
بسم الله الرحمن الرحيم

العنوان

Culture media
Sixth modular unit

Use of culture media

انـهذف

To ***isolate,**

***Identify**

***and Study the characteristic of microorganism**.

It is essential to grow on artificial media which gives artificial environments simulate conditions necessary for growth bacteria.

Basic requirements for bacteria culture media.

1- **Energy source**

2- Nutrition

A-Moisture

B-Carbon source

Ex: sugars, carbohydrate, CO2

C-Nitrogen source

Peptone

D-Others {like; Sulfurs, phosphorous, metal Salts, trace elements, vitamins, essential metabolites}

3- PH (acid, alkaline, natural).

4- O2 (aerobic or anaerobic).

5- Time for incubation.

6- Temperature: The optimum temperature of pathogenic bacteria is 37OC (Body temperature) well used the incubator

Media prepared in suitable containers according to solidity of culture media

- like **flasks** or **tubes** or **bottles** with suitable sizes
- **plugged with non-absorbent cotton , wool**, or in **screw capped bottles,**
- **Sterilized** by any method which will not destroy the nutritional properties of the medium (**moist temperatures Autoclaving or filtration).**

Media dispense in plate Petri-dish for solid media, **if colonial characteristics of an organism are to be examined**, the Petri dish is an excellent container for the medium. The dish should be flat-bottomed, and either of heat-resistance **glass or plastic**. The most commonly used Petri dishes are **90 mm diameter** disposable plastic. **Glass Petri dishes may be sterilized in copper tines, which have a deep lid to prevent air penetration on cooling.** They should be sterilized in a hot-air penetration on cooling. **They should be sterilized in a hot air oven for 1-2 h at 160-180 oC and allowed to cool slowly in the oven.**

Kinds of media

Media divided to **solid, liquid and semisolid** according their solidity .**In liquid media the bacteria are free to move** about, but when grown **in solid media they multiply at the site of inoculation and form colonies.** The appearance of these colonies is often typical of these species; this makes **possible the isolation of single species of bacteria from a mixture.**

Liquid media are solidified by the addition for example of **gelatin** or **agar.**

Agar is a long-chain carbohydrate obtained from sea algae it melt at 80 – 100 Co and solidified at 35- 42 Co which does not effect the nutrient properties of the original media (doesn‟t provided any nutrition to the bacteria). **It acts as solidifying agent**.

Gelatin is a protein derived from the collagen of bone, skin and sinew, melting point 24 Co.

Classification of media according to components

It may be **natural** or **synthetic**.

The artificial (synthetic) media classify to:

1-Simple 2- Enrichment 3- Differential 4- Selective 5- Special

Storage of culture media

Media may be dispensed with **rubber-lined screw caps**, **polypropylene caps** or **tubs plugged with non-absorbent cotton** wool. **Small bottles may be sterilized with their caps screwed down firmly, but not packet tightly in the baskets**. The large bottles should have their caps loosened before heating and subsequently tightened for storage.

Media for current use should always be **stored in a dust-free cupboard or in cool, moist atmosphere. For longer periods store at 4 – 6 oC (refrigerator).** Each batch should be tested before use and labeled with a batch number. The shelf life of media varies considerably. Poured plates will be used within a few days of preparation but **agar slops should be checked to ensure that moisture is still** the basal media should be such that each batch is renewed within 3 months of manufacture.**present**.

Culture technique:

In clinical laboratory indications for culture are:

1-To Isolation bacteria in pure culture

2-To demonstrate their properties

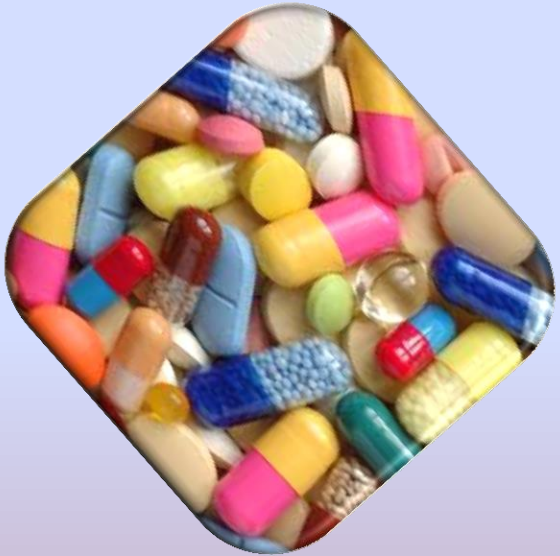
3-To obtain sufficient pure growth for preparation of antigen and for other test

d-For type isolation by method like bacteriophage and bacteriocin susceptibility.

4-To determine sensitivity to antibiotic

5-To estimate viable count

6-To maintain stock culture



Preparation of Culture media

Equipment:

1- Balance (2- pan Balance)

2- Conical flask.

3- Graduated cylinder.

4- Spatula.

5- Source of heat (Bunsen burner).

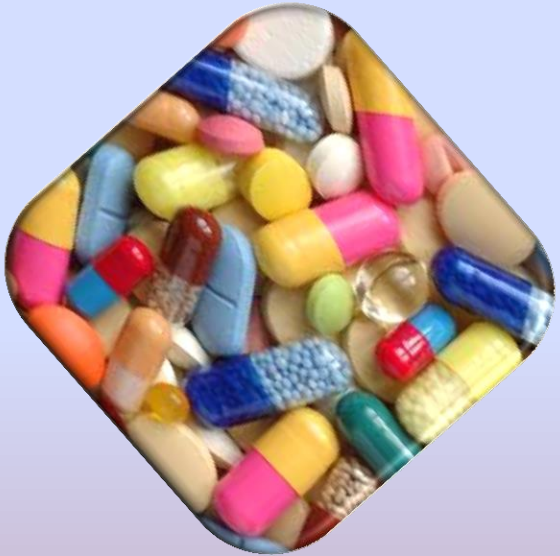
6- Filter paper

7- Autoclave

8- powder base of media

Procedure:

1-weight media powder by using a balance.
2-Disolve the powder in D. W
3- Using a heat to complete dissolving of powder.
4- Put cotton plug on 1 mouth of conical flask.
5-sterilize by using Autoclave.
6-cool the media to (45-50) C °
7-pour this media a Petri dish about 20 ml for each.
8- Let plate for some time to solidify a media.
9-Put plates in refrigerator upside down until using.



Inoculation on Solid media:

Streaking on Solid media

The purpose of streaking

- to get an isolated colony
- to know the type of Bacteria

Equipment: Spirit lamp, Bacteriological loop, Solid media

Procedure:

1-prepare Solid media in a Petri dish.
2-sterilize the loop by flaming.
3- Cool it by touching the loop on side of medium.
4-hold a piece of colony by loop and transfer it to a new media .as in **A**, this area termed **inoculum area** or **wed.**
5-Resterilizes the loop and repeat point 3.
6- Make 4 parallel lines as in B.
7- Repeat point (2and3).
8- Repeat point 6 as in **C**
9- Repeat point (2and 3).
10- Repeat point 6 as in **D**.
11- Repeat point (2and3).
12- From **D**, Make a **zigzag** line to the middle of medium to get an isolated colony

Typical Culture Laboratory Bench

1. The specimen should be properly labeled with the patient‘s name, hospital number, word and date of collection. This is essential in order to prevent confusion of specimens from patients similar.

2. The request from should state the provisional diagnosis and the nature of examination required. This facilitates selection of techniques; for example, if a specimen of sputum is sent to detection of *Mycobacterium Tuberculosis* (in a suspected case of pulmonary tuberculosis) detailed examination for other organisms is obviously not required.

3. Any information with regard to the chemotherapy should be noted. Certain precautions may be necessary; for example, specimens from patients receiving sulphonamides should be inoculated on to the media containing *p*-aminobenzoic acid, which prevents the bacteriostatic action of sulphonamides on the organisms. Similarly, the use of penicillinase may be required for the isolation of organisms from a patient receiving penicillin.

4. The correct container should be used. Most specimens for bacteriological examination should be received in a sterile container.

5. With all specimens extreme care must be taken with all manipulations and they should be carried out under the protection of an exhaust inoculating cabinet.

6. THE INOCULATING CABIENT (SAFTY HOOD)

All work involving the handling of such specimens and cultures should be performed under cover of an inoculating cabinet.

Incubation of culture media

Culture on Solid media

1 Bacterium one colony

37C° for (18-24) hr

In incubator

FURTHER PRECAUTIONS

Other precautions when dealing with all specimens or cultures are:

- Never lay a culture tube on the bench ; always place it in a rack or tin.

- Label clearly *every* tube or plate with the specimen's number.

- When finished always discard the cultures into an appropriate discard receptacle for sterilizing. Never remove the cultures once discarded until they have been sterilized.

- Keep the working space on the bench clear so that if an accident occurs the minimum number of articles will be involved.

- Handle all apparatus and materials carefully.

- Do not smoke when working with specimens or cultures.

- Never lay a pipette mouthpiece on the bench

- When pipetting always use a teat, never pipette by mouth.

- Do not lick gummed labels.

- Report any accident, however trivial, to the senior person in the laboratory.

- Always wash your hands with soap and water after handling cultures and specimens, and before going of duty. It is recommended that disposable paper or continuos roller towels be provided to minimize any possibility of cross infection.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