Culture Media

Culture Media used in Microbiology

Culture Media

- Considered as source of energy and provide certain environmental conditions in order to grow and produce bacteria.

- Depending on the physical states of the media and combination of nutrients, different categories of media can be made.

Why Using Culture Media?

The purpose of using cultural techniques is to:

- Demonstrate the presence of organisms in the specimens.

- Test the susceptibility of pathogens to antimicrobial agents.

Culture Media

A) Physiological requirements:

C.HOPKENS

- C= Source of carbon
- H= Hydrogen
- O= Oxygen
- P= Phosphorus
- K= Potassium
- E= Electrolytes
- N= Source of nitrogen
- S= Sulfur

Culture Media

B) Environmental conditions:

1- Oxygen (O2)

Obligatory aerobe, obligatory anaerobe, facultative anaerobe, Aerotolerant, and microaerophilic.

2- Temperature

Thermophile, mesophile, and psycrophile

3- PH

Neutrophile, basophile, and acidophile .

4- Osmotic pressure

Isotonic, hypotonic, and hypertonic

Common Contents of Culture Media

- **Water:** essential for bacterial growth.
- **Peptone:** it is hydrolyzed animal or plant protein, used as a source of nitrogen which help bacteria to make a.a. and proteins.
- **Meat extracts:** it provide the bacteria with amino acid, vitamins, mineral salts (phosphate and sulphate).
- **Yeast extract:** used to stimulate the growth of bacteria

- **Mineral salts:** media should contain little amount of: Mg, k, Fe, Ca>> which is essential for stimulate bacterial enzyme activity.

- **Carbohydrates:** to provide the bacteria with energy and carbon.

- **Agar:** its inert polysaccharide extracted from sea-weed or marine algae.

➢ Used as solidifying agent.

➢ Dissolves at 90-100 C

➢ Solidify at 45 C

Media Preparation

Equipment:

- Media powder.
- Water (100 ml).
- Balance.
- Flask (larger than the size of media volume).
- Weighing plate.
- Weighing spatula
- Cylinder.
- Bacti-incinerator.
- Autoclave.
- Sterile empty Petri dishes.
- Autoclave tape.
- Aluminum foil.

Procedure

- Measure out the required volume of the media,

➢ e.g. 40 gm of media for 1000 ml of water, let's say u want to prepare 100 ml of media

40 ↖↗ 1000

X ↙↘ 100

= 4 gm of media for 100 ml of water.

- Put the media powder in the flask.
- Add the water onto the media.
- Mix well.

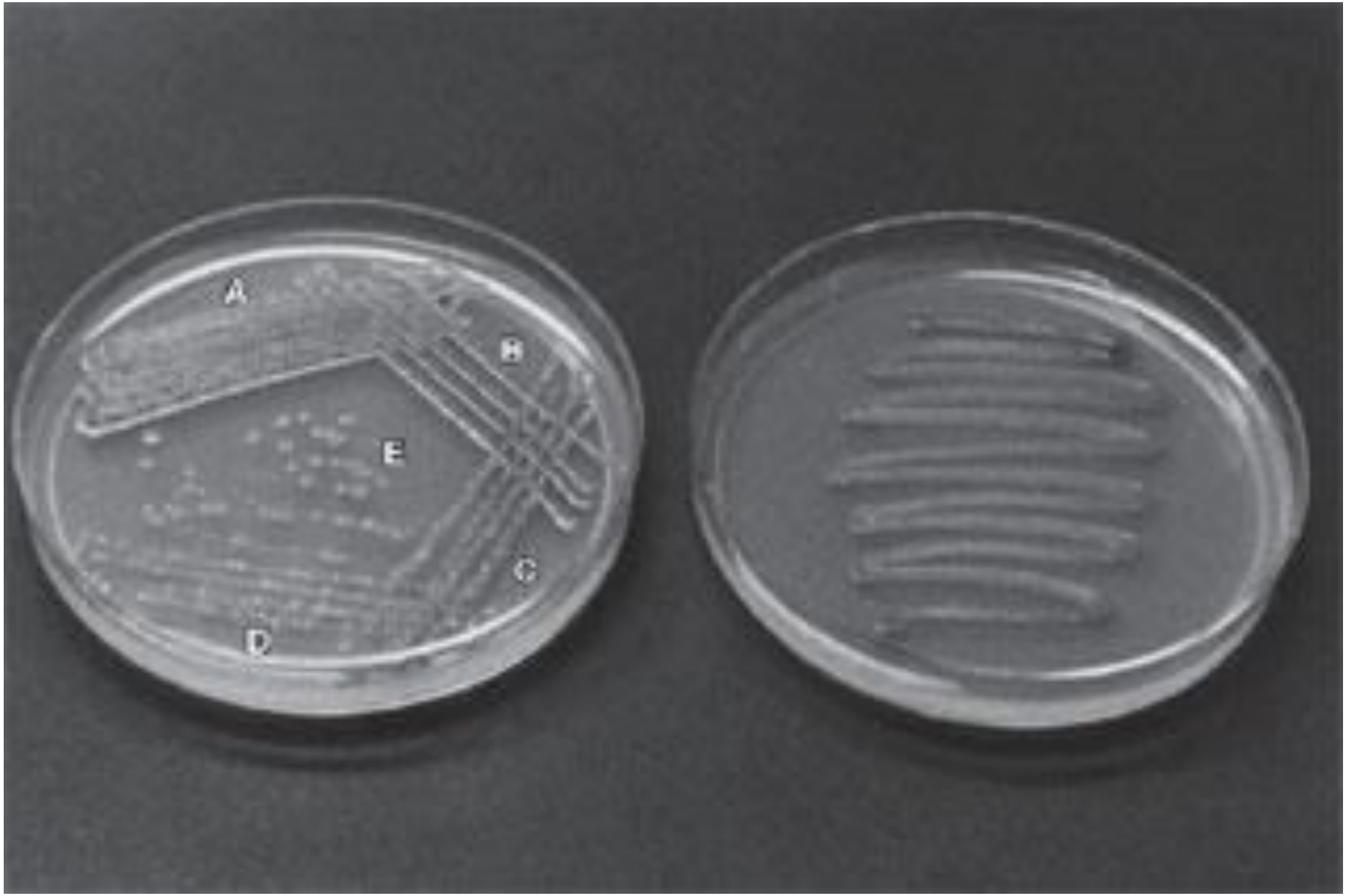
- Cover the flask with aluminum foil.
- Stick the autoclave tape on the flask.
- Sterilize the media in autoclave 15-20min at 121 °C
- Leave to cool at room temperature (to 45-50 °C).
- Pour the media in petri dish after labeling.
- Leave to solidified at RT.

Inoculation

It is streaking (putting or culturing) bacteria on agar plate, allow the bacteria to grow to produce isolated colonies, and pure culture.

- **Pure culture:** culture containing a single species of organism.
- **Mixed culture:** culture containing more than one species of organism.
- **Contamination culture:** a bacterial culture that has acquired unwanted organisms.

- In order to obtain well-isolated colonies, the **quadrant streak technique** should be used.


A
B
C
D
E

Quadrant Streak Technique

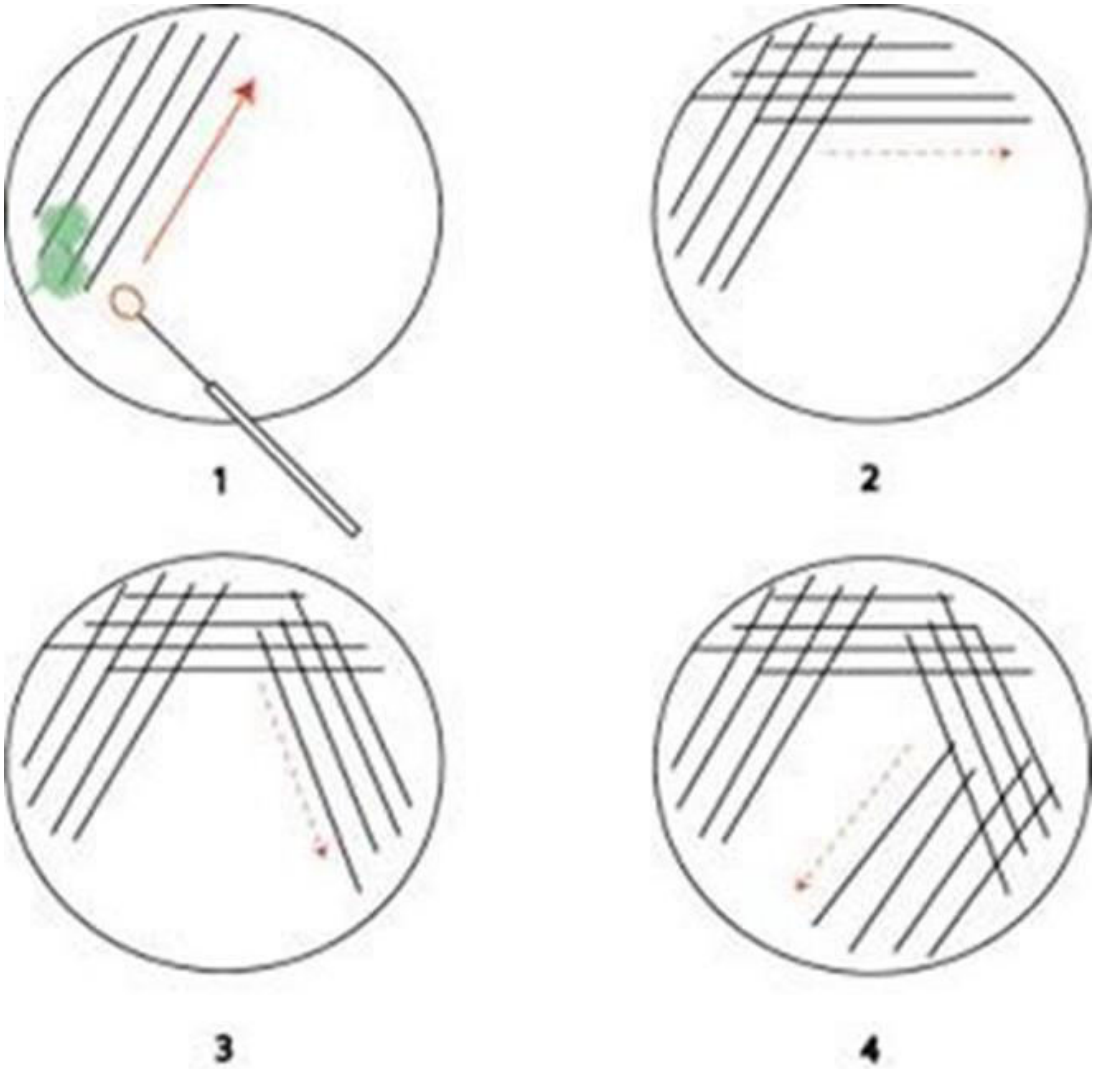
Equipment:

- Bacti-incinerator.
- Loop.
- Subculture media.
- Agar media plane.
- Marker.

PROCEDURE

- Sterilize the loop by bacti-cinerator until it is red then allow to cool.
- Take a loopful of bacteria from the subculture media
- Immediately streak the inoculating loop VERY gently over a quarter of the plate around 4-5 lines (quadrant 1).
- Sterilize the loop then allow to cool.
- Go back to the edge of the area 1, extend the streaks into the second quarter of the plate (quadrant 2).
- Sterilize the loop then allow to cool.
- Go back to the edge of the area 2, extend the streaks into the third quarter of the plate (quadrant 3).

- Sterile the loop then allow to cool.
- Go back to the edge of the area 3, extend the streaks into the forth quarter of the plate in zig zag lines (quadrant 4).
- let the bacteria to grow at 37 C° for 24 hr in the incubator.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