Practical Bacteriology
lab. 2

Types of Culture media, culturing, and pure culture methods

By:

Lecturer Shiama'a Al-Salihy

TYPES OF CULTURE MEDIA

- **Culture media** can be defined as artificial media contain basic requirements needed for microorganisms' growth. Can be contained either in test tubes, plates, flasks or screw-capped bottles…etc.
- **common ingredients for culture media:**

- Peptone
- Meat extract.
- NaCl: for isotonic environment
- H_2O. *
- Agar: it provides no nutritional benefits but used for solidifying purposes.*
- PH: neutral pH (7.2-7.4), obtained by adding of NaOH or HCL

Types of culture media:

Culture media can be divided according to:

- **physical state (consistency):**

1. **liquid media:** they do not contain agar, used for primary cultivation, suitable for blood culture, its disadvantage is the inability to observe colony morphology, e.g., nutrient broth, peptone water.
2. **Solid media:** they are made by adding a solidifying agent to a liquid media. Agar is added in percentage of 1.5-2 %, they are used for identification of colony morphology, e.g. blood agar.

Types of culture media:

3. **Semisolid media:** contain 0.4-0.8% of agar used for cultivation of spirochetes and studying motility, e.g. SIM (Sulfide, Indole, Motility)
4. **Biphasic media:** culture system composed of both liquid and solid media in the same bottle. The inoculum is added to a liquid medium, to subculture the inoculum, the bottle simply tilted to allow the liquid to flow over the solid medium, e.g. Castaneda system for blood culture.

Castaneda system

Semisolid medium (SIM)

Types of culture media:

➢ Uses of media:

A. Simple (basal) media: these media contain only common ingredients, used for cultivation of non fastidious microorganism, e.g. nutrient broth, nutrient agar.

B. Special media: these media contain common ingredients plus other substances. There are eight types:

1. **Enriched media:** prepared by adding blood, serum or vitamins to a basal media for supplying the growth of fastidious bacteria, e.g. blood agar
2. **Selective media:** contain inhibitory substances like bile salt, antibiotics or dyes, which favor the growth of certain microorganisms and inhibit growth of others, e.g. macConkey's agar and mannitol-salt agar

Types of culture media:

3. **Differential media:** contain substances which make certain species of bacteria to produce characteristic growth or effects in the medium that can easily recognized, e.g. MacConkey's agar, blood agar.
4. **Enrichment media:** this media allow the grow and enrich for one type of bacteria (fastidious one) and inhibits other, e.g. Selenite-f media.
5. **Transport media:** certain microorganism is weak and dies rapidly between time of specimen collection and examination so it needs a special media for transport, e.g. Stuart's medium.

Types of culture media:

6. **Indicator media:** use the usual changes in the color of an indicator due to microorganism metabolism as a diagnosis feature, e.g. sugar media, litmus milk.
7. **Sensitivity media:** a special media used to test antibiotic sensitivity for given microorganism, e.g. Mueller-Hinton media.
8. **Anaerobic media:** used for cultivation of anaerobic bacteria, contain reduced oxidation-reduction potential, e.g. thioglycollate medium

Pure Culture Methods

Pure culture methods:

Pure culture means a single kind of microorganisms growing alone in protected environment. Four methods are widely used for identification of pure culture from clinical specimens containing mixed microorganisms.

1) **Streak plate method:** this method is employed for the isolation of pure culture of bacteria from mixed population, e.g. sputum, urine, stool, pus from infected wound and abscess.

Pure culture methods:

❖Technique of Streak Plate Method:

- Sterilize the loop in a bunsen burner flame, cool it and streak the specimen over an area A
- Re-sterilize the loop, cool it and then streak over an area B.
- Continue the streak in the same manner to areas C&D.
- Incubate the plates at 37° C for 24 hrs.

Pure culture methods:

2) **Pour plate method:** this technique deals with the isolation of pure culture from mixed bacterial suspension by serial dilution and plating.

❖ **Technique of Pour Plate Method:**

- Make serial dilution of the specimen (food, stuff, water… etc.
- Mix the dilutions of the specimen with melted media in tubes
- Quickly pour the content of each tube into sterilized plates.
- Incubate the plates after solidification at 37° C for 24 hrs.

Pure culture methods:

3) Spread plate method:

- Dilute sample
- Pour on agar plate
- Spread by glass spreader.

4) Selective environment: application of selective conditions like high temperature and O2 or CO2, or use selective media like MacConkey's agar.

❖There are many ways to inoculate bacteria in the media:

1. Inoculation: loop
2. Pipetting: pipette
3. Stabbing: needle
4. Swabbing: cotton swab
5. Spreading: spreader
6. Streaking: loop


Thank You
For
Listening