STERILIZATION TECHNIQUES & MICROBIOLOGICAL CULTURE MEDIA PREPARATION

“ 240 MIC ”

نورة الكـبيسي
Nalkubaisi@ksu.edu.sa

2021

STERILIZATION OF MEDIA AND GLASSWARE

- **STERILIZATION:** is the process of rendering a medium or material free of all forms of life.
- There are three basic ways in which sterilization of media and supplies can be achieved.

THREE WAYS FOR STERILIZING CULTURE MEDIA AND SUPPLIES (GLASSWARE):

Steam under pressure (autoclaving)-121oC at 15 pounds of pressure for 15 minutes.	Dry heat-160 to 170oC for 2 hours.	Millipore filtration-passage through filters with pore sizes no greater than 0.22 lm.
		

AUTOCLAVING

The most useful approach is autoclaving**,** in which items are sterilized by exposure to steam at **121°C and 15 lbs** of pressure for **15 minutes** or longer, depending on the nature of the item. Under these conditions, microorganisms, even endospores, will not survive longer than about 12 to 13 minutes.

This method is rapid and dependable.

PROCEDURE FOR AUTOCLAVING

1. Load the autoclave with the freshly prepared culture media.
1. Close and lock the autoclave door.
1. Set the autoclave time for 15 minutes or longer.
1. Make certain that the autoclave temperature is set to 121°C.
1. Start the autoclave by pushing the start button or twisting the knob to the start position.
1. When the period of sterilization is completed and the pressure in the chamber reads 0.
1. Carefully open the door and remove the containers, using heat-proof gloves.

MICROBIOLOGICAL CULTURE MEDIA

- Microorganisms depend on a number of factors such as **nutrients**, **oxygen**, **moisture** and **temperature** to grow and divide.
- In the laboratory, except for the above factors, the culture medium should be **sterile** and **contamination** of a culture with other organisms **should be prevented**.
- The survival and growth of microorganisms depend on **available nutrients** and a **favorable growth environment.**
- In the laboratory, the nutrient preparations that are used for culturing microorganisms are called **MEDIA** (singular, medium).
- Depending on the type and combination of nutrients, different categories of media can be made.

CATEGORIES

1.**1. Complex media** are rich in nutrients, they contain water soluble extracts of plant or animal tissue (e.g., enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar, often glucose is added to serve as the main carbon and energy source.

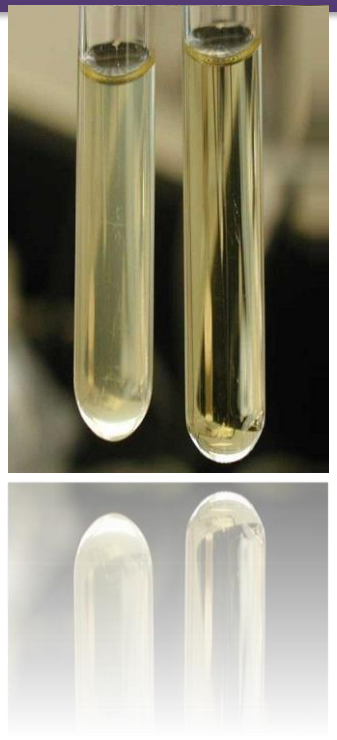
2.The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called **complex**.

1.**2. Defined media** are media composed of pure ingredients in carefully measured concentrations dissolved in double distilled water i.e., the exact chemical composition of the medium is known. Typically, they contain a simple sugar as the carbon and energy source, an inorganic nitrogen source, various mineral salts and if necessary growth factors (purified amino acids, vitamins, purines and pyrimidines).

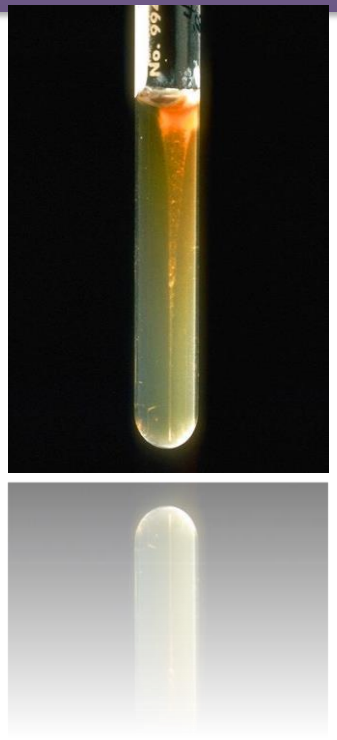
1.**3. Selective/differential media** are media based on either of the two categories above supplemented with growth-promoting or growth-inhibiting additives. The additives may be species- or organism-selective (e.g., a specific substrate, or an inhibitor such as cycloheximide (artidione) which inhibits all eucaryotic growth and is typically used to prevent fungal growth in mixed cultures).

THREE PHYSICAL FORMS OF MEDIA ARE USED

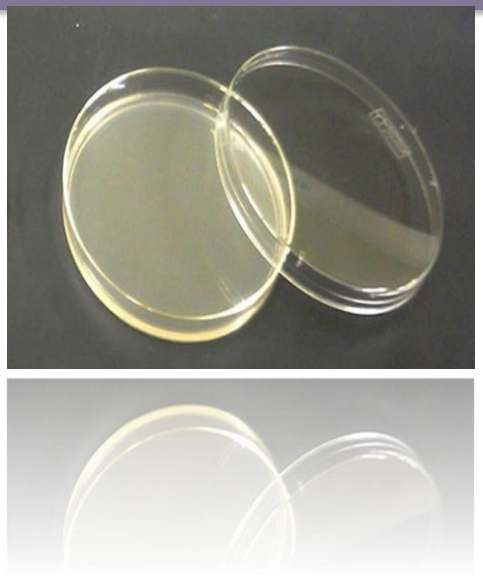
- 1. liquid, or broth media



- 2. semisolid media



- 3. solid media



The major difference among these media is that solid and semisolid media contain a solidifying agent (usually agar), whereas a liquid medium does not.

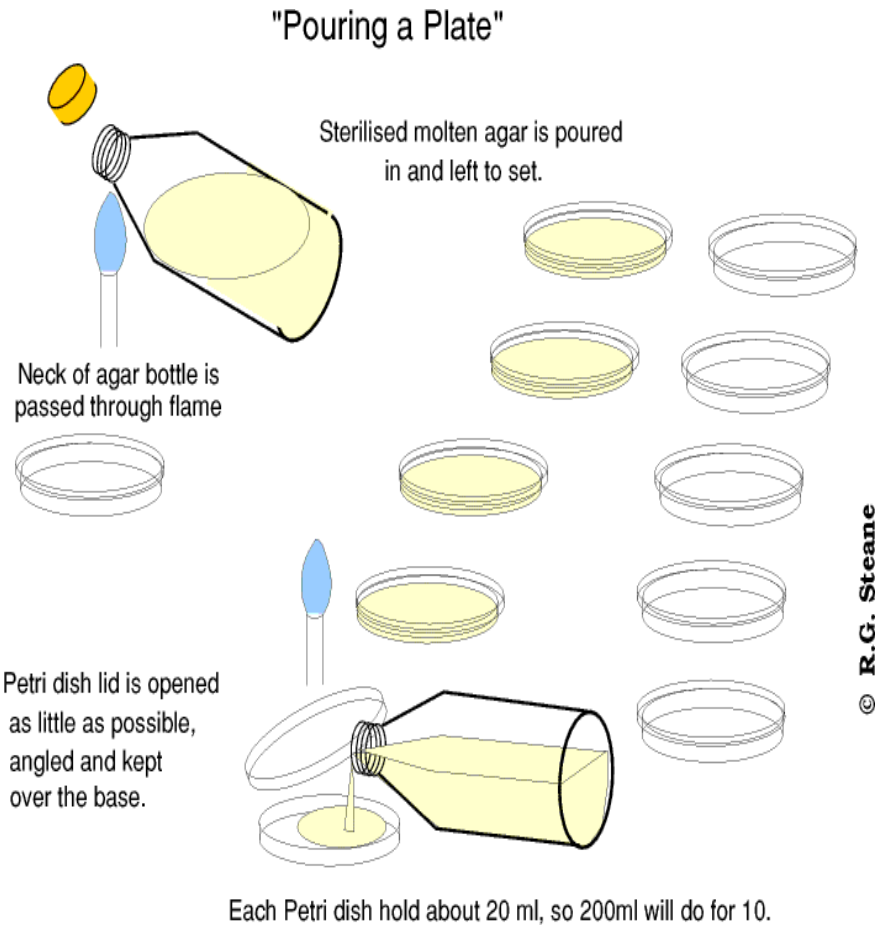
LIQUID MEDIA (BROTH)	SEMISOLID MEDIA	SOLID MEDIA
• Do not contain an agar component. • Example: nutrient broth, tryptic soy broth, or brain-heart infusion broth. • All above can be used to propagate large numbers of microorganisms in fermentation studies and for various biochemical tests.	• Can also be used in fermentation studies. • In determining bacterial motility. • Promoting anaerobic growth.	• Such as nutrient agar or blood agar. • **Can be used:** (1) for the surface growth of microorganisms in order to observe colony appearance. (2) Pure culture isolations. (3) Storage of cultures. (4) Observe specific biochemical reactions.

USES OF CULTURE MEDIA

BROTH	SLANT	PLATE	DEEP
To grow high concentration of bacteria.	Space saving solid culture.	Isolating individual colonies, can be used to count bacteria.	Look at motility & oxygen requirement.

POURING OF CULTURE MEDIA

- Using clear plastic disposable **petri dishes**, typically 95 or 100 mm in diameter, 20 per sleeve (packet).
- When prepared for inoculation, a plate contains **solid agar** to provide a surface for growth, mixed with nutrient materials.
- We prepare agar media either by mixing 1 to 2% agar with individual components or by using a pre-mixed powder.
- Either way, the dry components must be **heated to melt the agar and sterilized in a flask or bottle**, then **poured into the plates using aseptic technique**, preferably in a sterile cabinet (laminar flow hood).



HOW TO PREPARE A PETRI PLATE

- 1. Take liquid agar (in the water bath)
- 2. Pour aseptically into the base of the Petri plate
- 3. Wait until solidify (15 minutes) and invert
- 4. Plates are kept inverted so condensation does not drip onto the agar
- 5. To increase the shelf-life of the plates, store in a cool, dry environment until they are used (refrigerator).

LABELING PLATE

- **Make certain that all plates are labeled on the bottom half.**
- **Include the following:**

a. Your initials or identifying mark.

b. Date.

c. Type of specimen.

The End