

Al- Mustaqbal University College

First stage.
Department of Optometry(Optics)



جامعة المستقبل الاهلي
مرحلة الاولى
قسم التقنيات البصرية

Culture media and its preparation

Lab: 5

Dr:Zainab waddah naser

6/3/2022

Culture media and its preparation



What is culture medium

- The food material or substances liquid or gel designed to support the growth of microorganisms. *in vitro* (outside the body) is called **culture medium**.

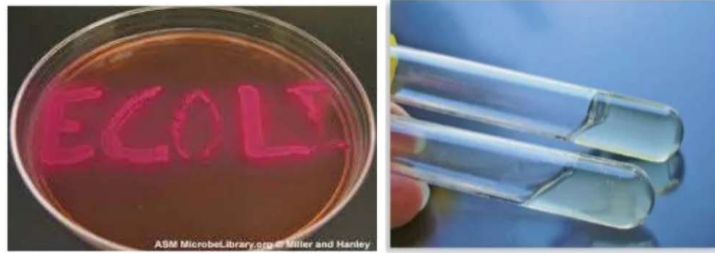


It is important to grow microorganisms outside the body for the following purposes:

1. to identify the cause of infection from the clinical sample, so that proper treatment can be given.
2. to study the characteristics or properties of microorganisms.
3. to prepare biological products like vaccines, toxoides, antigens...etc.

I. Classification based on physical state

- a) solid medium
- b) semi solid medium
- c) liquid medium



Solid medium

agar is the most commonly used solidifying agent.

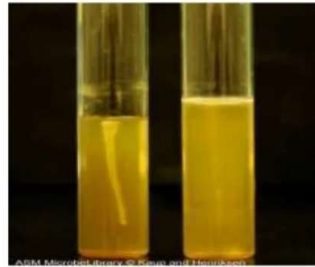
What is agar

- Golden-yellow granular powder
- Prepared from seaweeds.
- Not affected by the growth of the bacteria.
- Melts at 98C & sets at 42C



Semi-solid media

- Such media are soft and are useful in demonstrating bacterial motility and separating motile from nonmotile strains .



Liquid media

- are sometimes referred as “ **broth** “.
- bacteria grow uniformly producing general turbidity
eg. Nutrient broth



Simple media

- eg: Nutrient broth, N. agar
- NB consists of peptone, meat extract, NaCl,
- NB + 2% agar = Nutrient agar

**Complex media**

- such as blood agar, it has ingredients that exact components are difficult to estimate.



Synthetic or defined media

- specially prepared media from pure chemical substances for research purpose and composition of every component is well known
- eg: peptone water –
- 1% peptone + 0.5% NaCl in water.



Special media

Enriched media:

- Substances like blood, serum, egg are added to the simple medium Used to grow bacteria that are exacting in their nutritional needs. eg: Blood agar, Chocolate agar



Special media

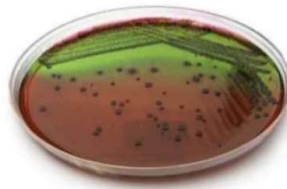
Selective media

- The inhibitory substance is added to a solid media to inhibit commensal or contaminating bacteria such as :
- Antibiotics
- Dyes
- Chemicals
- Alteration of pH



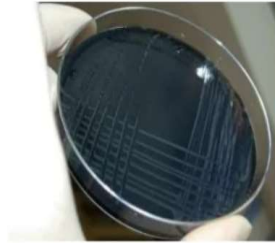
Eosin methylene blue

- selective for gram negative bacteria
- The dye methylene blue in the medium inhibit the growth of gram positive bacteria.



Campylobacter agar

- Is used for isolation of *Campylobacter jejuni* from fecal or rectal swab.



Lowenstein –Jenson medium

- is solid medium used for *Mycobacterium tuberculosis*.
- contain **penicillin, nalidixic acid** and
- **malachite green** to inhibit growth of gram positive and gram negative bacteria, in order to limit growth to Mycobacteria species only.



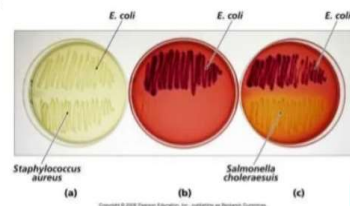
Differential media

- are designed in such a way that different bacteria can be recognized on the basis of their colony color.
- Dyes and metabolic substrates are incorporated so that those bacteria that utilize them appear as differently colored colonies.

Examples:

- **MacConkey agar**
- **CLED agar**
- **XLD agar**

- **MacConkey medium**
- Distinguish between lactose fermenters & non lactose fermenters.
- Lactose fermenters – **Pink** colonies
- Non lactose fermenters – colorless colonies



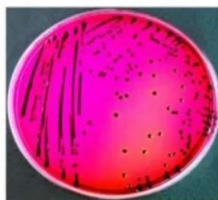


Xylose Lysine Deoxycholate Agar(XLD)

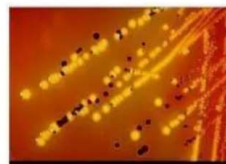
- Selective for gram negative bacteria
- Used for differentiation of *Salmonella* and *Shigella* species.



***Shigella* on XLD.**



***Salmonella* on XLD.**



XLD Agar

Escherichia coli (yellow colonies)
Salmonella sp. (black colonies)

Image Source: Faculty of Health and Medical Sciences - University of Copenhagen, Denmark

Cysteine Lactose Electrolyte Deficient Agar(CLED)

For cultivation of pathogen from urine specimen , inhibit swarming of *proteus sp.*



CLED , e-coli

Anaerobic media

- These media are used to grow anaerobic organisms.

Eg:

- Robertson's cooked meat medium.
- Thioglycolate broth medium.

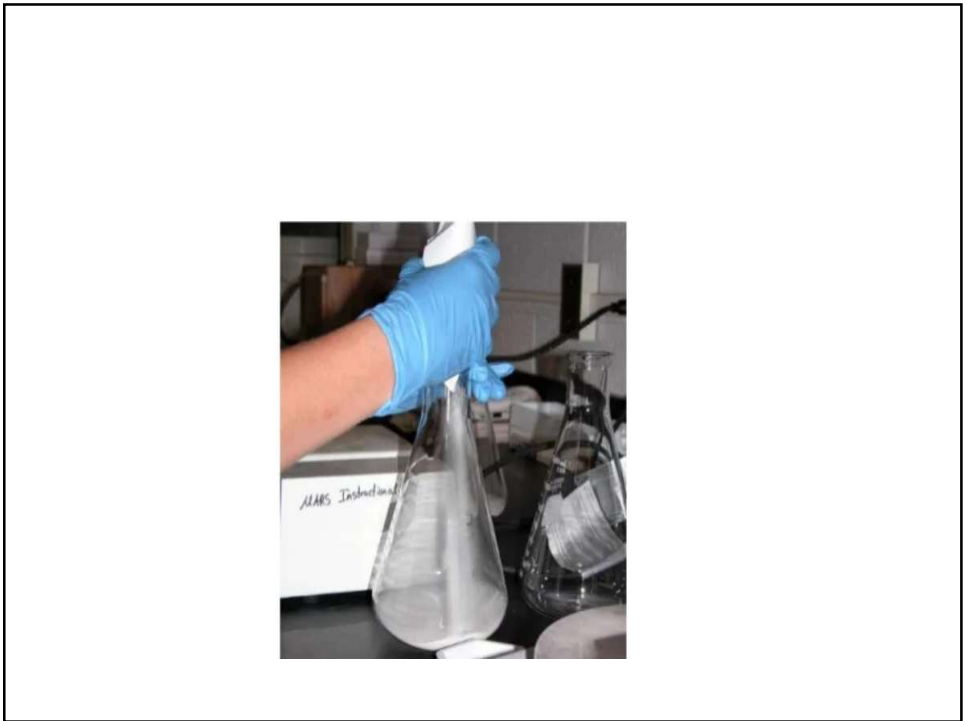
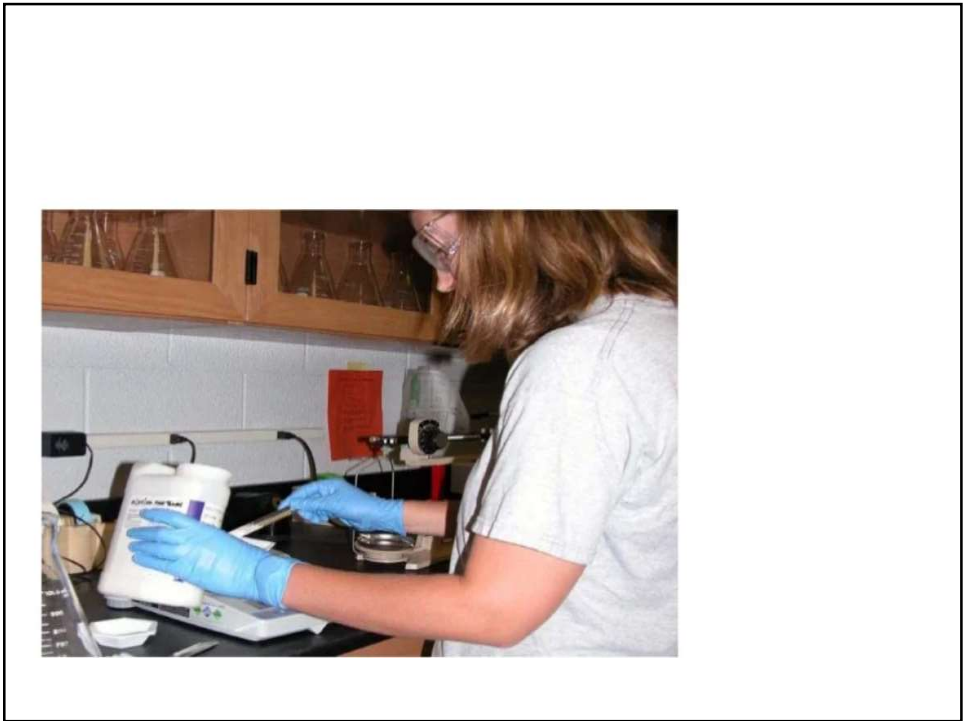


Cooked Meat Medium
for Anaerobic Bacteria



Re-hydrate powder according to manufacturer's instructions





Before sterilization, ensure ingredients are completely dissolved, using if necessary.



Pouring

1. Collect one bottle of sterile molten agar from the water bath.
2. Hold the bottle in the left hand; remove the lid with the little finger of the right hand.
3. Flame the neck of the bottle.
4. Lift the lid of the Petri dish slightly with the right hand and pour the sterile molten agar into the Petri dish and replace the lid.
5. Flame the neck of the bottle and replace the lid.
6. Gently rotate the dish to ensure that the medium covers the plate evenly.
7. Allow the plate to solidify.
8. Seal and incubate the plate in an inverted position.

