Preparation and sterilization Of culture media

Culture of bacteria (Streak plate method)

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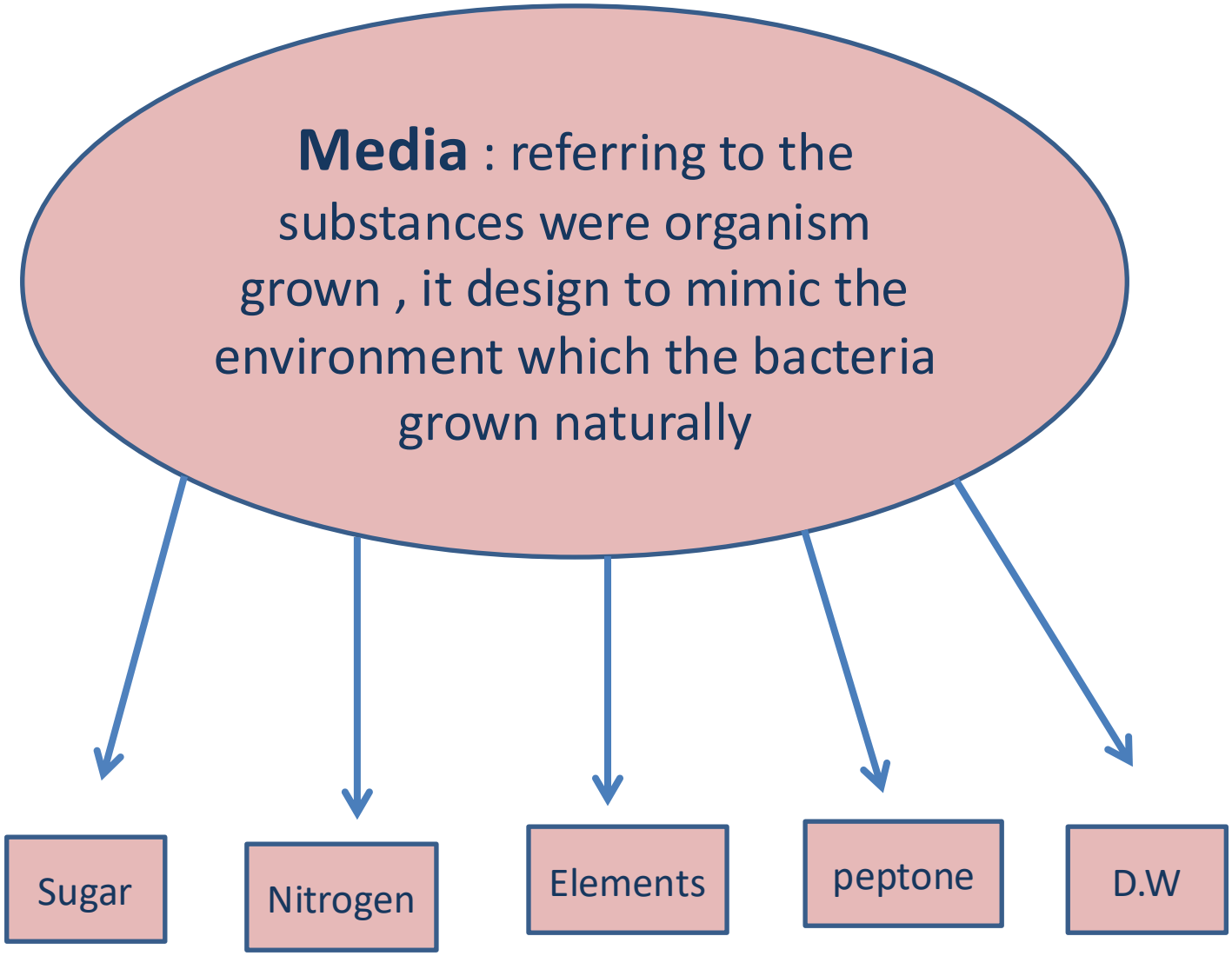
- In this lab we will talk about preparation and sterilization of culture media and explain how bacteria are cultured by **streak plate method.**

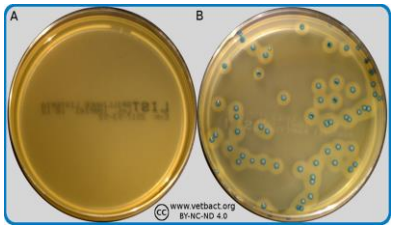
- **Culture medium** or growth medium is a liquid or gel designed to support the growth of micro-organisms, there are different types of media suitable for growing different types of cells.

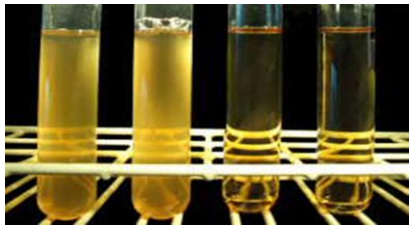


Microbiological cultures used for growing microbes

- Those pics show classification of bacteria culture media on the base of consistency: **Agar media, liquid media (broth)**

Agar media





Liquid media (broth)

❖ **solid medium**: contains agar at a concentration of 1.5-2%.

- solid medium has physical structure and allow bacteria to grow in physically informative or useful ways, examples: colonies or in streaks.
- solid medium is useful for isolating bacteria or for determining the colony characteristic of the isolate.

❖ **liquid medium (broth)** : this medium contain specific amount of nutrients but don't have a trace of gel agent such as a gelatin or agar.

- broth medium serves various purposes such as: propagation of a large number of organism, fermentation studies and various other tests such as sugar fermentation tests
- growth or presence of bacteria turn the liquid from clear to turbid .

- preparation of culture media formulation - including liquid growth media and cultured media based on agar- is a common procedure in any microbiology lab.
- the culture media formulation process involves many **steps** and must be carried out with care to avoid the cross contamination.

Steps

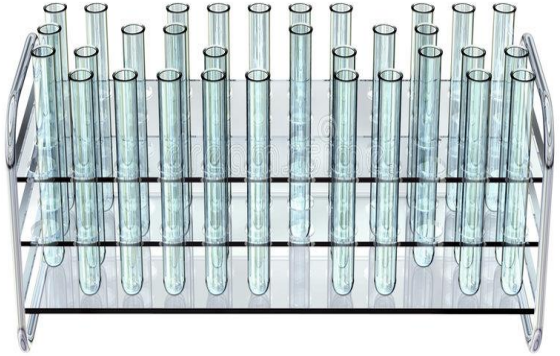
1. Select culture media from database.
2. Recalculate ingredients quantities according to the required culture media volume.
3. Weigh main ingredients into the container then weight trace ingredients on a high accuracy balance and add to the container.
4. Use deionized water up to around 80% of the required volume.
5. Mix to dissolve the ingredients, gentle heating may be required.
6. top up the culture media to the required volume.
7. label the container and at the end **sterilize in auto clave.**

Auto clave

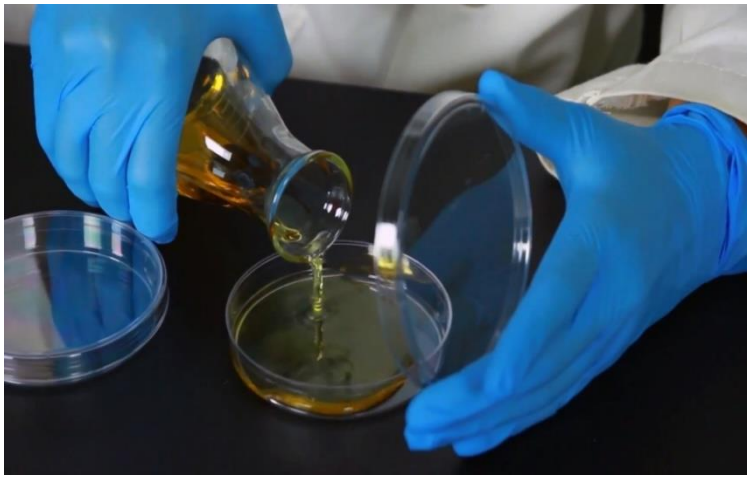
- is a machine that uses steam under pressure to kill harmful bacteria, viruses, fungi and spores on items that are placed inside a pressure vessels.
- the items are heated to an appropriate sterilization temperature for a given amount of time.
- many auto claves are used to sterilize equipment and supplies by subjecting them to a pressure at **15 pound/inch at 121 C or 250 F for around 15 to 20 min** depending on the size of the load and the contents.
- Take care that we can not sterilize all types of media by using autoclave because some of them include substance that getting denaturated with autoclave temperature and pressure.

- **Autoclave condition**:
- 121 C
- 30 Min
- 15 pound/inch

Liquid media (broth)



Agar media



Steps

8. After autoclave cycle finished get out old media flask and check for autoclave tape if it is changed to **a black lines**.

- autoclave tape: an adhesive tape used in autoclaving heating under high pressure with steam to sterilize, to indicate whether a specific temperature has been reached.
- autoclave tape works by changing color after exposure to temperature commonly used in sterilization process, typically **121 C** in a steam autoclave.

9. Place a sterile lab thermometers in the mixture and monitor until its temperature falls about **47 degrees**.
10. Pour melted agar into the patter dish to cover the bottom about a quarter and replace the lid immediately, if it is a liquid media pour it in a sterile tubes.
11. allow the agar plate to cool and set , the medium will set like gelatin at room temperature then ready for storage once it sets.

- during storage in a refrigerator but not to freeze the agar plate should be replaced in an inverted position with a lid at the bottom, this prevents the condensation from dripping down onto the surface of the agar, which may allow for the movement of the organism from one colony to another.

1-Enrichment media

- Enrichment media contain the nutrients required to support the growth of a wide variety of organisms including some **fastidious** ones, **by** the addition of a blood or serum (blood agar is an enriched bacterial growth medium)
- fastidious organisms such as **streptococcus** don't grow well in ordinary growth medium, so <u>blood agar</u> is a type of growth medium trypticase soy agar enriched with 5% sheep blood that encourage the growth of bacteria such as **streptococcus** that otherwise wouldn't grow.

1-Enrichment media

- on the other hand, chocolate agar or chocolate blood agar is a **nonselective enriched growth medium** used for isolation of fastidious bacteria such as **haemophilus influenzae and Neisseria species.**
- So, it is a variant of the blood agar plate containing red blood cells that have been lysed by slowly heating to 80 C.
- lysis of RBCs during the heating process release intracellular co-enzyme **nicotinamide adenine dinucleotide NAD** or **V factor** into the agar for utilization by fastidious bacteria.

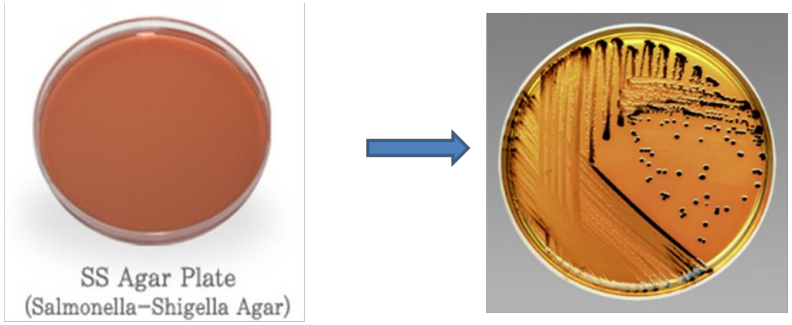
pics show a blood agar with and without bacterial growth on its surface

2-Selective media

- Selective media contain ingredients that inhibit the growth of some organism but allow others to grow, for example:

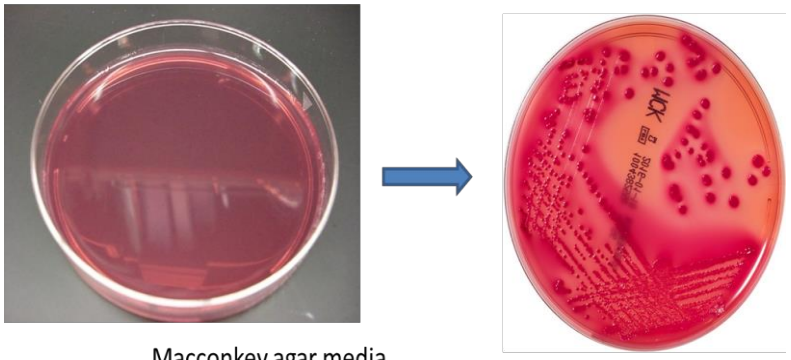
SS agar (salmonella-shigella agar) and Macconkey agar, both are considered as differential media besides, it's considered as a selective media.

- SS agar is a **selective and differential medium** which is used for the isolation, cultivation and differentiation of **gram negative**, enteric micro-organisms SS species which isolated from both clinical and nonclinical specimens such as from feces, urine and suspected blood items.
- colonies of **salmonella species** appear as transparent or translucent colorless colonies appear with black centers depending on the species isolated due to H2S production, while **shigella species** appear as transparent or translucent colorless colonies without H2S production.



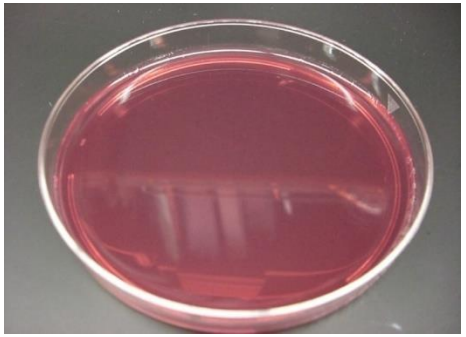
2-Selective media

- on the other hand, **macconkey agar media** is a selective and differential medium designed to isolate and differentiate enteric based on their ability to ferment lactose, bile salts and crystal violet inhibit the growth of gram-positive organisms.
- lactose provides a source of fermentable carbohydrate allowing for differentiation and at the same time it prevents forming of proteous species.
- lactose ferment bacteria <u>appear pink to rose red</u> colonies while non lactose ferment <u>appear colorless</u>.



Macconkey agar media

SS Agar Plate
(Salmonella–Shigella Agar)



This pic shows SS agar and Macconkey agar considered both as differential and selective media at the same time.

3-Differential media

- differential media are bacteriologically growth media that contains specific ingredients to allow one to distinguish selected species or categories of bacteria by visual observation.
- differential media are used to distinguish between closely related organism or group of organisms.
- an example of differential media is **CLED agar (cystine lactose electrolyte deficient agar)** that is used in isolating and enumerating bacteria from urine, it supports the growth of urinary pathogens and contaminants but prevent forming of proteus species due to its lack of electrolyte, so we use CLED and Macconkey to prevent swarming of proteus species.
- lactose fermented bacteria <u>appear yellow to green colony on CLED</u> and non lactose fermented bacteria <u>appear white colony.</u>

Streaking plate method

- streaking plate method is a technique used to isolate a pure strain from a single species of microorganism often bacteria.
- the inoculum is streaked over the agar surface in such a way that it thins out the bacteria , some individual bacterial cells are separated and well spaced from each other so that the organism can be identified, studied or tested.