

# CULTURE MEDIA

Bacteria have to be grown (cultured) for them to be identified.

By appropriate procedures they have to be grown separately (isolated) on culture media and obtained as pure for study.

## Agar

Used for preparing solid medium Obtained from seaweeds .

No nutritive value

Not affected by the growth of the bacteria.

Melts at 98°C & sets at 42°C

2% agar is employed in solid medium.

## **Types of culture media**

1. Based on their consistency .I

- a) solid medium
- b) liquid medium
- c) semi solid medium

2. Based on the constituents/ ingredients .II

- a) simple medium
- b) complex medium
- c) synthetic or defined medium
- d) Special media

## **Special media**

Enriched media

Enrichment media

Selective media

Indicator media

Differential media

Sugar media

Transport media

Media for biochemical reactions

## **Based on Oxygen requirement**

- Aerobic media
- Anaerobic media

## **Simple media / basal media**

- Eg: Nutrient broth, Nutrient Agar
- NB consists of peptone, meat extract, NaCl,
- NB + 2% agar = Nutrient agar .

## **Complex media**

Media other than basal media.

They have added ingredients.

Provide special nutrients

### **Synthetic or defined media**

Media prepared from pure chemical substances and its exact composition is known

Eg: peptone water – 1% peptone + 0.5% NaCl in water.

### **Enriched media**

Substances like blood, serum, egg are added to the basal medium.

Used to grow bacteria that are exacting in their nutritional needs.

Eg: Blood agar, Chocolate agar.

### **Enrichment media**

Liquid media used to isolate pathogens from a mixed culture.

Media is incorporated with inhibitory substances to suppress the unwanted organism.

Eg:

Selenite F Broth – for the isolation of Salmonella, Shigella

Alkaline Peptone Water – for Vibrio cholera.

### **Selective media**

The inhibitory substance is added to a solid media.

Eg: Mac Conkey's medium for gram negative bacteria

TCBS – for V.cholerae

Wilson and Blair medium – S.typhi

Potassium tellurite medium – Diphtheria bacilli

### **Indicator media**

These media contain an indicator which changes its colour when a bacterium grows in them.

Eg: Blood agar

Mac Conkey's medium

Christensen's urease medium

### **Differential media**

A media which has substances incorporated in it enabling it to distinguish between bacteria.

Eg: Mac Conkey's medium

Peptone

Lactose

Agar

Neutral red

Taurocholate

Distinguish between lactose fermenters & non lactose fermenters.

Lactose fermenters – **Pink** colonies

Non lactose fermenters – colorless colonies.

### **Sugar media**

Media containing any fermentable substance.

Eg: glucose, arabinose, lactose, starch etc.

Media consists of 1% of the sugar in peptone water.

Contain a small tube (Durham's tube) for the detection of gas by the bacteria.

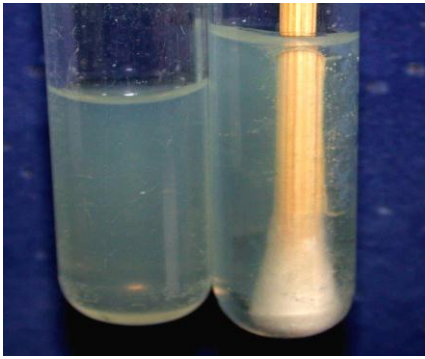
### **Transport media**

Media used for transporting the samples.

Delicate organisms may not survive the

time taken for transporting the specimen without

a transport media.



Eg:

Stuart's medium – non nutrient soft agar gel containing a reducing agent

Buffered glycerol saline – enteric bacilli .

# CULTURE METHODS

**Culture methods** include:

Streak culture

spreading 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.

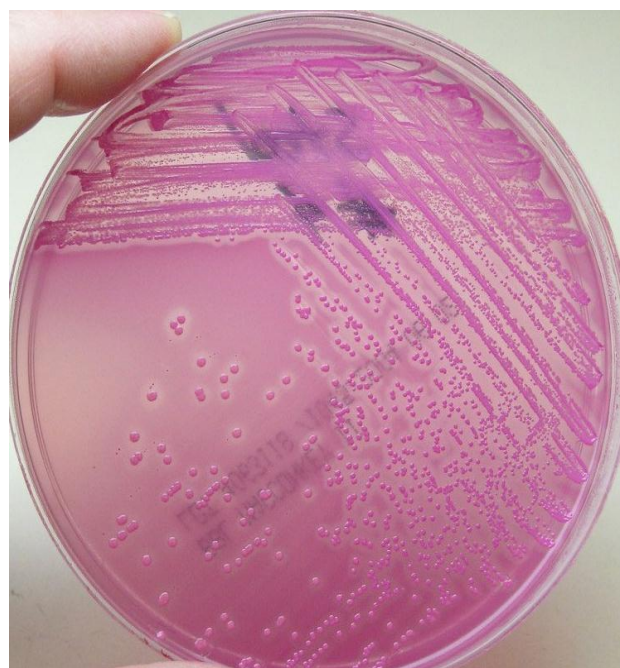
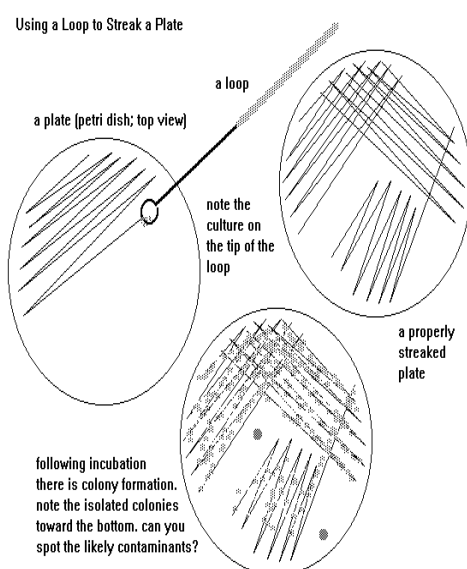
Platinum wire or Nichrome wire is used.

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.



## **SPRAEDING CULTURE**

Provides a uniform surface growth of the bacterium.

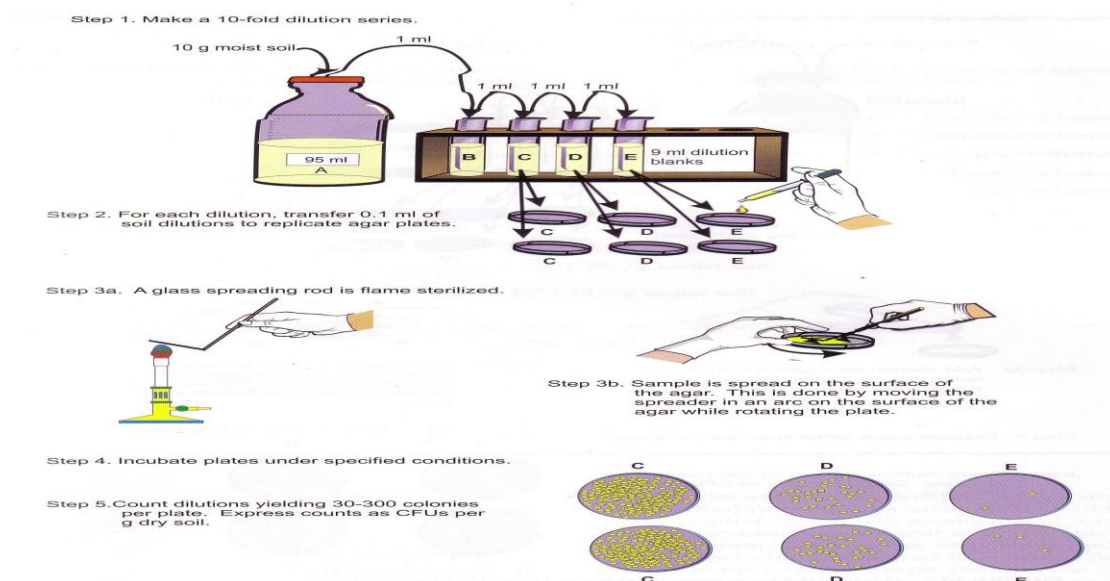
Uses:

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.



## **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 stroke 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.

### LIQUID CULTURES

Liquid cultures are inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.

Uses:

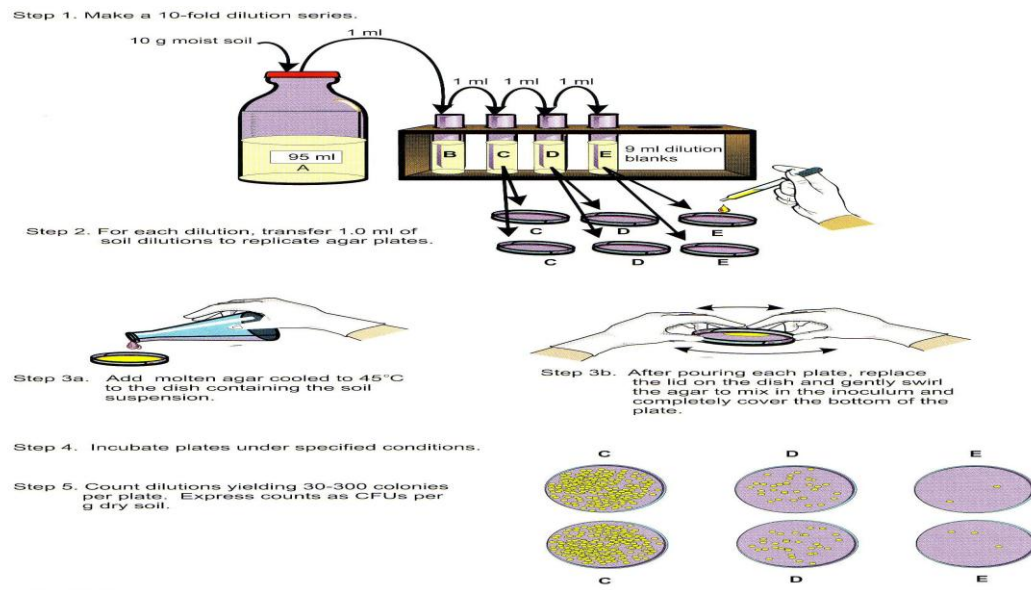
Blood culture

Sterility tests

Continuous culture methods

Disadvantage

It does not provide a pure culture from mixed inocula.



### ANAEROBIC CULTURE METHODS

Anaerobic bacteria differ in their requirement and sensitivity to oxygen.

*Cl. tetani* is a strict anaerobe – grows at an oxygen tension < 2 mm Hg.

#### Methods:

Production of vacuum

Displacement of oxygen with other gases

Chemical method

Biological method

Reduction of medium

#### Production of vacuum:

Incubate the cultures in a vacuum desiccators.

#### Displacement of oxygen with other gases

Displacement of oxygen with hydrogen, nitrogen, helium or CO<sub>2</sub>.

Eg: Candle jar