**Assignment for viva**

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**Section. :. A**

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

1. Analytical Balance

2. Autoclave

3. Bunsen burner

4. Centrifuge

5. Colony Counter

6. Deep Freezer

7. Homogenizer

8. Hot plate

9. Hot air oven

10. Incubator

11. Laminar Air Flow/ Laminar Hood

12. Magnetic Stirrer

13. Microscope

14. pH Meter

15. Spectrophotometer

16. Vortex Mixture/ Vortexer

17. Water Bath

18. Water Distiller

## **1. Analytical Balance**

1. An analytical balance is a type of balance that is commonly used for the measurement of mass in the sub-milligram range.

## **2. Autoclave**

1. An autoclave is a pressurized chamber used for the process of sterilization and disinfection by combining three factors: time, pressure and steam

## **3. Bunsen burner**

1. Bunsen burner is a standard tool used in laboratories, named after Robert Bunsen.
2. It is a gas-fueled single open flame.

## **4. Centrifuge**

1. A centrifuge is a device that allows the rotation of an object about a single axis, where an outward force is applied perpendicularly to the axis.
2. A laboratory centrifuge is motor-based and allows the rotation of a liquid sample resulting in the separation of the components of the mixture

## **5. Colony Counter**

1. A colony counter is used to estimate the density of a liquid culture by counting the number of CFU (colony forming units) on an agar or culture plates.

## **6. Deep Freezer**

### **Working  Principle**

1. Deep freezers are based on the principle that under extremely low temperatures, there is minimum microbial growth which allows for the protection and preservation of different substances.

## **7. Homogenizer**

1. Homogenizer is a device used in laboratories for the mixing of various liquids and materials like tissue, plant, food, soil, and many others.

## **8. Hot plate**

1. A hot plate is a stand-alone appliance used in microbiology laboratories as a tabletop heating system

## **9. Hot air oven**

1. A hot air oven is an electrical device that is used for sterilization of medical equipment or samples using dry heat.

## **10. Incubator**

1. An incubator is a device that is used in the laboratories for the growth and maintenance of microorganisms and cultures.
2. Incubator provides an optimal temperature, pressure, moisture, among other things required for the growth of microorganisms.

## **11. Laminar Air Flow/ Laminar Hood**

1. Laminar Hood is a closed device primarily for processes or instruments sensitive to microbial contamination.

## **12. Magnetic Stirrer**

1. Magnetic Stirrer is a device commonly used in microbiology laboratories for the purpose of mixing liquids.

## **13. Microscope**

1. Microscopes are devices that allow the observer to an exceedingly close view of minute particles.

## **14. pH Meter**

1. pH meter is a device used in laboratories that measure the H-ion concentration in water-based solutions to determine the acidity or alkalinity of the solution.
2. A pH meter is often termed as “potentiometric pH meter” as it measures the difference in electric potential between the reference and a pH electrode.

## **15. Spectrophotometer**

1. The spectrophotometer is an optical instrument for measuring the intensity of light in relation to the wavelength.
2. Based on the amount of light absorbed by a colored solution, a quantitative analysis of the solution can be done.

## **16. Vortex Mixture/ Vortexer**

1. A vortex mixture is one of the basic technologies used for the mixing of samples in glass tubes or flasks in laboratories.

## **17. Water Bath**

1. Water Bath is a conventional device that is used for chemical reactions that required a controlled environment at a constant temperature

## **18. Water Distiller**

1. A water distiller is a device that purifies water by the process of distillation.
2. This instrument is commonly used in medical laboratories, microbiology laboratories, organic chemistry laboratories and medical industries

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

**Sterilization**

It is the process by which an article, surface or medium is made free of all microorganisms either in vegetative or spore form.

**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.

**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.

**Sterilization control**

The spores of Bacillus subtilis subsp. Niger (NCTC 10075 or ATCC 9372) are kept inside the oven. These spores should be destroyed if the sterilization is proper.

Thermocouples may also be used.

Browne’s tube with a green spot is available. After proper sterilization, a green color is produced (after two years at 1600C).

**Chemical Method**

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

The chemical disinfectants need to have a broad spectrum of activity against all microorganisms such as bacteria, viruses, protozoa and fungi.

The chemical agents should act in the presence of organic matter.

High penetration power is an important property of the chemical agents

The chemical agent needs to be chemically stable under both acidic and basic environments.

The chemical substances should not have any corrosion activity in metals.

The disinfectants are needed to be non-toxic if absorbed into circulation.

Finally, the chemical agents are needed to be easily available and less expensive.

**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.

**Disinfection**

It refers to the destruction of all pathogens or organisms which can cause infection but not necessarily spores. All organisms may not be killed but the number is reduced to a level that no longer harmful to health.

**Chemical methods of disinfection**

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemical have very narrow spectrum of activity and some have very wide. Those chemicals that can sterilize are called chemisterilants. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.  
  
**ALCOHOLS**: Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.

**ALDEHYDES**: Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores.

**PHENOL**:  Act by disruption of membranes, precipitation of proteins and inactivation of enzymes

**HALOGENS**: They are oxidizing agents and cause damage by oxidation of essential sulfydryl groups of enzymes. Chlorine reacts with water to form hypochlorous acid, which is microbicidal.

**HEAVY METALS:**  Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic.

**SURFACE ACTIVE AGENTS:** They have the property of concentrating at interfaces between lipid containing membrane of bacterial cell and surrounding aqueous medium. These compounds have long chain hydrocarbons that are fat soluble and charged ions that are water-soluble. Since they contain both of these, they concentrate on the surface of membranes. They disrupt membrane resulting in leakage of cell constituents.

**DYES**: Acridine dyes are bactericidal because of their interaction with bacterial nucleic acids.

**ETHYLENE OXIDE** : It is an alkylating agent. It acts by alkylating sulfydryl-, amino-, carboxyl- and hydroxyl- groups.

**Physical Methods**

**Filtration**

Both live and dead microorganisms can be removed from liquids by positive- or negative-pressure filtration. Membrane filters, usually composed of cellulose esters (eg, cellulose acetate), are available commercially with pore sizes of 0.005 to 1μm. For removal of bacteria, a pore size of 0.2μm is effective because filters act not only mechanically but by electrostatic adsorption of particles to their surface.

**Pasteurization**

Pasteurization involves exposure of liquids to temperatures in the range 55 to 75°C to re-move all vegetative bacteria of significance in human disease. Spores are unaffected by the pasteurization process. Pasteurization is used commercially to render milk safe and extend its storage quality. With the outbreaks of infection due to contamination with en-terohemorrhagic E. coli , this has been extended (reluctantly) to fruit drinks.

**Microwaves**

The use of microwaves in the form of microwave ovens or specially designed units is an-other method for disinfection. These systems are not under pressure, but they but can achieve temperatures near boiling if moisture is present. In some situations, they are being used as a practical alternative to incineration for disinfection of hospital waste. These pro-cedures cannot be considered sterilization only because the most heat-resistant spores may survive the process.

**Chemical Methods**

Given access and sufficient time, chemical disinfectants cause the death of pathogenic vegetative bacteria. Most of these substances are general protoplasmic poisons and are not currently used in the treatment of infections other than very superficial lesions, having been replaced by antimicrobics .

Alcohol

The alcohols are protein denaturants that rapidly kill vegetative bacteria when applied as aqueous solutions in the range 70 to 95% alcohol. They are inactive against bacterial spores and many viruses. Solutions of 100% alcohol dehydrate organisms rapidly but fail to kill, because the lethal process requires water molecules. Ethanol (70 – 90%) and iso- propyl alcohol (90 – 95%) are widely used as skin decontaminants before simple invasive procedures such as venipuncture.

**Halogens**

Iodine is an effective disinfectant that acts by iodinating or oxidizing essential components of the microbial cell. Its original use was as a tincture of 2% iodine in 50% alcohol, which kills more rapidly and effectively than alcohol alone. This preparation has the disadvantage of sometimes causing hypersensitivity reactions and of staining materials with which it comes into contact. Tincture of iodine has now been largely replaced by preparations in which iodine is combined with carriers (povidone) or nonionic detergents.

**Hydrogen Peroxide**

Hydrogen peroxide is a powerful oxidizing agent that attacks membrane lipids and other cell components. Although it acts rapidly against many bacteria and viruses, it kills bacte- ria that produce catalase and spores less rapidly. Hydrogen peroxide has been useful in disinfecting items such as contact lenses that are not susceptible to its corrosive effect.

**Surface-Active Compounds**

Surfactants are compounds with hydrophobic and hydrophilic groups that attach to andsolubilize various compounds or alter their properties. Anionic detergents such as soaps are highly effective cleansers but have little direct antibacterial effect, probably because their charge is similar to that of most .

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