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**VIVA Assingnment**

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

**List of equipment / apparatus used in microbiology laboratory**

1. Autoclave

2. Incubator

3. Hot air oven

4. Inoculating loop

5. Vortex mixer / shaker

 6. Water bath

7. Heating mantle

8. Hot plate with magnetic stirrer

9. UV chamber

10. Inoculation chamber

11. pH meter

12. Colony counter

13. Microscope

14. Refrigerator

15. Bunsen burner

16. Spirit lamp

17. Micrometer (stage and ocular)

18. Balance (Digital and 4-beam)

19. Thermometer 20. Membrane filter set

**1.Autoclave**

It is a robust, electrically heated steam vessel meant for sterilizing ‘thermostable’ culture media, glassware, and other materials that are not spoiled by moist heat. Autoclave runs on the principle of pressure cooker. The moist heat (steam) has a very good penetrating power. Microorganisms / cells are killed as a result of denaturation of cellular constituents (protein and nucleic acids). In routine process, sterilization can be achieved by operating the autoclave at 121°C (15 psig) for 15 min. In its simplest form, the equipment has a removable lid for the delivery of materials to be sterilized. It is necessarily equipped with a gasket, pressure-cum-temperature gauge, a vent for letting out air or excess pressure, a safety valve, and a drain

**2. Incubator**

This an insulated, electrically heated cabinet meant for providing microorganisms with optimum temperature for growth. The cabinet is insulated and thermostatically controlled. For routine purposes, the temperature is maintained at 28-30°C for bacteria, about 25°C for molds, and 35-37°C for mesophilic bacteria. A temperature as high as 100°C can also be maintained for extremely thermophilic organisms (stereothermophiles).

**3. Hot air oven**

This is similar to incubator in make except that it can operate at temperatures up to 300°C and has a fan for circulating hot air. Hot air oven is used for sterilization of glassware and materials that are spoiled by moist heat. The death of cells occurs due to the oxidation of cellular constituents by the dry heat. For routine purpose, sterilization can be achieved by running the equipment at 180°C for 1.5 hours. Hot air oven is less effective than autoclave. Fig. 3 shows a typical hot air oven popular in microbiology laboratories.

 **4. Inoculating loop**

 This is a tool for transferring and streaking cultures. It consists of a thin nichrome wire whose one end is twisted into a small loop while the other end is fixed to a thermoset plastic handle. Sometimes, the looped end is straightened out to form what is called inoculating needle. Inoculating needles are used for preparing ‘stab’ cultures.

**5. Vortex mixer**

This equipment is used for mixing liquids kept in a test tube. It has one or more cup-like depressions at the top to receive the bottom of the test tube. The machine is electrically powered. When actuated, the machine moves the bottom of the test tube in a gyratory motion, thereby affecting a thorough mixing of the solution. The speed of the mixer can be varied..

 **6. Water bath / Boiling water bath**

Water bath is used for heating and melting of media, solutions, samples etc. at temperatures below 100°C. It can also be used to maintain constant temperature that is required in microbiology lab work. Several models and types of water bath are available a typical water bath commonly used in laboratories. It is electrically heated.

**7. Heating mantle**

 It is an electrically heated and thermostatically controlled unit used to heat or melt samples and reagents. The inner lining is made of asbestos and therefore gives an indirect heat to the materials to be heat. Hot plate with magnetic stirrer .This is an electrically powered equipment performs the dual function of heating and agitation. The agitation occurs by magnetic arrangement. Any type of glassware can be used for the heating and agitation. Magnetic beads are used for the agitation.

**9. UV chamber / UV viewing cabinet**

This equipment is used for analyzing fluorescent materials, spots in thin layer chromatography, etc. The equipment has two lamps for long- and short wavelength UV radiation. Since UV radiation is genotoxic (mutagenic) its exposure to skins and eyes must be avoided. A viewport with colored glass is provided for safety.

**11. Inoculation chamber / Sterile chamber**

This is an enclosed box in which culture transfers, plating, etc. can be carried out aseptically. The chamber is equipped with UV lamp for periodic disinfection of the chamber. While working, the UV light must be turned off and day-light bulb is turned on. Fig. 10 shows a locally fabricated inoculation chamber11 pH meter pH meter is an electrical instrument used for measuring hydrogen ion concentration of solutions and mixtures In microbiology lab, it is used for maintaining pH of the medium and diluents. The pH meter must be standardized with buffer solutions before operation. Since the instrument is very sensitive, it must not be used for stirring and it must not be dipped in hot or very cold solutions. The electrodes must always be kept immersed in suitable solutions. Read the manual carefully before using the instrument.

**12. Colony**

counter It is used for counting microbial colony (bacterial and yeast). The instrument is equipped with a backlight source, gridlines and a magnifying lens. It also has a sensor for digitally registering the number of colonies counted

**13. Microscope**

It is an instrument for observing microscopic items such as cells, crystals and cell organelles. It has the dual function of magnification and resolution. For routine microbiological works, bright field compound microscope with oil immersion objective is adequate

**14. Refrigerator**

This is a common household equipment for keeping foods and beverages cool. This equipment is used in microbiology laboratory for storing / preserving cultures, media, and many sensitive materials.

**15. Bunsen burner**

Bunsen burner is a common tool used in science lab. In microbiology lab, it is used for sterilizing inoculating loop, plating out cultures, transferring cultures, heat-fixing of smears and creating a sterile zone for aseptic operation.

**16. Spirit lamp**

The function of spirit lamp is the same as the Bunsen burner but is portable. It uses rectified spirit as the fuel (produces smoke-free flame). The lamp must be covered with a lid when not in use to prevent loss of spirit.

**17. Micrometers (stage and ocular)**

These are graduated glass pieces used for the measurement of size of the cells. Stage micrometer is a slide on which etching is done with 0.001 mm spacing. The ocular micrometer, which is place on the eyepiece, has an arbitrary scale and must be calibrated against the stage micrometer. During measurements, the ocular micrometer is retained while the stage micrometer is replace with the specimen slide.

**18. Balance**

Balance is needed in microbiology lab for weighing chemicals, samples, media, etc. Digital balances are fast to work with but needs frequent calibration

**19. Thermometer**

Thermometers are required to ensure the heating equipment is running at the correct temperature. The temperature of the medium, incubator, etc., need to be frequently checked. Mercury in glass thermometers are standard thermometers, the temperature measurement is based on the expansion of mercury present in the bulb. Digital thermometers use probes for measurement of temperatures.

**20. Coliform membrane filter**

This glass equipment is used for the testing of coliforms in water. 100 ml of test water is poured in the funnel and filtered through a special Millipore filter through external application of suction. The filter retains the microorganisms. The filter is then aseptically transferred to a selective-cum-differential semisolid medium kept in a petri dish. If there are coliforms, they will appear as pink dots after incubation at 35°C for 22 hrs.

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

**Ans: Sterilization involves both chemical and physical methods.**

**Physical methods** include moist heat in autoclaves, dry-heat in ovens, ionizing and non-ionizing radiation, filtration, and plasma sterilization.

 **Chemical methods** include glutaraldehyde, peracetic acid, ethylene oxide, formaldehyde gas, and hydrogen peroxid

**Chemical Method of Disinfection**

**ALCOHOLS -** Ethyl or isopropyl at a concentration of 50-70% are used for some surfaces where a rapid evaporation of the chemical and leaving no residue may be important, such as on laboratory equipment, etc. Alcohols are low in sporocidal activity and must remain wet on the surface for several minutes to achieve any reasonable disinfection.

**ALDEHYDES -** Formaldehyde or more usually glutaraldehyde are used as instrument and catheter disinfectants. The glutaraldehyde is the basic chemical for various trademarks, such as Cidex, or Sonacide and newer ones such as Sporocidin or Glutacide or Totacide, etc. These are used for disinfectants or sterilization of instruments and catheters but not for environmental surfaces. They may also be used for pipettes and clinical thermometers, etc.

 **DETERGENTS -** The term detergent refers to a removal of soil or dirt and various types of detergents are available. The anionic detergent such as soap and sodium lauryl sulfate and its close chemical relative which are the ingredients in the various dish and laundry detergents have very low level in antimicrobial activity and work by the removal of dirt and microorganisms and rinsing them down the drain.

 **GASEOUS AGENTS -** Ethylene oxide as a gaseous agent may best be used as a sterilant, but has been used in liquids for antimicrobial action. The use of formaldehyde as a liquid at the 8% level in alcohol (for many hours) may have sterilizing capability as well as 20% 0 aqueous formalin and formaldehyde with low temperature steam (75 C).

**HALOGENS -** Chlorine and iodine are the usual halogens used as antimicrobial chemicals. The chlorine is used as a gas for disinfection of water and swimming pools. (It is used as the hypochlorite (Clorox) for sanitizing.) Chlorine dioxide has been utilized for disinfection and is a rapid oxidizing sterilant when used under certain conditions. Iodine as the tincture is probably the best of the skin antiseptics, but is more frequently used as the iodophor which is a so-called tamed iodine which releases iodine slowly to the environmental surfaces. It is used as a sanitizer in food preparation areas and also as a skin antiseptic, a surgical scrub, etc.

 **HEAVY METALS -** The mercurial salts and other heavy metal preparations have lost favor in the laboratory and hospital scene since they are more bacteriostatic than bactericidal and may be extremely toxic. This would include trademark names such as Mercurochrome, Merthiolate (Thiomerosal), Merbak, Metaphen and others.

**PEROXIDES -** The use of weak peroxides on skin wounds of various small area has negligible antimicrobial activity and its effect probably due to a washing away of extraneous dirt and microorganisms. A newer, highly concentrated peroxide at a low pH has been proposed as a disinfectant-sterilant, under the trademark of Sterisyl and may disinfect very rapidly.

**PHENOLICS -** The chemicals based on phenol, i.e., a benzene ring with hydroxyl (OH) group are among the more common disinfectants for environmental surfaces. Instead of phenol or cresol, today it is more common to use a mixture of highly substituted phenolics (such as orthophenylphenol) which may be diluted out further (1:128-1:256) to achieve their bactericidal activity.

 **PHYSICAL METHODS OF DISINFECTION**

 **PASTEURIZATION**

 A modification of the 0 procedure utilized by Pasteur for wine and dairies for milk (62+ C for 30 minutes) has been adapted for disinfecting laboratory and health care facilities heat labile instruments that might be damaged by some temperatures. This consists, essentially, of a washing process to remove 99+% of the organic 0 matter and organisms followed by immersing into water at 60 C-65 C for at least 30 minutes.

**RADIATION Ultra-violet**

lights as it is used in laboratories and health care facilities may reduce the number of organisms to low level in the air and on surfaces but it is not a sterilizing process. It must be well engineered and installed very carefully to prevent burning of skin and eyes.

 **FILTRATION**

The filtration in the laboratory of fluids that are heat labile such as antibiotics, vitamins and other growth factors may be carried out with very fine pore membrane filters below 0.45 microns in diameter. These fluids should be checked for sterility by subculture before use. Filtration of air as carried out in the health care facilities is not a process of sterilization or even disinfection. The HEPA filtration reduces the number of organisms on dustladen particles by 99.9+% down to 0.3 micron size, etc., but does not achieve real sterilization. The use of 6-10 or more air changes per hour in a non-infectious facility helps dilute out the numbers of organisms in the air.

 **THE END**