milk for chamberlyophilization,and
- sucrose for manifoldlyophilization

ADVANTAGES

- Removal of wateratlow temperature
- Thermolabilematerialscanbe dried.
- Sterilitycanbemaintained.
- Reconstitutioniseasy

DISADVANTAGES

- Many biological molecules are damaged by the stress associated with freezing, freeze- drying, orboth.

sive damage to molds, protozoa,

be stored by thismethod

high porosity and la urface area.