

**COVENANT UNIVERSITY
NIGERIA**

*TUTORIAL KIT
OMEGA SEMESTER*

PROGRAMME: MICROBIOLOGY

COURSE: MCB 121

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MCB 121: Laboratory Techniques in Microbiology

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1. Differentiate between a liquid medium and a solid medium
2. Discuss the major types of media used in the microbiology laboratory.
Give examples of the different types
3. What is a pure culture?
4. Write a short note describing a mixed culture
5. Stock cultures are.....
6. Write short notes on the different culturing techniques used in the
Microbiology laboratory for culturing microorganisms
7. Briefly describe the biochemical test that is used for the following:
 - a. Identification of a motile bacterium.
 - b. Differentiating between pathogenic and non-pathogenic Staphylococci.
 - c. Differentiating between a bacterium that converts glucose to acidic products and one that converts glucose to acetoin.

Answers

1. There are two main groups of culture media
 - Liquid Medium also referred to as broth
 - Solid Medium

The difference is basically on whether or not a solidifying agent is added

- **Liquid medium** - This is just water to which the appropriate kinds and concentrations of nutrients have been added.
 - **Solid Medium** - This is a liquid medium to which a solidifying agent is added. The solidifying agent is agar. It is also called agar agar
 - **Agar or Agar agar** - Agar agar is a polysaccharide extract derived from seaweed, and it has gelling property. It has a molecular weight of between 11,000 KD and 100,000kD. Agar can remain solid at temperatures as high as 100°C but when molten it resolidifies below 45°C. It is added to media at a final concentration of between 1.2% and 2%. There are other gelling agents. Gelatin is another example. The advantage of agar over gelatin is that gelatin readily becomes liquid at 28°C
2. The Major groups of media are the following:
 - Routine Laboratory Medium
 - Differential Medium
 - Selective Medium
 - Enriched Medium
 - Enrichment Medium

Routine Laboratory Medium

- This medium is used for the cultivation of common microorganisms' e.g. nutrient agar or broth, peptone water, meat and yeast extract

Differential Medium

- Contains substances which enable a particular organism to differ visually from other organisms based on certain physiological reactions. Example is the Deoxycholate –citrate medium which contains neutral red indicator. On this medium, colonies of lactose fermenting organisms are red while those of non-lactose fermenters appear colorless

Selective Medium

- This medium consists of substance which inhibits the growth of certain organisms but not of others. Almost all media are selective because no single culture medium favors and supports the growth of a wide range of microbial groups.
- The MacConkey medium is used mainly for gram -negative and enteric bacteria because the bile salt and crystal violet in it inhibits the growth of Gram –positive bacteria and it is therefore selective for Gram –negative and enteric bacteria. The azide- dextrose broth is also selective for streptococci

Enriched Medium

- An enriched medium has certain substances such as blood and serum which is necessary for the growth of some fastidious organisms.
- Example is the fildes' broth. It is used for the cultivation of organisms requiring easily assimilated X and V factors, and they are present in blood contained in the medium.

Enrichment Medium

- This consist of substances which are capable of inhibiting the growth of one kind of organism while allowing unrestricted, growth of the desired organism which may be present in small numbers.
- These substances are therefore said to be enriching the population e.g. Salmonella and some strains of shigella will grow but most of the colliform and other bacteria which are inhibited by selenite will not grow in selenite broth.
- It is thus used in the enrichment and subsequent isolation of the organism from faeces or other sources suspected of faecal contamination.

3. **The pure culture Technique:** This is a term used to describe the technique employed by microbiologist to isolate, grow and maintain organisms of interest. A pure culture is therefore the organism of interest.

The pure culture technique used in the microbiology laboratory consists of the following steps.

- ❖ Preparation of a suitable medium for the organisms. This is so essential because many microorganisms grow in some laboratory basic media while others require specific and special media for their growth and reproduction.

- ❖ Sterilization of a medium is to remove or destroy all living organisms already present
Isolation of a pure strain of the organism
- ❖ Isolation of a pure strain of the organism
- ❖ Inoculation of sterile medium with the pure strain. Care should be taken to work strictly under aseptic conditions as contaminations particularly with similar organisms are not easily detected and in liquid cultures contaminations are hardly detected.
- ❖ Incubation of the inoculated plates

4. **A mixed culture:** In Microbiology, a mixed culture refers to a laboratory culture that contains more than one species of organism that are grown in a medium. Most pure cultures are usually derived from mixed cultures by methods of separating individual cells so that, when they multiply, each will form an individually distinct colony.

5. **A stock Culture:** A culture of a microorganism maintained solely for the purpose of keeping the microorganism in a viable condition by subculture, as necessary, into fresh medium.

6. Microorganisms are cultured in various ways, especially bacteria and therefore there are the following types of culture techniques.

- ✓ Broth Cultures
- ✓ Slant Cultures
- ✓ Stab Cultures
- ✓ Shake Culture
- ✓ Plate Culture
- ✓ Pour Plates
- ✓ Streak Plates

Broth Cultures: Broth cultures are also called liquid cultures. These are cultures or organisms grown in water to which nutrients have been added.

Slant (Agar Slope) Cultures: These consist of test tubes or small bottles to which molten solid medium (e.g. nutrient agar) has been added and allowed to set in a slanting or slopping position. The inoculum may be spread or streaked over the surface of the slope.

Stab Cultures: This is similar to the slant cultures except that the tubes containing solid media are allowed to cool at an upright (vertical) position. It is inoculated by plunging a long straight wire (carrying the inoculum) vertically into the middle of the tube.

Shake Cultures: Test tubes or bottles containing a solid medium are employed. The medium is warmed to 45°C, inoculated and mixed thoroughly by rotating the tube between the hands. The tube is then allowed to cool in an upright position.

Plate cultures are of two types

- Pour Plate

- Streak Plate
- For pour plate - The inoculum is added to a tube or flask of molten medium (45°C), mixed well and it is then aseptically poured into sterile plates.
- Streak Plate - The molten solid medium is poured into sterile plate and allowed to set. The surface of the medium is then streaked.

7. Motility test

The motility test is not a biochemical test but rather tests for the ability of bacteria to migrate away from a line of inoculation with physical features like flagella. To perform this test, the bacterial sample is inoculated into SIM or motility media using a needle to stab the medium in a straight line. After incubating the sample for 24-48 hours observations are made to see if the bacteria have migrated away from the original line of inoculation. If an organism is motile then the growth will radiate from the stab mark and make the entire tube appear turbid (positive test). Lack of migration away from the stab mark indicates a lack of motility (negative test result). *Pseudomonas aeruginosa* and *Proteus mirabilis* are motile.

3b. Coagulase Test

Coagulase test depends on the ability of an organism to produce coagulase enzyme. Coagulase is an enzyme that clots blood plasma notably human and rabbit plasma. This test is performed on Gram-positive, catalase positive bacteria to identify the coagulase positive *Staphylococcus aureus*. It is useful in differentiating the pathogenic from non-pathogenic staphylococci. Coagulase is a virulence factor of *S. aureus*. The formation of clot around an infection caused by this bacterium protects it from phagocytosis.

The test reagent is usually human or rabbit plasma. Rabbit plasma is placed in a tube and inoculated with one single colony. The colony is emulsified in the plasma and incubated at 37 degrees C for 24 hours.

- If the organism has coagulase it will clump the plasma.
- If the organism does not have coagulase it will not clump the plasma.

3c. MRVP (Methyl Red-VogesProskauer) Test

This test is used to determine two things. The MR portion (methyl red) is used to determine if glucose can be converted to acidic products like lactate, acetate, and formate. The VP portion is used to determine if glucose can be converted to acetoin. Both tests are used to differentiate species of the family *Enterobacteriaceae*. Two tubes of

glucose broth are inoculated with a loop. After 48 hours of incubation, add a few drops of MR to one tube, and VP reagents to the other tube.

- MR— a + result is red (indicating pH below 6) and a – result is yellow (indicating no acid production)
- VP—A + result is red after VP reagents are added (indicating the presence of acetoin) and a – result is no color change.