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Section: A

Basic microbiology

Q1. Write the names and function of different equipments used in microbiology lab?

Answer:

1. Hot Air Oven for Sterilization:

It is used for sterilization of glassware’s, such as test tubes, pipettes and petri dishes. Such dry sterilization is done only for glassware’s. Liquid substances, such as prepared media and saline solutions cannot be sterilized in oven, as they lose water due to evaporation.

2. Drying Oven:

For preparation of certain reagents, the glassware’s, after proper cleaning and rinsing with distilled water, are required to be dried. They are dried inside the drying oven at 100°C till the glassware’s dry up completely.

3. Autoclave:

Autoclave is the nucleus of a microbiology laboratory. It is used not only to sterilize liquid substances such as prepared media and saline (diluents) solutions, but also to sterilize glassware’s, when required. It has the same working principle as a domestic pressure cooker. The maximum temperature that can be obtained by boiling water in an open container is 100°C (boiling point of water).

4. Microbiological Incubator:

Profuse growth of microbes is obtained in the laboratory by growing them at suitable temperatures. This is done by inoculating the desired microbe into a suitable culture medium and then incubating it at the temperature optimum for its growth. Incubation is done in an incubator which maintains a constant temperature specifically suitable for the growth of a specific microbe. As most of the microbes pathogenic to man grow profusely at body temperature of normal human being (i.e. 37°C), the usual temperature of incubation is 37°C.

5. BOD Incubator (Low Temperature Incubator):

Some microbes are to be grown at lower temperatures for specific purposes. The BOD low temperature incubator which can maintain temperatures from 50°C to as low as 2-3°C is used for incubation in such cases.

6. Fridge (Refrigerator):

It serves as a repository for thermo labile chemicals, solutions, antibiotics, serums and biochemical reagents at cooler temperatures and even at sub-zero temperatures (at less than 0°C). Stock cultures of bacteria are also stored in it between sub-culturing periods. It is also used for the storage of sterilized media, so as to prevent their dehydration.

7. Deep-fridge:

It is used to store chemicals and preserve samples at very low sub-zero temperatures.

8. Electronic Top-pan Balance:

It is used for weighing large quantities of media and other chemicals, where precise weighing is not of much importance.

9. Electronic Analytical Balance:

It is used to weigh small quantities of chemicals and samples precisely and quickly.

10. Double-pan Analytical Balance:

It is used to weigh chemicals and samples precisely. Weighing takes more time, for which it is used in emergency only.

11. Distilled Water Plant:

Water is used in the preparation of media and reagents. If the media are prepared using tap water, the chemical impurities present in it may interfere with the growth of the microorganisms in the media. Moreover, the higher is the bacteria content of the media, the longer is the time required for their sterilization and greater is the chance of survival of some bacteria.

12. Ultrapure Water Purification System:

For precision analytical works, now-a-days, instead of using double- or triple-distilled water, micro- filtered water is used. In case of distilled water, there is chance that, few volatile substances present in the water get volatilized during heating of the water and subsequently get condensed into the distilled water collected.

13. Homogeniser:

For microbiological analysis, liquid samples are directly used, whereas solid samples have to be mixed thoroughly with a diluents (usually physiological saline), so as to get a homogenous suspension of bacteria. This suspension is assumed to contain bacteria homogenously.

14. pH Meter:

A pH meter is an instrument for determining the pH of liquid media, liquid samples and buffers. It has a glass pH electrode. When not in use, it should be kept half immersed in water contained in a small beaker and preferably be covered by a bell jar to avoid dust accumulation in the water and loss of water through evaporation.

15. Hot Plate:

Hot plate is used to heat chemicals and reagents. The hot plate is made of an iron plate, which gets heated by an electric heating element from below. The required degree of heating is obtained by a regulator.

16. Shaking Water Bath:

Sometimes, heating at very precise temperatures is required. Such precise temperatures cannot be obtained in an incubator or oven, in which temperature fluctuates, though slightly. However, precise temperatures can be maintained in a water bath, which provides a stable temperature.

17. Quebec Colony Counter:

In enumeration of bacteria in samples, it is assumed that a single bacterium gives rise to a single visible colony, when grown on a plate of solidified nutrient medium. Thus, by counting the number of colonies, the number of bacteria in a sample can be estimated.

18. Electronic Colony Counter:

Electronic colony counter is of two types:

(1) Hand-held electronic colony counter and

(2) Table-top electronic colony counter.

The hand-held electronic colony counter is a pen-style colony counter with an inking felt-tip marker. For counting of colonies of bacteria grown in a petri dish, it is kept in an inverted position, so that the colonies are visible through the bottom surface of the petri dish.

19. Magnetic Stirrer:

In the preparation of solutions, certain chemicals require stirring for long time, to be dissolved in certain solvents. Magnetic stirrer is used to dissolve such substances easily and quickly. A small Teflon- coated magnet, called ‘stirring bar’, is put into a container containing the solvent and the solute.

20. Sonicator:

It is used to rupture cells using high frequency waves.

21. Vortex Mixer:

It is an instrument used for thorough mixing of liquids in test tubes. It has a rotor, whose speed can be controlled. On the tip of the rotor is a foam-rubber top. When the bottom of a test tube is pressed upon this foam-rubber top, the rotor starts rotating, thereby rotating the bottom of the test tube at high speed.

22. Electronic Cell Counter:

It is used to directly count the number of bacteria in a given liquid sample. An example of electronic cell counter is the ‘Coulter counter’. In this equipment, a suspension of bacteria cells is allowed to pass through a minute orifice, across which an electric current flows.

23. Membrane Filtration Apparatus:

Certain substances like urea disintegrate and lose their original properties, if sterilized by heat. Such substances are sterilized by membrane filtration apparatus. In this apparatus, the solution of the substance to be sterilized is filtered through a membrane filter, which does not allow bacteria cells to pass down. Filtration is done under suction pressure to increase the rate of filtration

24. Microscopes:

Different types of microscopes are used for visual observation of morphology, motility, staining and fluorescent reactions of bacteria.

25. Computers:

Computers are generally used for analysis of results. They are also used for identification of bacteria easily within few hours. Otherwise, identification of bacteria is a tedious process and takes days together to identify one bacteria species.

26. Spectrophotometer:

It is an instrument for measuring the differences in color intensities of solutions. A beam of light of a particular wavelength is passed through the test solution and the amount of light absorbed (or transmitted) is measured electronically.

27. Electrical Devices:

A fluctuation of electric voltage in the laboratory is one of the most important reasons, which reduces the longevity of the equipments and sometimes damage them. Therefore, all the voltage-sensitive equipments should be provided with voltage protection devices like stabilizers, servo stabilizers or constant voltage transformers (CVT) as per the recommendations of the manufacturers of the equipments.

28. Automatic Bacteria Identification System:

It is an instrument used for automatic computer-assisted identification of bacteria. The conventional method of identification of bacteria is very lengthy and cumbersome.

29. PCR Thermocycler, Refrigerated Centrifuge, Ultra-centrifuge, Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), Paper Chromatography, Column Chromatography and Electrophoresis Unit:

These are instruments used for isolation, purification and identification of biochemical substances, such as bacterial DNA, plasmids, microbial toxins etc. Polymerase chain reaction (PCR) is an important tool in nucleic acid based methods. It is a workhorse in modern microbiology and biotechnology laboratories

Q2. What are the different chemical and physical methods of sterilization and disinfection?

Answer:  
Methods of Sterilization

Physical Methods

Sunlight

Sunlight has an active germicidal effect due to the presence of ultraviolet rays. It is a natural procedure of sterilization which reduces the number of microorganisms in water tanks, lakes, etc.

Heat

Heat is a mostly used method of sterilization. Moreover, it is a highly effective and most reliable process. There are two major methods of using heat in sterilization which are dry heat and moist heat. The principle behind both of these methods is similar. Dry heat induces the denaturation of protein, oxidative damage and toxic effect due to the high level of electrolytes. Moreover, the dry heat can also damage the DNA of the microorganism. As a result, the microorganism got killed. Moist Heat kills the microorganisms by denaturation and coagulation of proteins. There are several factors that can influence the heat killing procedure.

Red Heat

Inoculation loops, wires, forceps tips, needles are needed to be sterilized to inhibit microbial contamination. These instruments are held in the flame of a Bunsen burner until they become red hot.

Flaming

Glass slides, scalpels, and mouths of culture tubes or conical flasks are passed through Bunsen flame without allowing them to become red hot.

Incineration

This procedure is used to reduce the infective material into ashes by burning. The incinerator is used for the process. Soiled dressings, animal carcasses, bedding, and pathological materials are dealt with this method.

Hot Air Oven

It is a widely used method of sterilization by dry heat. The heat inside the oven is maintained by electricity and a fan fitted inside it provides the adequate distribution of hot air inside the chamber. A thermostat is also connected which maintains the temperature inside the chamber. 1600C for two hours is required for sterilization. There are also some alternative temperatures and holding time which include 1700C for 1 hour and 1800C for 30 minutes.

Inspissation

Media like Lowenstein- Jensen’s and Loeffler’s serum are required to sterile at 80-850C for 30 minutes daily on three consecutive days. This process is known as inspissation and the instrument used is called inspissator.

Vaccine bath

It is used for sterilization of bacterial vaccines at 600C for one hour. Serum or other body fluids can be sterilized by heating in a water bath at 560C for several successive days.

Low-temperature steam formaldehyde sterilization (LTSF)

This method is applicable for materials that cannot withstand 1000C temperature. In this method, steam at subatmospheric pressure at 750C with formaldehyde vapor is used. Bacillus stearothermophilus plays an important role as a biological control to test the efficacy of the test.

Boiling

It is an effective method that can kill vegetative cells. Boiling for 10-30 minutes can kill most of the vegetative cells; however, many spores can withstand this temperature. Boiling can be employed when adequate methods are not available to sterilize glass syringes, rubber stopper, etc.

Tyndallisation

In this case, steam at 1000C for successive 3 days is used. It is also known as intermittent sterilization. In this case, the first exposure kills the vegetative forms, and in the intervals between the heating and remaining spores germinates into vegetative forms which are killed on subsequent heating.

This process is applied for sterilization of egg, serum or sugar-containing media which can be damaged due to exposure in high temperature for a longer period.

Steam sterilizer

Koch’s and Arnold’s steam sterilization is usually used for media which can easily decompose due to the high temperature in the autoclave. Those media are kept on a perforated tray and steam at 1000C and at atmospheric pressure passes through the media for 90 minutes. It is an effective method to kill vegetative cells.

Autoclave

Steam above 1000C or saturated steam has a better killing capacity than dry heat. Bacterial proteins coagulate rapidly at moist heat. Saturate steam has the ability to penetrate any porous material. When steam comes into contact with the cooler surface it condenses into water and releases its latent heat to the surface. The large reduction in volume sucks in more steam to the same site and the process continues until the temperature of the substance raised to that of steam. The condensed water produced moist conditions for killing the microbes present.

Radiation

Ionizing radiation

Ionizing radiations such as gamma rays, X rays, and cosmic rays are used for sterilization process. Due to the high penetrating power, these radiations are lethal for cells. The bacterial cells are killed by damage in the DNA. Gamma radiations from a cobalt 60 source are commercially used for sterilization of disposable items. This procedure is also known as cold sterilization.

Non-ionizing radiation

Infrared radiation and UV radiation comes under this of radiation. Infrared radiation is used for mass sterilization of syringes and catheters. UV radiation with a wavelength of 240nm to 280nm has bactericidal capacity. The UV radiation causes protein denaturation and interferes with DNA replication of bacteria. UV radiations are used for sterilization of close areas, surfaces, operation theaters, laminar airflow, etc.

Chemical Method

Several chemical agents are used as antiseptic and disinfectants. The properties of a chemical antiseptic or disinfectant are following

Alcohols

Ethyl alcohol and isopropyl alcohol are frequently used as chemical agents for disinfection. Both of the chemicals facilitate the protein denaturation of bacterial proteins. 70% ethyl alcohol is the standard concentration which is used for disinfection. These are used as skin antiseptics. Apart from this methyl alcohol has activity against fungal spores and used to disinfection of inoculation cabinets.

Aldehydes

Formaldehyde

It is known for its bactericidal, sporicidal and virucidal activities. It can be used in both aqueous and gaseous form. A 10% formalin solution is a standard chemical disinfectant. It is used for

* Prevention of tissues for histological examinations.
* Sterilization of bacterial vaccines
* Preparation of toxoids from toxins.

Glutaraldehyde

It has its activity against bacteria (Mycobacterium tuberculosis), fungi and viruses (including HIV, hepatitis B, etc). It can also kill spores and is known for its less toxic nature. It is used as a 2% buffered solution. Glutaraldehyde is used for

Sterilization of cystoscopes, endoscopes, and bronchoscopes

Sterilization of plastic endotracheal tubes, face masks, metal instruments, etc.

Orthophathalaldehyde

Orthophathalaldehyde (OPA) is a high-level disinfectant and is known for its stability during storage. It has bactericidal effects against mycobacteria. 0.5% OPA is slowly sporicidal and OPA vapors irritate the respiratory tract and eyes, therefore, it must be handled with appropriate safety.

Phenols

Lister (father of antiseptic surgery) used phenol for the first time in the sterilization of surgical instruments. Phenols work as a disinfectant and kill microorganisms by cell membrane damage. It is toxic for the skin. Different derivatives of phenol are used as antiseptics which are following

Cresols

An example of cresol is Lysol which is mostly used for sterilization of infected glasswares, floors, etc.

Chlorhexidine

Savlon is an example of a chlorhexidine solution which is widely used in wounds, preoperative disinfection of the skin. It is bactericidal at high dilution. Moreover, it also has fungicidal activity.

Chloroxylenol

Dettol is commercially available as a chloroxylenol solution. It is less toxic and less irritant.

Hexachlorophene

It is bacteriostatic at very high dilution.

Halogens

Chlorine and iodine are commonly used disinfectants. Chlorine is used in water supplies, swimming pools, food, and dairy industries. Chlorine compounds in the form of bleaching powder, sodium hypochlorite, and chloramines. The disinfection action of all the chlorine compounds is due to the release of free chlorine which becomes a strong oxidative agent.

Iodine in alcoholic and aqueous solution is used as a skin disinfectant. It is active against M tuberculosis and slightly active against spores. Compounds with iodine with surface-active agents known as iodophors are claimed to be more active than aqueous or alcohol solution.

Oxidizing agents

Hydrogen peroxide

It is effective against most organisms in the concentration of 3-6 %. However, it kills spores at higher concentrations (10-25%). The mode of action is by the liberation of free hydroxyl radical on the decomposition of hydrogen peroxide. These free radicals are active ingredients in the disinfection process.

Peracetic acid

It is an oxidizing agent and is a more potent germicidal agent than hydrogen peroxide.

Salts

Slats of heavy metals have a toxic effect on bacteria. The salts of copper, silver, and mercury are used as a disinfectant. They are protein coagulant ant act by combining with sulphydryl groups of bacterial proteins and other essential intracellular compounds. Merthiolate (sodium ethyl mercurithiosalicylate) is used in a dilution of 1:10000 for the preservation of sera.

Dyes

Two groups of dyes, aniline and acridine dyes have been used as a skin and wound antiseptics. Both the dyes have bacteriostatic activity. Aniline dyes include crystal violet, brilliant green, and malachite green. Acridine dyes include acriflavine, cuflavin, proflavin, and aminacrine.

Vapor phase Disinfectants

Ethylene Oxide (ETO)

It is a colorless liquid with a boiling point of 10.70C. It is effective against all types of microorganisms including viruses and spores. It acts by alkylating the amnio carboxyl, hydroxyl and sulphydryl groups in protein molecules. In addition, it reacts with DNA and RNA. It is specially used for sterilizing plastic and rubber articles, respirators, heart-lung machines, dental equipment, etc.

Betapropilolactone (BPO)

This is a condensation product of ketane and formaldehyde. It has rapid action and used in0.2%. It is more efficient in fumigation than formaldehyde. BPO is used for the inactivation of vaccine

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