FE 204 Experiment 1

Preparation of Bacteriological Media and Sterilization

Contents

- Media and types of media
- Preparation of General Purpose Media
- Procedure
- Sterilization techniques
- Working principle of autoclave

Media and Types of Media

Definition: Any material which supports the growth and replication of microorganisms is called **Medium (pl. media).**

- Liquid Media

Generally called as **Broth**.
For ex: Nutrient broth

- Solid media

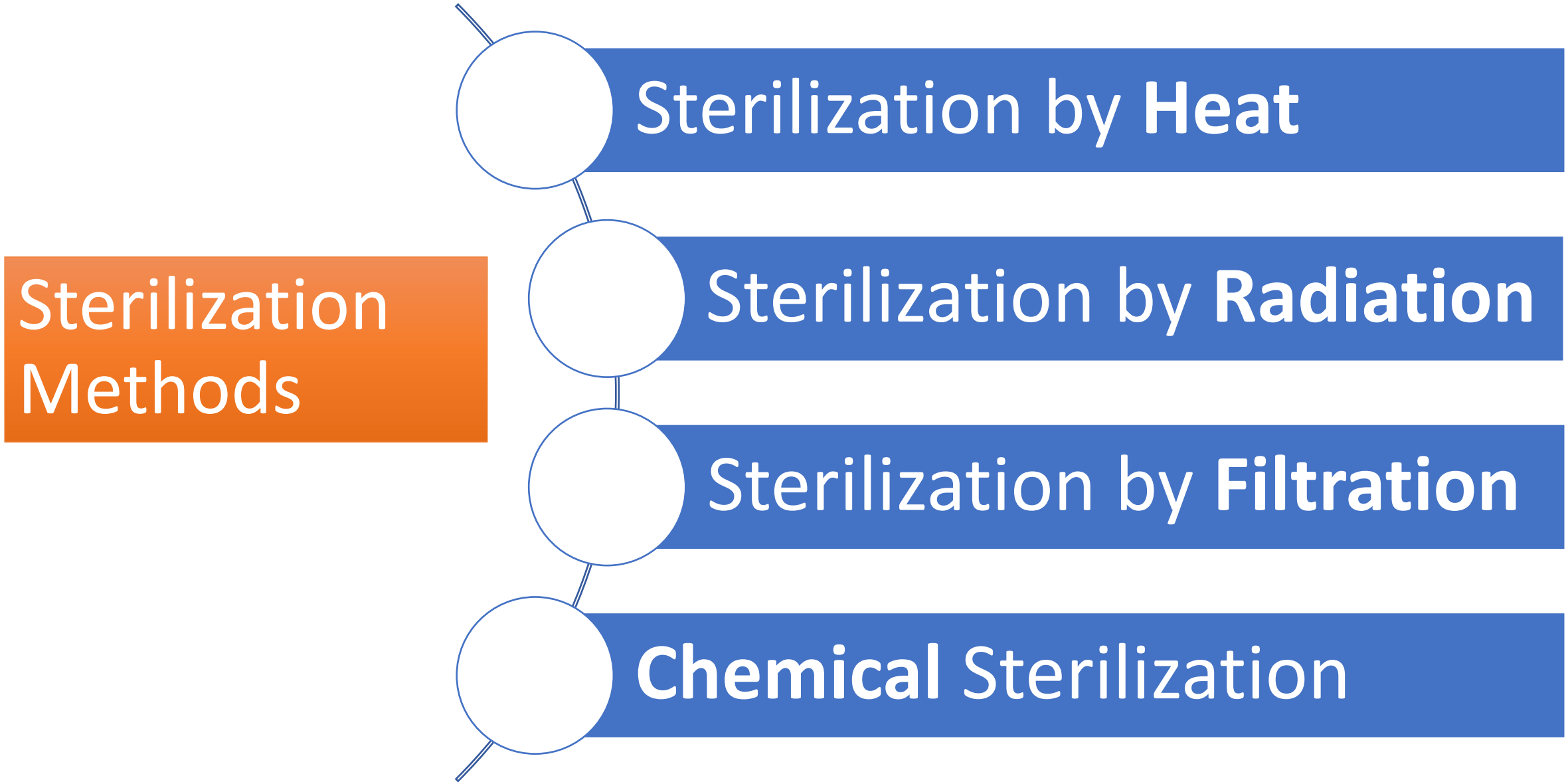
Generally called as **Agar**.
For ex: Nutrient agar

Any broth media can be turned into a solid media by adding agar agar into broth with a 2.5% ratio of volume.

Special Purpose Media

- Anaerobic media
- Synthetic media
- Transport media
- Enriched media
- Selective media
- Differential media
- Selective & Differential media
- Microbiological assay media

- **Anaerobic media**: Media supporting growth of only anaerobic m/os O2 is removed from the media.
- **Synthetic media**: referred to as chemically defined medium which their concentrations are known.
- **Transport media**: are used for temporary storage maintaining the number of m/os during trnsportation.
- **Enriched media**: contain nutrients which supports all types of microorganisms.
- **Selective media**: support the growth of a certain type of m/o while inhibiting the growth of others.
- **Differential media**: contain indicators that distinguish between organisms on the basis of their appearance on the medium.
- **Selective & Differential media**: hold the properties of both selective and differential media.
- **Microbiological assay media**: are used to measure concentrations of substances such as antibiotics and vitamins. For ex: Muller-Hinton Agar.


Sterilization Methods
Sterilization by **Heat**
Sterilization by **Radiation**
Sterilization by **Filtration**
Chemical Sterilization

Autoclave

- For sterilization of materials and media
- 121 C for sterilization of media
- 134 C for sterilization of waste and dish

Oven and Incubators

- Dry air sterilization Oven 180 C

- Incubator
- 25, 37 and 45 C

Materials used in LAB

- 250 ml beaker
- 500 ml flask
- 10 ml pipettes
- Sterile petri dishes
- Nutrient agar
- Nutrient broth
- Sensitive balance
- Magnetic stirrer
- Peptone
- Electrical heater
- Clean spatula

Dehydrated media

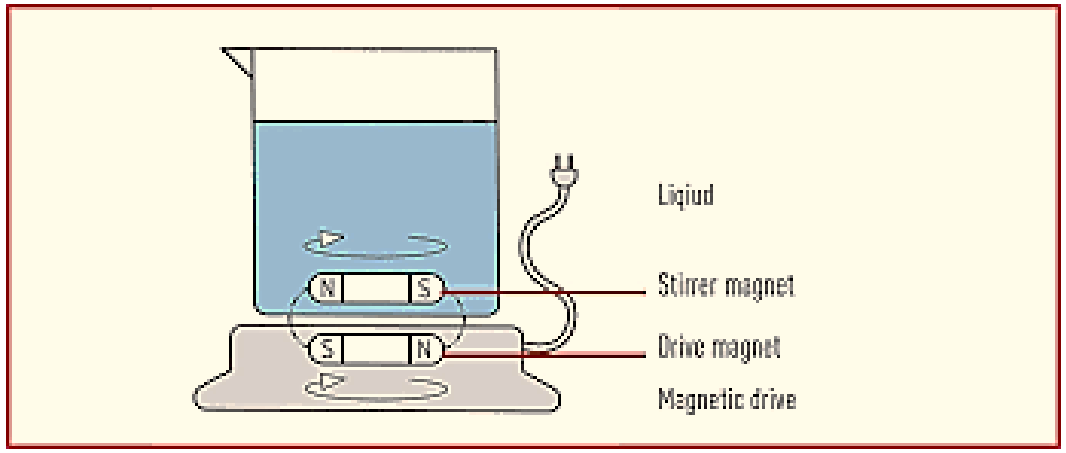
Preperation Materials

- Balance
- Spatules and spoons

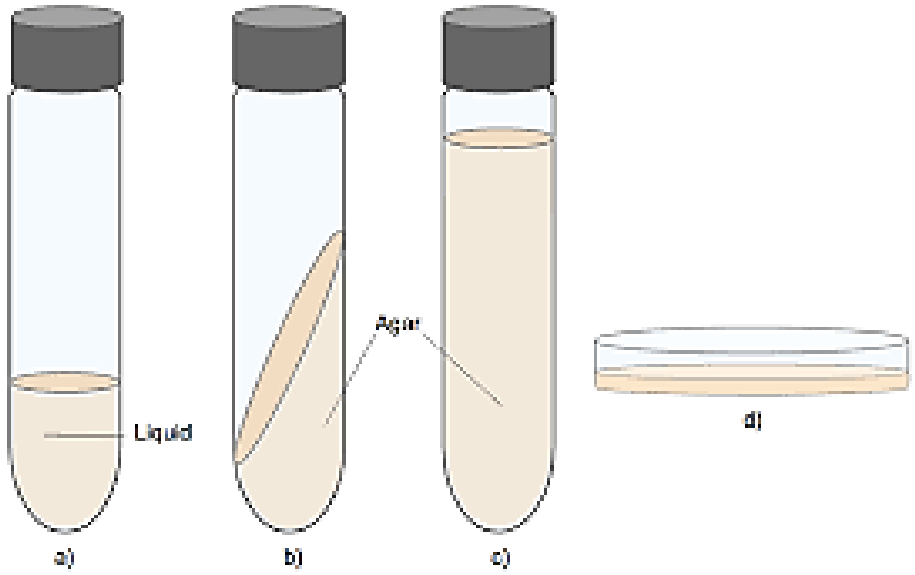
Pipette and Petri Dishes

Magnetic Stirrer

- Mixing and heating solutions homogenously.



Procedure



Medium	Amounts	Container	Sterilization
a) Broth	5-10 ml	Test Tube	In Autoclave
b) Agar Slants	10 ml	Test Tube	In Autoclave
c) Agar Deep	7 ml	Test Tube	In Autoclave
d) Agar Petri Plates	20 – 25 ml	Petri Dish	Media → In autoclave Petri Dishes → In Oven

Procedure

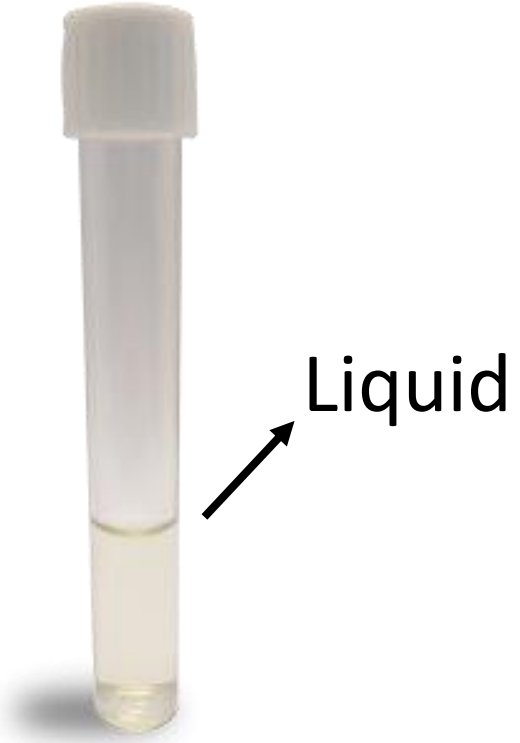
- Preparation of Nutrient broth:
- Calculate ingredients to make 100 ml of Nutrient broth by looking the formula given on the box of media.

Preparation

Suspend 25 g Standard I Nutrient Broth/litre, autoclave (15 min at 121 °C).

pH: 7.5 ± 0.2 at 25 °C.

The prepared media are clear and yellowish-brown.



Procedure

- Preparation of Nutrient broth:
- Weigh the required calculated amount on balance and put inside a 250 ml beaker

- Add 100 ml distilled water and mix by magnetic stirrer until getting a clear solution without any suspended powder.

Procedure

- Preparation of Nutrient broth:
- Dispense into test tubes of 5ml amount and place inside a tube-rack.

- Sterilize all of the tubes in autoclave at 121 C for 20 min. After sterilization they are ready to use.

Procedure

- Preparation of Nutrient agar:
- Calculate ingredients to make **200 ml of Nutrient agar** by looking the formula given on the box of media.

- Weigh the required calculated amount on balance and put inside a 250 ml baloon/flask.

- Add 200 ml of distilled water, place magnetic stirrer and mix the ingredients and heat by electrical heater.

Agar Slant

Agar Deep

Agar Petri Plate

Heat until boiling

Procedure

- Preparation of Nutrient agar:
- Continue to mix and shake until boiling the whole suspension in the flask. Careful about not spilling around the neck.
- After a clear solution obtained. Distribute into test tubes for;
- Agar slant and agar deep
- Keep rest in flask for petri plates.
- Sterilize in autoclave at 121 C for 20 min.

Mix and heat

Pour 10 ml of hot molten agar for agar slant

Agar Slant

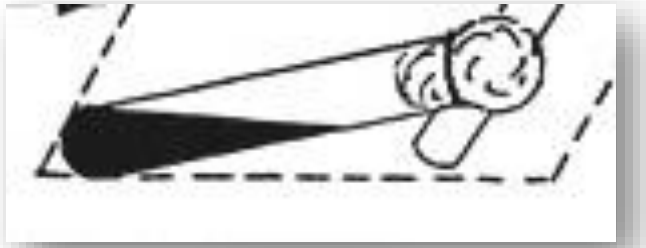
Pour 7 ml of hot molten agar for agar deep.

Agar Deep

Procedure

After sterilization of agar tubes and flask.

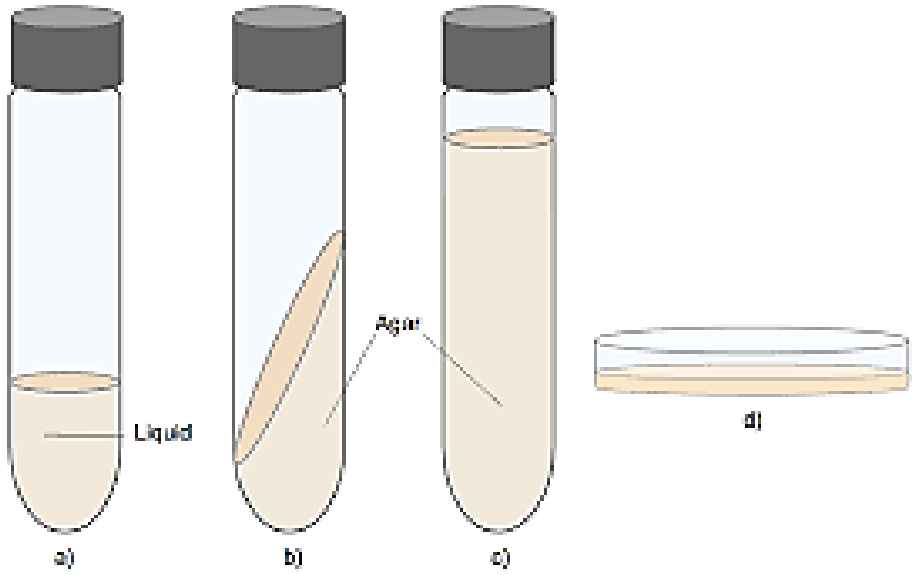
- For Agar slant;
  - Keep the tubes at slant position lay down horizontally at room temperature to solidify.



- For Agar Deep;
  - Keep the tubes at vertical position until solidify.

- For Agar Petri Plates;
  - Pour 20-25 ml of Molten Agar in Sterile Petri Plates at aseptic conditions and keep them at room temperature to solidify.

Result



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