UNIT- I STERILIZATION

A **Petri dish** (alternatively known as a **Petri plate** or **cell-culture dish**) is a shallow transparent lidded dish that biologists use to hold growth medium in which cells can be cultured, originally, cells of bacteria, fungi and small mosses The container is named after its inventor, German bacteriologist Julius Richard Petri. It is the most common type of culture plate.

Petri dish was originally developed by a German physician Robert Koch in his private laboratory in 1881, as a precursor method. Petri, as assistant to Koch, at Berlin University made the final modifications in 1887 as used today.

Petri dishes are usually cylindrical, mostly with diameters ranging from 30 to 200 millimetres (1.2 to 7.9 in), and a height to diameter ratio ranging from 1:10 to 1:4. Petri dishes were traditionally reusable and made of glass; often of heat-resistant borosilicate glass for proper sterilization at 120–160 °C.

Since the 1960s, plastic dishes, usually disposable, are also common.

The dishes are often covered with a shallow transparent lid, resembling a slightly wider version of the dish itself. The lids of glass dishes are usually

loose-fitting. Plastic dishes may have close-fitting covers that delay the drying of the contents. Alternatively, some glass or plastic versions may have small holes around the rim, or ribs on the underside of the cover, to allow for air flow over the culture and prevent water condensation.

Some Petri dishes, especially plastic ones, usually feature rings and/or slots on their lids and bases so that they are less prone to sliding off one another when stacked or sticking to a smooth surface by suction.

Small dishes may have a protruding base that can be secured on a microscope stage for direct examination.

Some versions may have grids printed on the bottom to help in measuring the density of cultures.

Microplate

It is a single container with an array of flat-bottomed cavities, each being essentially a small Petri dish. It makes it possible to inoculate and grow dozens or hundreds of independent cultures of dozens of samples at the same time. Besides being much cheaper and convenient than separate dishes, the microplate is also more amenable to automated handling and inspection.

Significance of design of Petri dish /plate

1. It provides more surface area with little media to develop isolated colonies.
2. It provides head space air for growth of aerobic microorganisms.
3. The dish is used to culture cells and preventing them from getting contaminated.
4. Since the dish is transparent, it is easy to observe the growth stages of microorganisms clearly.

History

The Petri dish was developed by German physician Julius Richard Petri (after whom the name is given) while working as an assistant to Robert Koch at Berlin University. Petri did not invent the culture dish himself; rather, it was a modified version of Koch's invention, which used an agar medium that was developed by Walther Hesse. He described a new bacterial culture method that used a glass slide with agar and a container. A bacterial culture was spread

on the glass slide, and then placed in the moist chamber with a small wet paper. Bacterial growth was easily visible.

Petri made changes in how the circular dish was used. Instead of using a separate glass slide or plate on which culture media were placed, Petri directly placed media into the glass dish, eliminating unnecessary steps such as transferring the culture media, using the wet paper, and reducing the chance of contamination.

Petri dishes are widely used in biology to cultivate microorganisms such as bacteria, yeasts, and molds. It is most suited for organisms that thrive on a solid or semisolid surface.

The culture medium is often an agar plate, a layer a few mm thick of agar or agarose gel containing whatever nutrients the organism requires and other desired ingredients. The agar and other ingredients are dissolved in warm water and poured into the dish and left to cool down. Once the medium solidifies, a sample of the organism is inoculated.

The dishes are then left undisturbed for hours or days while the organism grows, possibly in an incubator. They are usually covered, or placed upside-down, to lessen the risk of contamination from airborne spores.

Virus or phage cultures require that a population of bacteria be grown in the dish first, which then becomes the culture medium for the viral inoculum.

Types of Petri dishes

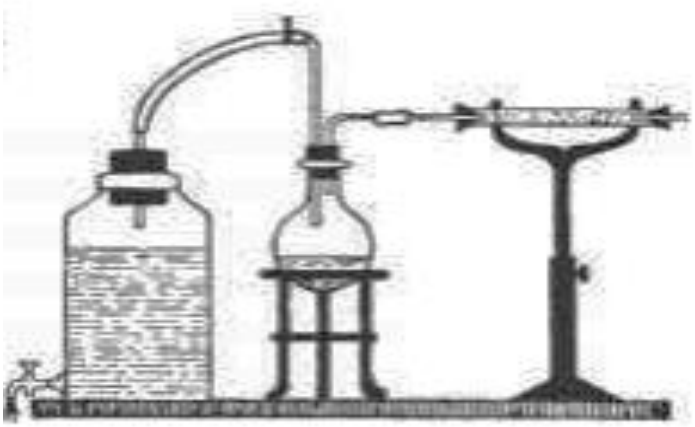
In the field of microbiology, petri dishes are used in particular to culture cells, cultivate microorganisms like bacteria and detect contamination. However, there are differences among the laboratory dishes. Not every type of petri dish is suitable for every application. Certain requirements should be considered selecting a petri dish:

Size	Depending on the application, select a petri dish with a suitable diameter. Petri dishes are available in diameter sizes of 35, 60, 94, 100 and 140 mm.	Petri dish 35 mm Petri dish 60 mm Petri dish 100 mm
Temperature	When working with hot agar, special attention must be paid to the material of the petri dish. Heat-resistant, clear polystyrene is very suitable for high temperatures.	
Gas exchange	For optimum gas exchange, petri dishes with vents are recommended.	Petri dish with vents
Multi-parameter measurement	If several parameters have to be observed simultaneously during an application, petri dishes with two or three compartments are ideal.	
Application: Germ counting	Petri dishes with 10 mm calibration grids for quick evaluation are recommended for germ counting.	
Application: Hygiene control	Contact plates are recommended for hygiene control. They usually have calibration grids and vents.	

❖ **Schroder and von Dutch: Cotton Plug**

In the 1850s, with Heinrich G. F. Schröder (1810-1885), he demonstrated that a filter made of cotton-wool was effective in removing microbes such as bacteria from air. Dusch was the author of influential works involving thrombosis of cerebral sinuses , heart disease and diseases of the endocardium and myocardium . Theodor von Dusch, was a German physician. He, along with Georg Schroeder, performed what was thought to be a convincing experiment that basically cancelled the theory that living organisms could rapidly arise from non-living organisms, otherwise known as Spontaneous Generation. Schroeder and von Dusch were the first to present the idea of using cotton plugs for plugging microbial culture tubes.

Schroder and von Dusch (1854) successfully used a layer of cotton as a filter for sterilization. First, the organic substance in the middle glass was heated and after that a flow of air was adjusted by opening the tap and emptying the left, large bottle slowly. Since then, layers of cotton have been used frequently to sterilize air in microbiology and biotechnology.



The Schröder and von Dusch experiment with a glass tube filled with cotton

Preparation of Temporary Cotton Plugs

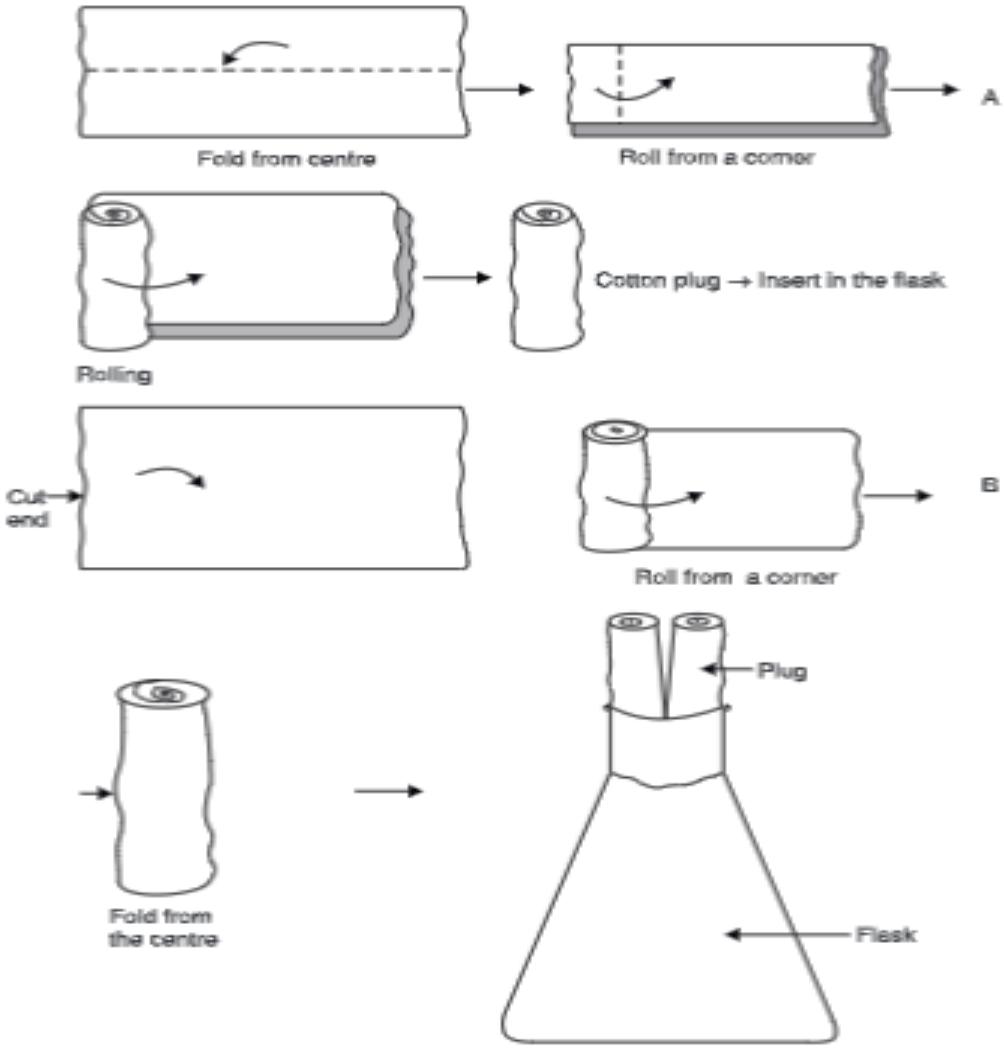
Microorganisms are everywhere in their distribution. In any given environment, many microorganisms are present at any given time. It is difficult to identify a specific kind of microbe until we understand their ecological needs. For instance, anaerobic microbes do not require oxygen those that are aerobic require oxygen micro-aerophiles to have oxygen however, in a small amount. So, we require cotton plugs to provide conditions that are aerobic and keep the growing culture uninvolved with unwanted microbes. Cotton plugs are made of cotton along with air pours. Air can flow through air pours, but not the bacteria that cause microbial contamination. They are fixed to the surface of cotton fibres, and prevent them from being able from getting into flasks, tubes, etc. Air aids in for the development of microorganisms within glass equipment.

Requirements

1. Non-absorbent cotton rolls
2. Culture tubes
3. Flasks
4. Scissors

Procedure

1. Get a non-absorbent cotton roll and then remove the packing
2. Roll up the roll according to the requirements (i.e. size, number and type)
3. Cut a small amount of cotton using scissors. In accordance with the specifications, the two varieties of cotton plugs can be made.



Preparation of Temporary Cotton Plugs

A. Cut, fold and rolled plug

1. Cut a small portion of cotton sheets using scissors according to the requirements i.e. flask/ culture tube, etc.
2. Fold the cut piece away from the centre.
3. Fold the sheet starting from one of its ends. The opposite side, cotton fibres are released freely.
4. Inject the rolled and folded side into the neck or mouth of a flask/culture tube and the opposite end of the plug is covered with cotton fibres.
5. If it's not tight, remove the cap and remove the cotton from one side and then adjust as before.

B. Cut rolled and fold plug

1. Cut the appropriate cotton with the aid of scissors.
2. The piece is rolled from one end to the other
3. Remove the rolled piece away from the middle.
4. Place the folded end into the mouth/neck of the flask/culture tube and the other with cotton fibres is positioned in the air.

Preparation of Permanent Cotton Plugs

Temporary cotton plugs made in the above manner are not recommended for use repeatedly since they can be contaminated by microorganisms due the adhesion to growth media. To avoid the need to prepare plugs of cotton more often permanent plugs must be made.

Requirements

1. Non-absorbent cotton rolls
2. Culture tubes
3. Flasks
4. Scissors
5. Muslin cloth

Procedure

1. Make temporary cotton plugs according to the procedure previously described.
2. Cut small squares (4-6″) from the muslin cloth.

3. Place the folded edge of the plug into the middle in a piece muslin cloth
4. Securely tie all four edges of the muslin at the unrolled free end on the plug. It's a durable cotton plug that is reusable.

Autoclave

An autoclave is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure. Autoclave sterilizes the materials by heating them up to a particular temperature for a specific period of time. The autoclave is also called a steam sterilizer that is commonly used in healthcare facilities and industries for various purposes. The autoclave is considered a more effective method of sterilization as it is based on moist heat sterilization**.**

Principle and Working of autoclave

- The autoclave works on the principle of moist heat sterilization where steam under pressure is used to sterilize the material present inside the chamber.
- The high pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.
- Water usually boils at 100°C under normal atmospheric pressure (760 mm of Hg); however, the boiling point of water increases if the pressure is to be increased.
- Similarly, the high pressure also facilitates the rapid penetration of heat into deeper parts of the material, and moisture present in the steam causes the coagulation of proteins causing an irreversible loss of function and activity of microbes.
- This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.
- When this steam comes in contact with the surface, it kills the microbes by giving off latent heat.
- The condensed liquid ensures the moist killing of the microbes.

- Once the sterilization phase is completed (which depends on the level of contamination of material inside), the pressure is released from the inside of the chamber through the whistle.
- The pressure inside the chamber is then restored back to the ambient pressure while the components inside remain hot for some time.

STERILIZATION CONDITIONS FOR AUTOCLAVE

S.No	Temperature	Time required	Pressure
1.	100°c	90 min	0 lbs
2.	110°c	60 min	5 lbs
3.	116°c	30-40 min	10 lbs
4.	121°c	15-20 min	15 lbs
5.	126°c	10 min	20 lbs
6.	135°c	3 min	30 lbs

$$\text{Temperature} \propto \frac{1}{\text{Time}}$$

The simplest form of the autoclave is the pressure cooker type or laboratory bench autoclaves. The following is the detailed description of different components/ parts of an autoclave:

a. Pressure Chamber

- The pressure chamber is the main component of a steam autoclave consisting of an inner chamber and an outer jacket.
- The inner chamber is made up of stainless steel or gunmetal, which is present inside the out chamber made up of an iron case.
- The autoclaves used in healthcare laboratories have an outer jacket that is filled with steam to reduce the time taken to reach the sterilization temperature.
- The inner chamber is the case where the materials to be sterilized are put.
- The size of the pressure chamber ranges from 100 L to 3000 L.

b. Lid/ Door

- The next important component of an autoclave is the lid or door of the autoclave.
- The purpose of the lid is to seal off the outside atmosphere and create a sterilized condition on ht inside of the autoclave.
- The lid is made airtight via the screw clamps and asbestos washer.
- The lid consists of various other components like:

Pressure gauge

- A pressure gauge is present on the lid of the autoclave to indicate the pressure created in the autoclave during sterilization.
- The pressure gauge is essential as it assures the safety of the autoclave and the working condition of the operation.

Pressure releasing unit/ Whistle

- A whistle is present on the lid of the autoclave is the same as that of the pressure cooker.

- The whistle controls the pressure inside the chamber by releasing a certain amount of vapour by lifting itself.

Safety valve

- A safety valve is present on the lid of the autoclave, which is crucial in cases where the autoclave fails to perform its action or the pressure inside increases uncontrollably.
- The valve has a thin layer of rubber that bursts itself to release the pressure and to avoid the danger of explosion.

c. Steam generator/ Electrical heater

- An electrical steam generator or boiler is present underneath the chamber that uses an electric heating system to heat the water and generate steam in the inner and the outer chamber.
- The level of water present in the inner chamber is vital as if the water is not sufficient; there are chances of the burning of the heating system.
- Similarly, if the water is more than necessary, it might interfere with the trays and other components present inside the chamber.

Procedure for running Autoclave

In general, an autoclave is run at a temperature of 121° C for at least 30 minutes by using saturated steam under at least 15 psi of pressure. The following are the steps to be followed while running an autoclave:

1. Before beginning to use the autoclave, it should be checked for any items left from the previous cycle.
2. A sufficient amount of water is then put inside the chamber.
3. Now, the materials to be sterilized are placed inside the chamber.
4. The lid is then closed, and the screws are tightened to ensure an airtight condition, and the electric heater is switched on.
5. The safety valves are adjusted to maintain the required pressure in the chamber.
6. Once the water inside the chamber boils, the air-water mixture is allowed to escape through the discharge tube to let all the air inside to be displaced. The complete displacement can be ensured once the water bubbles cease to come out from the pipe.

7. The drainage pipe is then closed, and the steam inside is allowed to reach the desired levels (15 lbs in most cases).
8. Once the pressure is reached, the whistle blows to remove excess pressure from the chamber.
9. After the whistle, the autoclave is run for a holding period, which is 15 minutes in most cases.
10. Now, the electric heater is switched off, and the autoclave is allowed to cool until the pressure gauge indicates the pressure inside has lowered down to that of the atmospheric pressure.
11. The discharge pipe is then opened to allow the entry of air from the outside into the autoclave.
12. Finally, the lid is opened, and the sterilized materials are taken out of the chamber.

❖ SOLOMONSEN HOT AIR OVEN

- A hot air oven is laboratory instrument that uses dry heat to sterilize laboratory equipment and other materials.
- That equipment cannot be wet or material that will not melt, catch fire, or change form when exposed to high temperatures are sterilized by using the dry heat sterilization method.
- Hot air oven also known as forced air circulating oven.
- Some examples of material which cannot be sterilized by employing a hot air oven such as surgical dressings, rubber items, or plastic material.
- We can sterilize Glassware (like petri dishes, flasks, pipettes, and test tubes), Powder (like starch, zinc oxide, and sulfadiazine), and Materials that contain oils, Metal equipment (like scalpels, scissors, and blades) by using hot air oven.
- To destroy microorganisms and bacterial spores, a hot air oven provides extremely high temperatures over several hours.
- The widely used temperature-time relationship in hot air ovens to destroy microorganisms is 170 °C for 30 minutes, 160 °C for 60 minutes, 150 °C for 150 minutes.

- Most of the medical industries use hot air ovens to sterilize laboratory instruments and material due to its simple standard operating procedure and low price. It also provides quick-drying processes.
- The process of dry heat sterilization using a hot air oven originally developed by Louis Pasteur.
- The temperature range of a hot air oven is 50 to 300°C. It can be controlled by using a temperature regulator.
- The forced air circulation provided by the oven ensures the temperature uniformity throughout the oven.
- In a hot air oven first, the surface of the material is sterilized then the temperature slowly enters the center of the item.

Working Principle of Hot air oven

Sterilization by dry heat is performed by conduction. The temperature is consumed by the surface of the objects, then moves towards the core of the object, coating by coating. The whole object will ultimately attain the temperature needed for sterilization to take place.

Dry heat causes most of the injury by oxidizing particles. The primary cell components are damaged and the organism dies. The temperature is kept for about an hour to eliminate the most ambitious of the resistant spores.

It is originally used in a hot air oven. This method is proper for items like glassware, powders, oil containing materials, metal material. Hot air oven acts in a manner so that items stored inside the oven don’t grab fire or meltdown. It primarily acts on the principle of structure where heat moves on the outside of the substance and then to the core of the item. It is termed a dry sterilization process because the procedure is performed by employing the hot air.

While the air shifts hot, it becomes thinner and transfers towards the ceiling of the chamber. If air hits the roof of the chamber it travels towards the ground of the chamber. It helps in the circulation of air within the chamber. This current flow assures the proper and uniform heating everywhere in the chamber. This is approaching a longer method than autoclaving or moist sterilization. Hence, a few organizational facilities are needed if it is practiced in the medical laboratory, so that workers can create a schedule of the sterilization method.

Hot air oven Parts and Functions

1. **External cabinet:** The External cabinet is made of stainless sheets. It covers the inner chamber.
2. **Glass wool insulation:** The space between the inner chamber and external cabinet is filled with Glass wool. It provides insulation to the hot air oven.
3. **Inner chamber:** The inner chamber of the hot air oven is made of Stainless steel.
4. **Tubular air heaters:** They help to generate heat within the inner chamber. Two Tubular air heaters are located on both sides of the inner chamber.
5. **Motor-driven blower:** It helps in uniformly circulating the air within the chamber.
6. **Temperature sensor:** It measures the temperature within the hot air oven and displays it on the controller screen.
7. **Tray slots**: The inner wall of the chamber contains several try slots that hold the trays.
8. **PID temperature controller:** It maintains the accurate temperature during the entire cycle. It also controls the temperature and also displays the temperature values.
9. **Load indicator:** it indicates the hot air oven is overloaded.
10. **Mains on/off switch:** It helps to turn on/ turn off the hot air oven.
11. **Safety thermostat:** It is also known as an over-temperature protection device. It keeps your oven and specimen safe in case of controller malfunction.

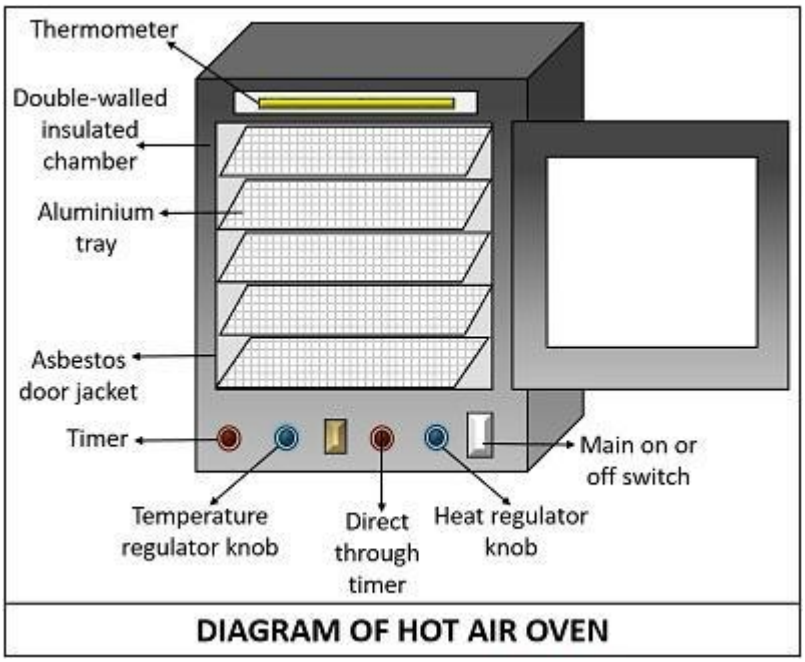


DIAGRAM OF HOT AIR OVEN

❖ INOCULATION LOOP

An inoculation loop is a simple tool used mainly by microbiologists to pick up and transfer a small sample of microorganisms called inoculum from a microbial culture, e.g. for streaking on a culture plate. This process is called inoculation.

The tool consists of a thin handle with a loop about 5 mm wide or smaller at the end. It was originally made of twisted metal wire (suchas platinum, tungsten or nichrome),butdisposable molded plastic versions are now common.

Platinum loops and needles are used for transferring bacterial cultures. These loops and needles are made of B&S platinum wire with 15% iridium added for rigidity, permitting a thinner wire for delicate work. Loops are fused to form a "perfect circle" with an inside tolerance of +/-0.02 mm to ensure accurate delivery.

Platinum Inoculating Loops are used for bacteriological and other microbiological applications. They will better resist constant exposure to acid and flame than the nichrome wire inoculating loop.

It consists of a looped nichrome wire fixed into a brass handle. The handle is 8 in. long and 1/4 in. diam. The durable nichrome wire is 24 gauge and 2-3/4 in. long.

Microbiologists use inoculating loops to transfer microorganisms to growth media. It is easy to sterilize and reuse because nichrome wire resists deterioration with repeated heat/cooling cycles.

Flame sterilization is a very simple method of killing microorganisms on an inoculating loop. Hold the wire loop end inside an alcohol burner or Bunsen burner flame for a few seconds to bring it to redness and then remove for 15-30 seconds to cool. When cooled, it is ready for culture inoculations.

❖ **Roux and Yersin**

Diphtheria is an example of the toxigenic infectious diseases. In 1883, **Klebs** demonstrated that *Corynebacterium diphtheriae* was the agent of diphtheria. One year later, **Loeffler** found that the organism could only be cultured from the nasopharyngeal cavity, and postulated that the damage to internal organs resulted from a soluble toxin. By 1888**, Roux and Yersin** showed that animals injected with **sterile filtrates** of *C diphtheriae* developed organ pathology indistinguishable from that of human diphtheria; this demonstrated that a potent exotoxin was the major virulence factor.

Diphtheria is most commonly an infection of the upper respiratory tract and causes fever, sore throat, and malaise. A thick, gray-green fibrin membrane, the pseudomembrane, often forms over the site(s) of infection as a result of the combined effects of bacterial growth, toxin production, necrosis of underlying tissue, and the host immune response. Recognition that the systemic organ damage was due to the action of diphtheria toxin led to the development of both an effective antitoxin-based therapy for acute infection and a highly successful toxoid vaccine.

Although toxoid immunization has made diphtheria a rare disease in those regions where public health standards mandate vaccination, outbreaks of diphtheria still occur in nonimmunized and immunocompromised groups. In marked contrast, widespread outbreaks of diphtheria reaching epidemic proportions have been observed in those regions where active immunization programs have been halted.

❖ Agar

Beginning in the late 19th century, agar began to be used as a solid medium for growing various microbes. Agar was first described for use in microbiology in 1882 by the German microbiologist Walther Hesse, an assistant working in Robert Koch's laboratory, on the suggestion of his wife **Fanny Hesse**. Agar quickly supplanted gelatin as the base of microbiological media, due to its higher melting temperature, allowing microbes to be grown at higher temperatures without the media liquefying.

Agar consists of a mixture of two polysaccharides: agarose and agaropectin, with agarose making up about 70% of the mixture. Agarose is a linear polymer, made up of repeating units of agarobiose, a disaccharide made up of D-galactose and 3, 6-anhydro-L-galactopyranose. Agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts, and is made up of alternating units of D-galactose and L-galactose heavily modified with acidic side-groups, such as sulphate and pyruvate.

Agar exhibits hysteresis, solidifying at about 40 °C but melting at 100°C. This property lends a suitable balance between easy melting and good gel stability at relatively high temperatures. Since many scientific applications require incubation at temperatures close to human body temperature (37 °C), agar is more appropriate than other solidifying agents that melt at this temperature, such as gelatin (melts at 28^0C).

Preparing and using agar slant tubes

An agar slant tube is a screw-capped culture tube partly filled with an agar mix such as nutrient agar. To make it a slant tube the agar is allowed to cool with the tube laying at an angle, resulting in a large surface area for spreading a culture.

Agar slants are used for storing pure cultures for a moderately long term. There is minimal risk of contamination or of losing the culture due to the medium drying out because the small volume of air inside the tube and narrow opening combine to limit water loss and exposure to outside air, including dust and other particles.

Preparing an agar slant

We prepare slants by preparing agar in a beaker, distributing it into tubes, sterilizing the capped tubes, and laying them at an angle to make a slanted surface as they cool.

1. Determine the volume of agar needed. We have found that 5-7 ml per 16 x 125 mm tube gives us a slanted surface with good surface area and a "butt" about 1 inch deep.
2. Place the desired number of tubes in a rack and obtain an equal number of screw caps.
3. Weigh out and mix the desired volume of agar in a beaker. The beaker should be close to double the volume of agar to prevent spilling.
4. Add a stir bar, place the beaker in a microwave and heat until the agar begins to foam. Watch carefully so that the agar does not boil over.
5. While stirring to keep the agar mixed, use a large syringe (w/o needle) to distribute the required volume into each tube.
6. Place a cap on each tube but do not screw them down. That way the tubes are able to vent.
7. Sterilize on a liquid cycle, remove the rack safely, and then tilt the tubes to allow the agar to cure.

Inoculating an agar tube

Aseptically remove the cap and transfer material from a single isolated colony to the agar surface. To transfer material to a slant we hold the tube at an angle, loosen the cap so that it can be pulled straight off, and pick up some culture from a single colony. With the colony material on the loop, remove the cap using the little finger of the hand holding the loop, pass the mouth of the tube quickly through the flame, then insert the loop or stick into the tube.

With the end of the loop or stick touching the agar surface near the bottom, move it back and forth slightly as you gradually pull it up toward the top of the slant. Remove it, again pass the mouth of the tube through a flame, and replace the cap.

Different investigators and technicians will conduct the inoculation differently, as can be seen in videos on the web. Some do not flame the mouth of the tube, others use different ways of transferring material to the agar surface.

❖ AGAR SLANT

Introduction In microbiology it is common practice to culture and preserve microbes on agar slants in test tube. The test tubes with appropriate medium is sterilised and then allowed to solidify at a particular angle. The angular position causes the media to solidify with angular surface to thereby increase the usable surface area. The angles at which media solidify vary with the different cultures and desire of the person, hence for this reason it desires a suitable rack that will provide the different angular slants. In 1964 Abraham Jacob Brook gave a design of culture tube design to be used in cultivating microbes like bacteria, fungi, etc. In 1972 D. J. Artie employee of NASA invented a device with variable angle tube holder to enable laboratory persons to make agar slants at any angle from horizontal to vertical. Harold F. DeGraff, Jr., in 1983 invented slant culture tube racks; see figure, 1, 2 & 3. Glaser et al in 2010 gave a method of making sampling tubes having culture media. Definition Slant is defined as the slope or lean in a particular direction or oblique direction with and fixed angle. Slanting means slope or oblique direction. Agar slant racks are made up of iron or plastic. A test-tube filled with liquid agar and allowed to solidify at a particular angle is called agar slant. 5ml or 10ml of agar medium is added to test tubes for preparing the slants. Angles used in slant preparations three angles commonly used are 5°, 11° and 20°. D. J. Artie invented a rack called as variable angle tube holder that holds the test tubes in angle from 0° to 90°. Agar slant tubes are used in growing and storing both aerobic and anaerobic microorganisms. The aerobic microbes require exposed surface area to grow while anaerobic do not requires the exposed area as they are throughout the medium. This desired distribution of microbes is achieved by tilting or angular positioning of the test tube rack. The agar medium is added in the test tube, and the tubes are kept in the rack having 5° tilt angles, but this angle does not give more exposure area. Hence the test tube rack is having front edges upwardly and outwardly at 20° from the vertical and rear edges extending upwardly and outwardly at 5° from the vertical, so that the rack can be positioned upright or at 5° or 20° slant setting. One side leg of the rack extends at 5°angle from the vertical and other side of the leg extends at 20°angle from the vertical. The inclined edge extends beyond the centre of gravity of the rack

with the test tube mounted in it. The racks can also be used to cultivate the anaerobic microbes. The figure indicates the resting at the 20°angle from the horizontal provides the greater depth of culture to facilitate the growth of anaerobic microorganisms. The large depth of medium is provided by the 20°slant helps it to prevent from drying out during the long term storage. The slant of 5°angle is used for the aerobic organisms, for daily use. The slant of 20°angle is used for the storing the cultures and for anaerobes.

Fig., 1: showing the rack for the slant

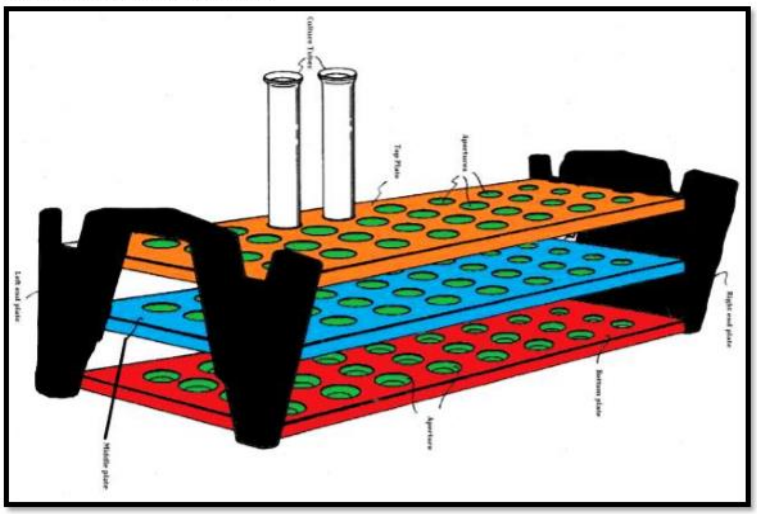


Fig. 2: Showing the angles in rack

Showing the angles in rack

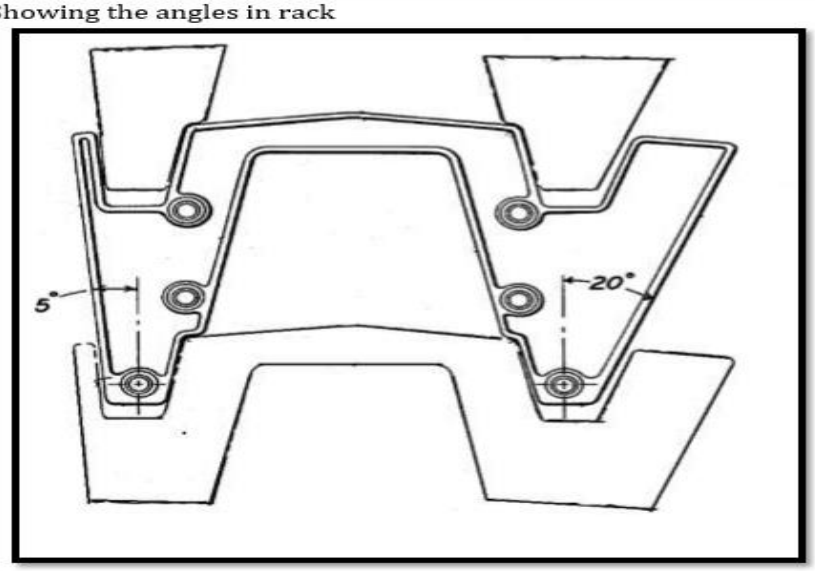


Fig. 3: Rack resting at position 5° angle

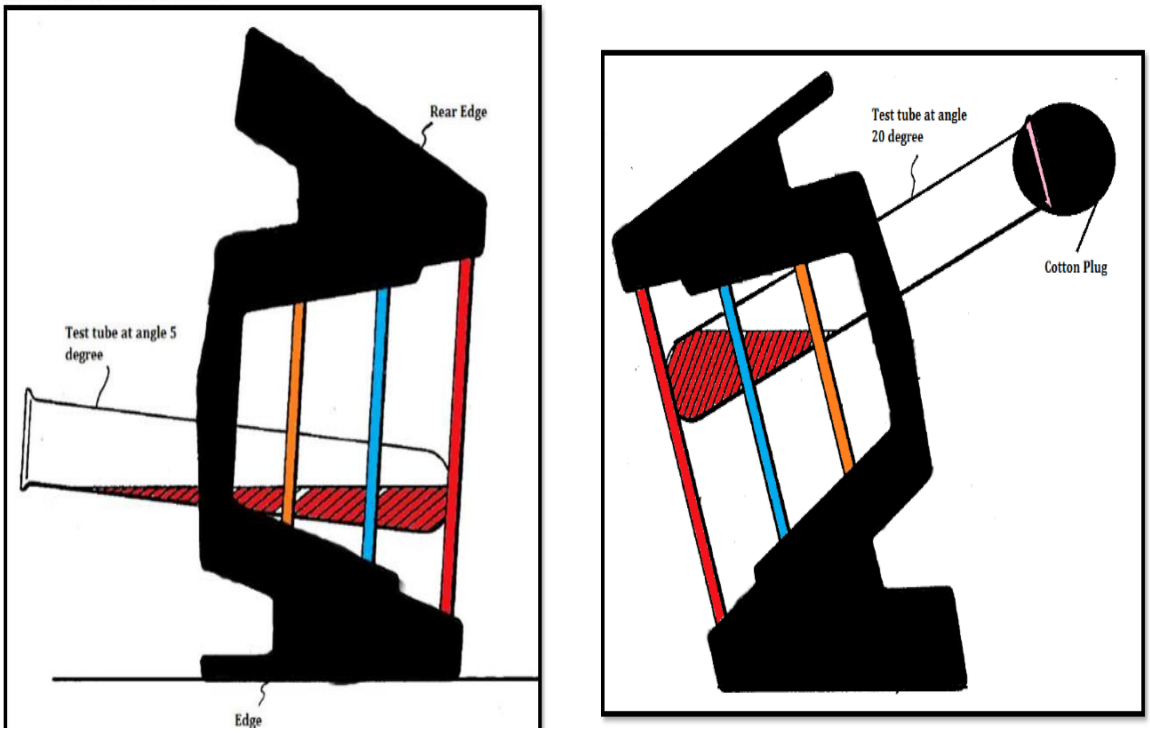
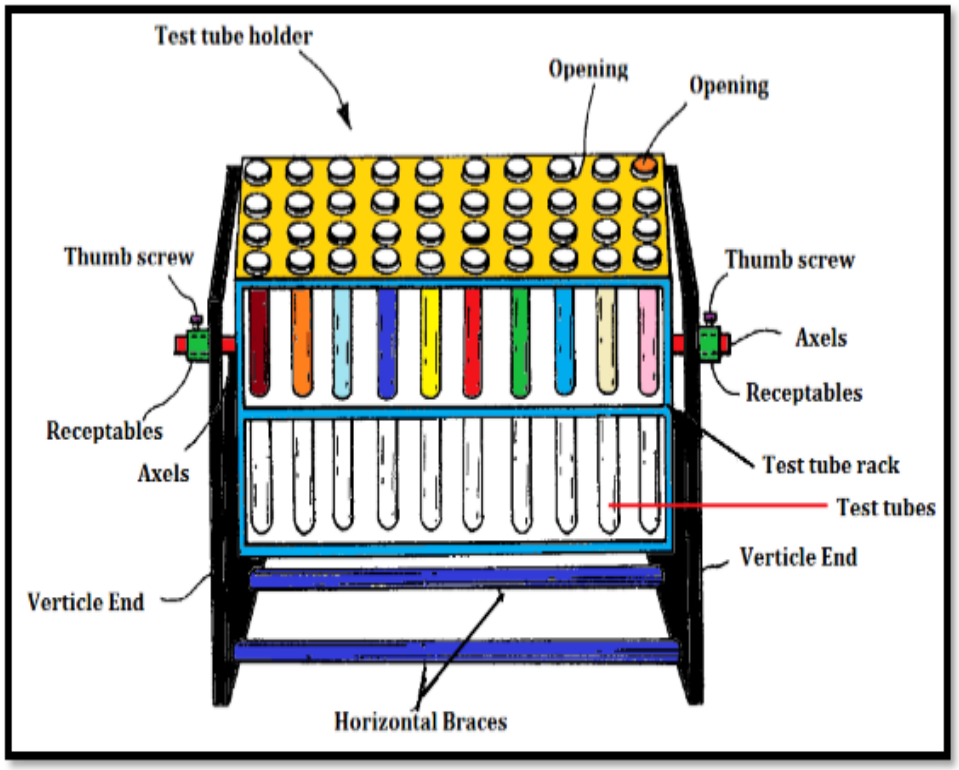
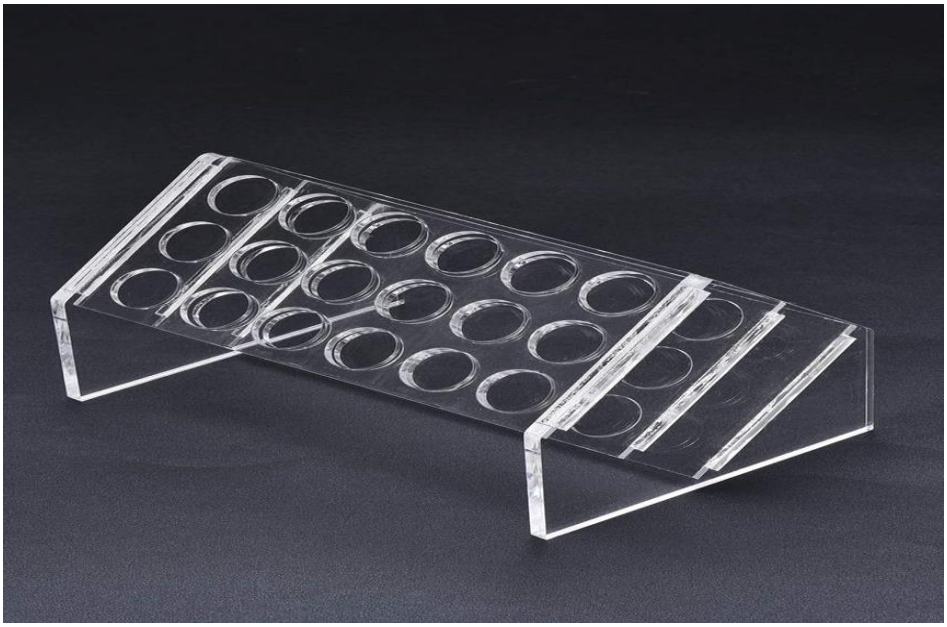


Fig. 5: Showing variable angle tube holder of A.D. Jessup.





Incubator

Definition

An incubator is an insulated and enclosed device used in biological laboratories. It creates an optimum environment which is required for the growth of microorganisms by providing optimum temperature, humidity, and other environmental conditions such as the CO2 and oxygen content inside's atmosphere.

In laboratories, it is used to grow and maintain microbiological cultures or cell cultures. Both bacterial and eukaryotic cell organisms are cultivated by using an incubator.

Working Principle of Incubator

All incubators work based on a simple principle that microorganisms require an optimum environment for their growth and development. An incubator provides an optimum level of temperature, humidity, oxygen, and carbon dioxide so that the microorganism can multiply and increase their numbers.

Incubator contain a thermostat which maintains the inside temperature of the incubator. We can monitor this temperature from the outside via the

thermometer. By utilizing the heating and no-heating cycles we maintain the inside temperature of the incubator.

Components of an Incubator

An incubator is made up of different parts or components which help to maintain the optimum condition required for the bacteria growth. The parts of an incubator are;

i) Cabinet

- The cabinet is made of a double-walled cuboidal enclosure. It has a capacity of 20 to 800L.
- It consists of an inner wall which is made of aluminum and an outer wall which is made of stainless steel sheets.
- To provide insulation to the incubator the inner gap between the inner wall and outer wall is filled with glass wool. It prevents heat loss from the incubator.

ii) Door

- The incubator contains an insulated door that encloses the insulated cabinet.
- There also presents a glass door that enables the visualization of the interior of the incubator.

iii) Control Panel

- It is located at the outer of the incubator. It consists of many switches and indicators which monitor the different parameters of the incubator.
- It also controlled the thermostat device.

iv) Thermostat

- It is used to achieve the desired temperature within the incubator.
- It constantly maintains the specified temperature within the incubator by the heating and no-heating cycles.

v) Perforated shelves

- It is located on the inner wall of the incubator. The plates of culture media are placed over it.
- These shelves are removed, which means we can clean them properly.
- The shelves contain perforations which help in the movement of hot air throughout the inside of the incubator.

vi) Asbestos door gasket

- It provides an airtight seal between the door and the cabinet.
- It separates the hot environment of the cabinet from the external environment and prevents the outside air from entering the cabinet.

vii) L-shaped thermometer

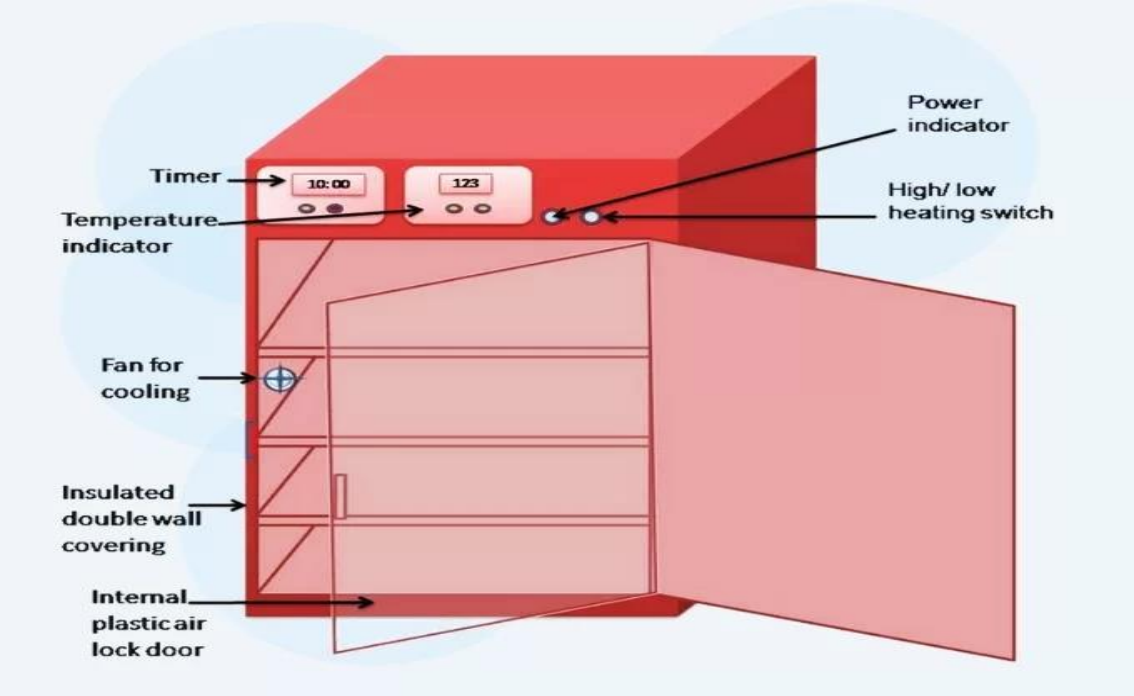
- It is located on the top part of the outer wall of the incubator.
- One end of this thermometer contains gradations which indicate the inside temperature of the incubator.
- Another end of this thermometer contains a mercury bulb which is located within the incubator.

viii) HEPA filters

- Some incubators also contain HEPA filters; it helps to reduce the contamination which is created by the airflow.
- It is connected with an air-pump which circulates the air within the incubator so it creates a closed-loop system to reduce the chances of contamination.

ix) Humidity and gas control

- A water source or reservoir is located underneath the chamber. This water is vaporized to control the relative humidity inside the incubator.
- It also contains a gas chamber that maintains the CO_2 concentration within the incubator.



Types of Incubator in laboratory

There are different types of incubators in microbiology. These are classified based on the types of parameters or the purpose of the incubator.

1. **Bench top incubators:** Most of the laboratories contain Benchtop incubators. It has a basic function of temperature control and insulation.
2. **Shaker incubator:** It contains a shaker which continuously shakes the culture media for proper aeration. It transfers the heat uniformly within the incubator.
3. **CO_2 incubators:** This type of incubator automatically controls the CO_2 and humidity within the chamber.
4. **Cooled incubators:** It is also known as a cooling incubator. They contain a refrigeration system with heating and cooling functionality. It is used to incubate the culture media at temperatures below the ambient. The heating and cooling controls of this incubator should be appropriately balanced.

Operating Procedure of an incubator

After preparation of culture, it needs to be stored in an incubator at the desired temperature, CO_2 concentration for a period of time.

1. Before run an incubator makes sure there are no remaining items present from the previous cycles.
2. If different organisms require the same parameters then keep them in the same incubator.
3. Then close the door and switch on the incubator. Make sure the door is properly closed.
4. Now set the desired temperature in the controller and keep monitoring it through the thermometer.
5. If any organism requires a specific concentration of CO2 or a specific humidity then we also can set them in the controller.
6. After setting the parameters, place the culture plates on the perforated shelves upside down.
7. It is necessary to seal the plates with adhesive tapes or are placed in plastic bags to prevent contamination.

Application of Incubator

In laboratories incubator is used for different purpose such as;

1. Used to maintain the growth of different microbial cultures or cell cultures, which we can use in later.
2. Sometimes they are used to enhance the growth rate of organisms.
3. Some advanced incubators are used for the reproduction of microbial colonies and subsequent determination of biochemical oxygen demand.
4. In zoology, they are also used for the breeding of insects and hatching of eggs.
5. It also used to store different medical samples.

Precautions

1. Before run an incubator makes sure there is enough water present underneath the shelves, otherwise the culture media will be drying out.
2. Clean the incubator regularly, otherwise organisms will settle down on the shelves or the corners of the incubator.
3. To avoid the condensation of water onto the media placed the plates upside down with the lid at the bottom.
4. Before placing the culture plates inside the incubator make sure the desire parameters are achieved.
5. Avoid repeated opening of the door when the incubator is running.

❖ **Anaerobic incubator**

Anaerobic incubators provide a non-oxygen environment to cultivate and handle anaerobic microbe. They usually form a part of the chambers or workstations that are used in laboratories specializing in anaerobic culture work. The incubator is provided in the workspace chamber whose temperature is controlled by using a solid state temperature sensor probe attached to the incubator body and a heating element. The anaerobic atmosphere in the chamber is initially achieved through removal of air of the workspace by flowing anaerobic mixed gas (AMG), which pushes normal atmosphere out of the chamber. Anaerobic incubators are used for anaerobic microbiology assays, clinical microbiology anaerobic tissue culture research, anaerobic bacteriology, industrial research, and quality control applications. They find usage in large clinical or research laboratories for cell culture applications where accurate control of oxygen, carbon dioxide, temperature, and humidity are required.

❖ **Perfusion incubator**

Liver chips have been developed to recapitulate in vivo physiological conditions to enhance hepatocyte functions for assessing acute responses to drugs. To develop liver chips that can assess repeated dosing chronic hepatotoxicity, we need to ensure that hepatocyte functions be maintained at constant values over two weeks in stable culture conditions of sterility, temperature, pH, fluidic-flow of culture media and drugs. Design of perfusion-incubator-liver-chip (PIC) for 3D cell culture assures a tangential flow of the media over the spheroids culture. Rat hepatocyte spheroids constrained between

a cover glass and a porous-ultrathin Parylene C membrane experienced optimal mass transfer and limited shear stress from the flowing culture media; maintained cell viability over 24 days. Hepatocyte functions were significantly improved and maintained at constant values (urea, albumin synthesis, and CYP450 enzyme activities) for 14 days. The chip acts as an incubator, having 5% CO_2 pressure-driven culture-media flow, on-chip heater and active debubbler. It operates in a biosafety cabinet, thus minimizing risk of contamination. The chronic drug response to repeated dosing of Diclofenac and Acetaminophen evaluated in PIC were more sensitive than the static culture control.

PIC Structure and Functionality

Device structure and microfluidic setup

The perfusion incubator liver chip (PIC) was designed combining a rigid, well defined and reusable glass/silicon structure with the advantages of PDMS assemblies (elasticity and gas permeable). The chip structure consists of three main elements illustrated in Fig. 1a:

A glass / silicon structure containing a 3D microfluidic circuit, the cell culture chamber, a bubble trap chamber and a heater (Fig. 1b). Nanoport microfluidic connectors were mounted on the glass/silicon die. The microfluidic circuits were engraved on both sides of silicon die while the glass die assures the sealing of the bottom microfluidics circuit. On the other side of the glass die a heater is printed.

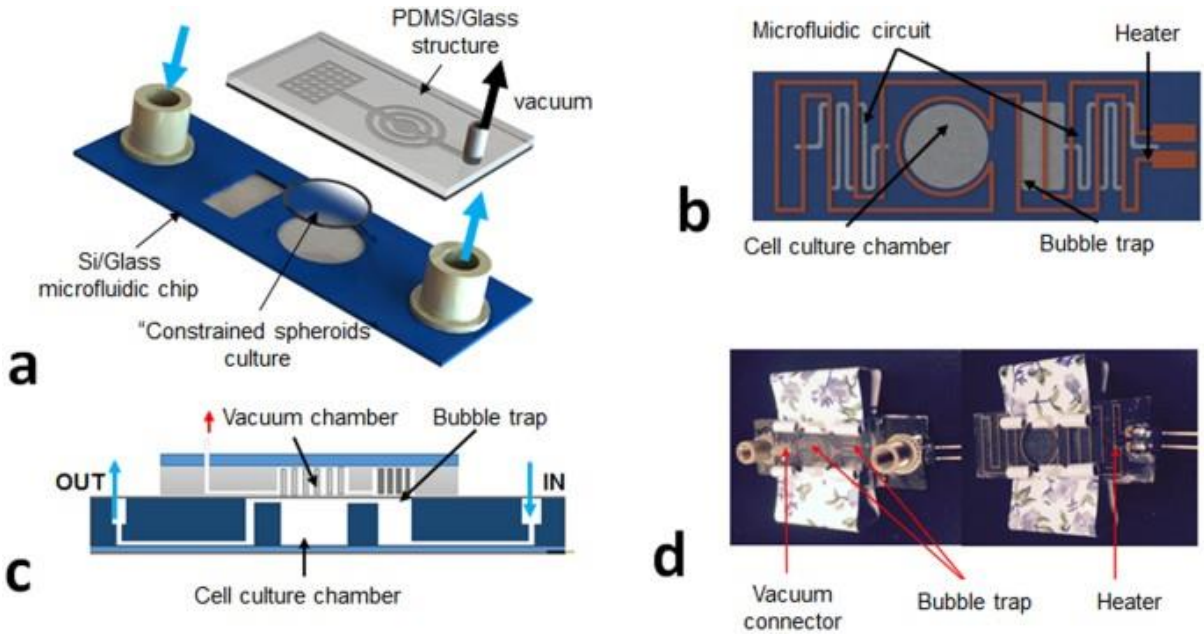
A PDMS/glass sealing structure closes the microfluidic circuit. The PDMS structure also acts as a debubbler to absorb the air bubbles trapped when assembling the chip and generated during the culture. A cross-section of the chip illustrating the functions of the PDMS / glass die is presented in Fig. 1c.

Schematic of the PIC chip.

a. 3D view with the PIC. A glass/silicon structure containing a 3D microfluidic circuit, the cell culture chamber, a bubble trap chamber as well as a heater
b. bottom view of the chip's layout illustrating the microfluidic circuit, the cell culture chamber, the bubble trap and the heater,
c. cross-section of the PIC illustrating the structure of the bubble trap. It consists of a 70 μm-thick PDMS membrane (gas permeable) bonded to a PDMS molded chamber with pillars that support the membrane. The PDMS structure is connected to external vacuum (through a pressure controller). The gas bubbles trapped in the microwell can diffuse through the PDMS membrane

due to negative pressure in the vacuum chamber while culture media remains inside the culture chamber.

d. Top and bottom view of the PIC



❖ **Pocket incubator**

This invention comprises a transparent and unbreakable pocket incubator of such constructional characteristics that micro-organisms may be deposited therein, sealed air tight against contamination, guarded against lethal ultraviolet rays, and also cultured and developed in situ to a point where they may be identified and counted.

An important field of use of the incubator of this invention is in water analysis as conducted by health officials to guard against epidemics originating from pollution of water supply or from infection by sabotage or in biological warfare. In accordance with the present practice, liter samples of the water to be treated must be obtained, sealed, refrigerated, and transported to the analysing laboratory. The samples are there subjected to filtering and the residue eluted and cultured in suitable nutrient. The whole process usually requires three or four days and this period, of course, may be at a critical stage in the development of epidemic conditions.

The object of the present invention is to provide a pocket incubator which will permit the filtered micro-organisms to be obtained in the field so that

culture may be immediately initiated and identification of the organism made within a few hours. It has been discovered that these desirable results may be obtained by employing a flat covered receptacle molded of transparent polystyrene and resembling in its general shape the well-known Petri dish. Polystyrene, or its equivalent, is an essential feature of the present invention since it is tough, flexible, resilient, and practically unbreakable. It may be molded so as to provide a hermetic (air tight) seal between the trays and cover portions. It has the unique property of being impervious to ultraviolet rays in the range which is lethal to micro-organisms, that is to say, from 560 to 3000 A. It is, moreover, transparent and has a rate of heat transmission only about one-quarter that of glass so that condensation within the incubator is eliminated to all practical intent. Its contents, therefore, are not obscured by a film of moisture and can, if desired, be directly subjected to microscopic examination without any necessity for opening the incubator in the meantime.

Going more into detail, the pocket incubator comprises a tray portion having an upstanding continuous wall and a cover portion having a flat top merging into a continuous wall tapered to fit telescopically with the upstanding wall of the tray and forming a hermetic seal therewith, the cover portion comprising transparent shatterproof polystyrene.

POCKET INCUBATOR

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Fig. 1

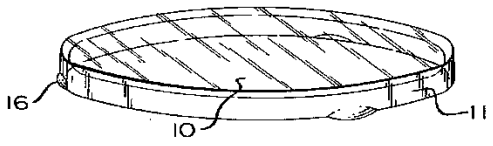
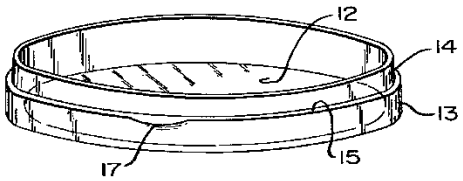


Fig. 2



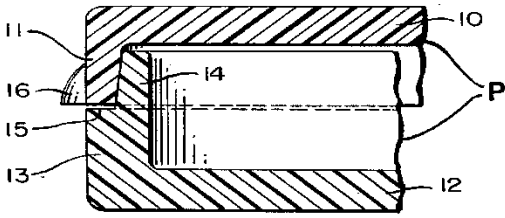


Fig. 3

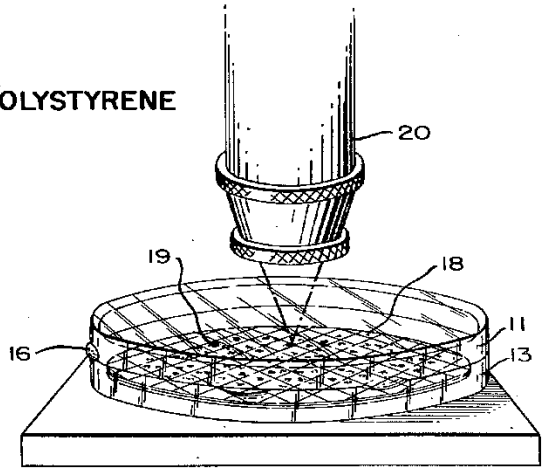


Fig. 4

Fig. 1 is a view in perspective of the cover portion,

Fig. 2 is a view in perspective of the tray portion,

Fig. 3 is a fragmentary sectional view on an enlarged scale, and

Fig. 4 is a view in perspective suggesting the microscopic inspection of bacteria contained in the incubator.

The cover and tray portions of the incubator are molded of transparent polystyrene which a resinous material is having characteristics that are essential in the incubator of this invention. In the first place, polystyrene is slightly flexible, resilient, non-shatterable and so practically unbreakable as employed in the present structure. These characteristics make possible the desired telescopic hermetical seal between the cover and tray portions of the device. In the second place, polystyrene is impervious to ultraviolet rays to an

extent which protects micro-organisms enclosed within the incubator from damage from a source which would otherwise be fatal. Further, as already noted, polystyrene has a low thermal conductivity and this tends to reduce internal condensation and to stabilize the internal temperature of the incubator. Finally, polystyrene is substantially unaffected by any change of temperature encountered in the normal use of the pocket incubator.

The incubator is designed to contain a circular filter sheet and has the property of permitting rapid passage of liquid and surface retention of micro-organisms or bacteria. The filtering step may be carried out in the field and the filter disk 18 immediately placed within the pocket incubator in contact with an underlying absorbent pad impregnated with suitable nutrient. This nutrient may be standard wet media or the pad may be impregnated with dehydrated nutrient. Activation and culturing then requires only the addition of distilled Water. If the sealed incubator is now transported to the analysing laboratory in the pocket of the analyst, his body heat will warm the incubator and its contents sufficiently to cause immediate culture of the contained bacteria and coliform organisms so that within perhaps two hours the colony will have developed sufficiently for identification.

The bacteria appear on the surface of the filter disk. These may be readily counted in the sealed incubator, the ruled area of the filter disk assisting in this respect. It will be observed that the incubator has remained hermetically sealed from start to finish, from the insertion of the filter disk, during incubation and development, and during the whole process of examination and identification, and that all of these operations have been carried out without interruption and with the highest possible expedition.

Thermal Gradient incubator

The temperature-gradient incubator consists of an aluminium bar with channels for media, with controlled temperatures at each end, and with insulation to prevent heat transfer. The resulting linear temperature gradient provides a means for determining minimal or maximal growth temperatures of microorganisms in any desired range and at any desired gradient. The operation of the incubator is unaffected by line-voltage variations or by ambient temperature. Media do not dehydrate seriously even during prolonged periods of operation.

Applications

1. Validation of optimal culture temperature for mammalian cells or microorganisms.
2. Germination and growth testing of seeds, bulbs, etc.; plant tissue culture, breeding experiment/research.
3. Developmental research or breeding of insects.
4. Chemical reaction experiments; quality verification of pharmaceuticals, foods, or cosmetics; and enzyme research.

Temperature can be controlled between +5 and +50^{o}C. When lighting, temperature should be set from 10^{o}C to 40 ^{o}C. If the lowest temperature is not less than 5 ^{o}C and less than 10oC, the temperature difference between shelves should be 5 ^{o}C. If the lowest temperature is not less than 10oC, the temperature difference between shelves should be 5- 10^{o}C. The temperature difference between adjacent shelves should be not less than 5 ^{o}C and not more than 10 ^{o}C. Temperature should be set to be lower in the shelf below and to be higher in the shelf above.

❖ Pasteurizer: Beer Pasteurizing Apparatus

Pasteurization is the process of heat treating beer to inhibit the growth of potential beer spoilage microorganisms and prolong the shelf life of the beer. Named after the great French scientist Louis Pasteur, who was able to prolong the drinking quality of beer by holding the beer at 55°C–60°C (131°F–140°F) for a short time, pasteurization is used in the production of most of the draught and bottled/canned beers throughout the world. Pasteurization is often confused with sterilization. In the former, the beer is subject to sufficient heat processing to render the beer free from microbial spoilage during the course of its shelf-life. However, low levels of some microorganisms might still survive the heating, albeit without causing spoilage of beer. In sterilization, the heat treatment applied is of such intensity that it kills all microorganisms present.

Initially, based on a largely empirical observation, holding beer at a temperature of 60°C for a few minutes was deemed sufficient to maintain microbiological integrity over its shelf-life of several months. The brewing industry uses this temperature of 60°C as a basis for quantifying the extent of the pasteurization process. For every minute the beer is held at 60°C it is said to be subject to one pasteurization unit (PU). Holding for 15 min at 60°C, therefore, is 15 PUs of treatment.

There are two major methods of beer pasteurization. Bottled and canned beers are pasteurized by passing the filled containers through a long, relatively narrow chamber in which hot water is sprayed over the containers for a fixed time before cooling. The spraying chamber is called a “tunnel” and the process is therefore called “tunnel pasteurization.” For large containers of beer such as kegs, heating the contents of the keg in a tunnel is impractical. Instead, beer is heat treated by being passed through a heat exchanger (hot water gives up its heat by exchanging with cold beer over a large surface area) where the beer will be heated to 70°C–72°C (158°F–162°F) for as little as 30 sec. See heat exchanger.

This is known as “high-temperature/short-time” processing or, more commonly, “flash pasteurization.” It is calculated that 72°C (162°F) for 30 sec is equivalent to 15 min at 60°C, or 15 PUs. Beer that has been flash pasteurized is then cooled rapidly before being filled into sterile containers. Depending on the perceived risk of microbiological spoilage of the beer, the number of PUs applied will vary but for most beers the range is 5–25 PUs, with 15 PUs the

approximate industry standard. Beers with very low alcohol contents will tend to receive more PUs.

Although pasteurization is effective in preventing microbial spoilage in beer, it can adversely impact flavour by accelerating the “staling” or “aging” of beer. Staling is a natural phenomenon, the result of relatively slow chemical changes to the beer components with time, particularly if any quantity of oxygen is present in the beer at the time of filling the beer container. The application of heat during pasteurization increases the rate of the chemical changes. Brewers endeavour to minimize oxygen pickup during packaging and reduce PU application to a minimum to maintain the freshness of the beer for as long as possible.

Flow Pasteurization of Beer

Beer is led from the inlet tank by a high pressure pump into the saturator tank. This tank is filled with carbon dioxide (CO_2) and has a steady constant pressure of 7-10 bar. The degree of saturation can be regulated by pressure. Beer is driven by the pressure of the saturator tank into the regenerative heat exchanger where the beer is preheated. After that the beer is directed to heat pasteurization and holding exchanger, where the pasteurization takes place. Subsequently, the beer is conducted into the second of the regenerative heat exchangers. In the next stage, the beer is kept in refrigeration units where is cooled to a temperature suitable for the filling.

The advantage of the flow pasteurizer is lower heat consumption due to efficiency of the recovery, and also it requires less space which pasteurization equipment occupies.

Tunnel Pasteurization of Beer

Bottles are shipped through special insulated tunnel conveyor where they are showered by hot water in three bands (preheating zone, pasteurization zone and cooling zone). Under the conveyor belt there are tubs of water. During preheating and cooling heat recovery is performed. Heating can be either electric or steam. The speed of the conveyor tunnel is adjustable, pasteurization temperature is maintained automatically.

The advantage of tunnel pasteurizer is current sterilization of not only beverages, but also the surface of the bottles. The disadvantages are higher heat consumption and the destruction of large amounts of beverages (bottled, which

are currently in pre-heating a pasteurization zone) when the line stops due to a fault.

Sterile filtration

In recent years, instead of pasteurizing the beer, some brewers attempt to exclude beer spoilage microorganisms by a process of microfiltration known as "sterile filtration." This method can be highly effective, but it also tends to strip away flavor, aroma, body, and even color.

❖ STERILIZATION BY RADIATION(COLD STERILIZATION)

Radiation is currently used for sterilization and decontamination in the medical supplies (surgical supplies, vaccines, and drugs) and food industries. Two types of radiations are available; **ionizing and non-ionizing.**

Radiation sterilization is not widely used in food industries as people have concerns about radioactive contamination, production of toxic or carcinogenic products, change in nutritional value, and taste alteration, etc.

❖ Ionizing radiations

Ionizing radiation is an excellent agent for sterilization/disinfection; it kills organisms without increasing the temperature; so aptly called cold sterilization. It destroys bacterial endospore and vegetative cells, both eukaryotic and prokaryotic; but not always effective against viruses.

Mechanism of Sterilization by Ionizing Radiations

When ionizing radiation collides with particles, they produce electrons (e^-) and other reactive molecules such as hydroxyl radicals (•OH), and hydride radicals (H•). Each of these reactive molecules is capable of degrading and altering biopolymers such as DNA and protein. Breakage of DNA and degradation of enzymes lead to the death of the irradiated cells.

Several sources of ionizing radiation are available, including X-ray machines, cathode ray tubes (electron-beam radiation), and radioactive nuclides (sources of gamma/x-rays).

X-Rays

X-rays are lethal to microorganisms and higher forms of life but are rarely used in sterilization because their production is expensive and efficient utilization is difficult (since radiations are given off in all directions from the point of origin).

Gamma Rays

Gamma radiations are high-energy radiations emitted from certain **radioisotopes such as Caesium-137 (^{137}Cs) and Cobalt-60 (^{60}Co),** both relatively inexpensive by-products of nuclear fission. Gamma rays are similar to x-rays but are of shorter wavelength and higher energy. They are capable of great penetration into the matter, and they are lethal to all life, including microorganisms. Gamma rays are attractive for use in commercial sterilization of materials of considerable thickness or volume, eg. packaged food or medical devices.

Cathode Rays (Electron-Beam Radiation)

Cathode rays or electron-beams can sterilize materials at room temperature with brief exposure. They have limited penetrating power and are used for the sterilization of surgical supplies, drugs, and other materials.

Uses of Ionizing Radiations

The major method in use for radiation sterilization is gamma irradiation. Gamma radiation is used in the sterilization of;

- Disposables such as plastic syringes, infusion sets, catgut sutures, catheters, gloves, and adhesive dressings before use.
- Bone, tissue grafts, antibiotics, and hormones.
- Irradiation of food (permitted in some countries).

Advantages of Ionizing Radiations

1. **High penetrating power:** products can be processed in their fully sealed, final packaging thus limiting the risk of contamination following sterilization.
2. **Rapidity of action**: saves and efforts.

3. **Temperature is not raised**: compatible with temperature-sensitive materials, such as pharmaceuticals and biological samples.
4. **Flexibility:** can sterilize products of any phase (gaseous, liquid, or solid materials), density, size, or thickness.

Disadvantages

- Capital costs are high and specialized facilities are often needed e.g. for gamma irradiation
- Use of gamma radiation requires handling and disposal of radioactive material.
- Not compatible with all materials and can cause breakdown of the packaging material and/or product. For example, Common plastics such as polyvinyl chloride (PVC), acetal, and polytetrafluoroethylene (PTFE) are sensitive to gamma radiation.

Sterilization/disinfection control: Efficacy of ionizing radiation is tested by using *Bacillus pumilus.*

❖ **Non-ionizing Radiation**

Non-ionizing radiations are quite lethal but do not penetrate glass, dirt, films, water; hence their use is restricted for disinfection of clean surfaces in operation theatres, laminar flow hoods as well as water treatment. The recommended dose is 250-300 nm wavelengths, given for 30 minutes.

Examples of non-ionizing radiation include **infrared and ultraviolet radiation**.

Infra-Red Radiation

Infra-red rays are low energy type electromagnetic rays, having wavelengths longer than those of visible light. They kill microorganisms by oxidation of molecules as a result of heat generated. Infra-red rays are used for the rapid mass sterilization of syringes and catheters.

Ultraviolet Light (UV) Sterilization

Sunlight is partly composed of UV light but shorter wavelengths of light are filtered out by the ozone layer. There are three types of UV radiation; UVA, UVB, and UVC, classified according to their wavelength. Short-wavelength UVC is the most damaging type of UV radiation.

Mechanisms of UV Sterilization

Many cellular materials including nucleic-acids absorb ultraviolet light. It causes bonding of two adjacent pyrimidine i.e. the formation of pyrimidine dimer (TT Dimer), resulting in the inhibition of DNA replication. This leads to mutation and death of exposed organisms.

Uses of UV Sterilization

UV lights are useful for disinfecting surfaces, air, and water that do not absorb the UV rays. Certain types of UV lights can kill the flu (influenza) virus. Ultraviolet radiation is used for disinfecting enclosed areas such as bacterial laboratory, nurseries, inoculation hood, laminar flow, and operation theatres. For example, laboratory biological cabinets all come equipped with a "germicidal" UV light to decontaminate the surface after use.

While UV sterilization is on-going, the area should be closed and UV lamps must be switched off immediately after use.

Effects of UV light in SARS-CoV-2 (COVID-19)

UV radiation kills viruses by chemically modifying their genetic material, DNA, and RNA. The most effective wavelength for inactivation, 260 nm, falls in the UVC range. Though we do not have much research regarding the effect of UVC in SARS-CoV-2, concentrated form of UVC is now on the front line in the fight against COVID-19. UVC light is being used to sterilize buses, UVC-emitting robots to sterilize hospital floors and even banks are using UV light to disinfect money.

Disadvantages

1. Damages skin and eyes: Conventional UV light can penetrate and damage skin and also cause cataracts.
2. Does not penetrate paper, glass, and cloth.