
بسم الله الرحمن الرحيم

BLOCK -G
FOUNDATION MODULE-II
PRACTICAL NO 1
BIOSAFETY PROCEDURES/PRECAUTIONS IN MICROBIOLOGY LAB

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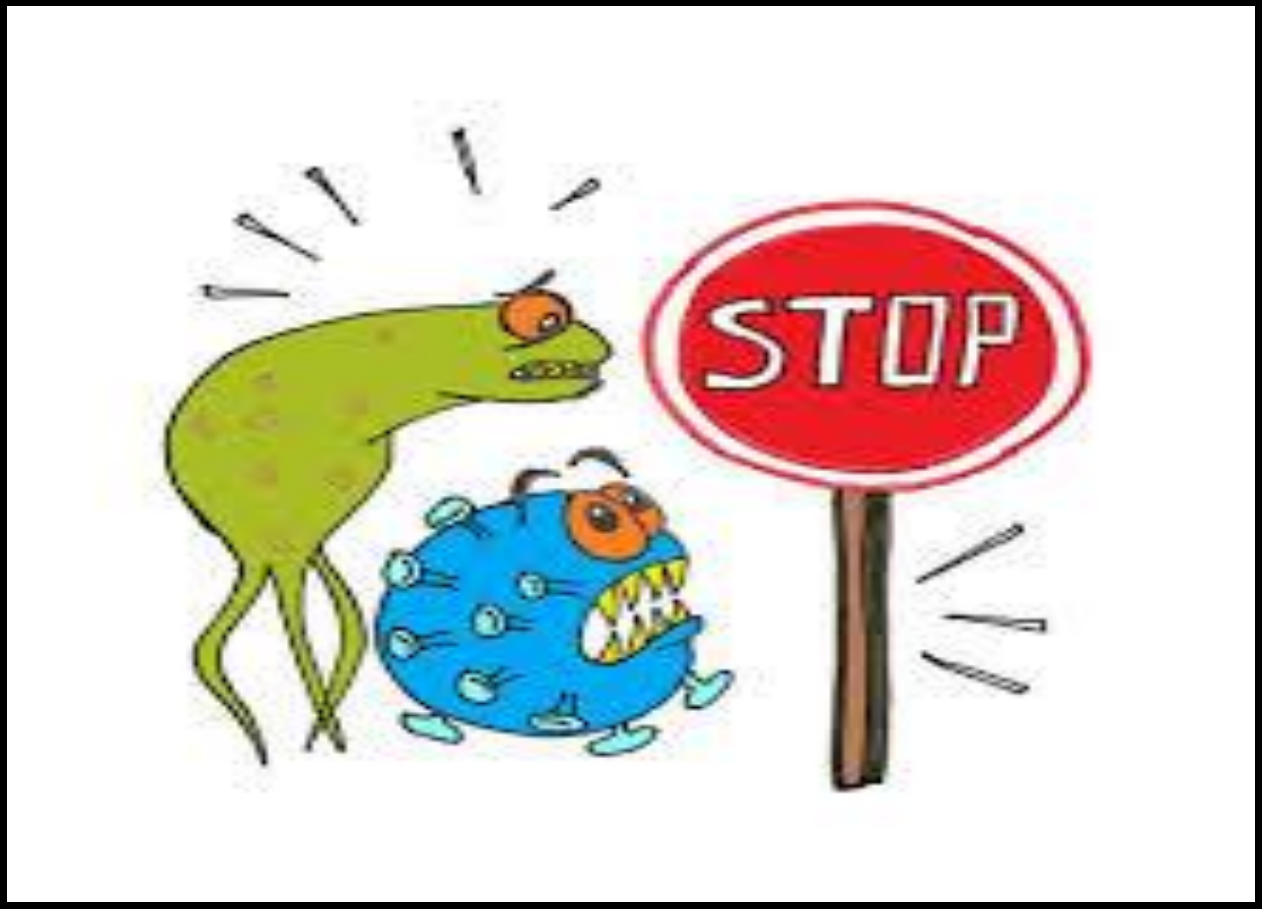
BIOSAFETY

The use of biosafety principles and specific practices in laboratories to reduce the health related risks associated with handling infectious agents, toxins and other biological hazards arising from an accidental exposure or unintentional release.


STOP

STERILIZATION

Is the process by which an article, surface or medium is made free of microorganism either in the vegetative or spore form.(bacteria, fungi, virus)

DISINFECTION

It means the destruction of all pathogen or organisms. Capable of producing infection but not kill spores. All of microorganism may not be killed but the number is reduced that is no longer harmful to health.

PHYSICAL AND CHEMICAL METHODS OF STERILIZATION AND DISINFECTION

(I)PHYSICAL METHODS OF STERILIZATION AND DISINFECTION)

The process in which we use physical agents to control microorganism is known as physical sterilization.

DRY HEAT(Flaming)

Heating over fire till they become red hot instruments 'point of forceps, spatulas, inoculating loops and wires

HOT AIR OVEN

Operated at 50 to 300c˚ (160c˚ for 1 hour) glass wares, forceps, scissors, scalpels, syringes, swabs, liquid paraffin and dusting powder

MOIST HEAT

- Pasteurization holder method: hold for 30 mints at 60c˚.
- Flash method: flash for 15 to 20 seconds at 72c˚ then quick cooling at 13c˚.

FILTRATION

Filtration is the easiest way to remove any microbes present in the fluids. When the fluid passes through the filter, bacteria and microorganism are trapped in the filter. However it take more time to clean the liquid.

BOILING

Boiling an instrument / article immersed fully in boiling water at (100c˚) for 10mints.Will kill most of the pathogenic organisms. Sterilization may be promoted by addition of 2% sodium bicarbonate in water.

AOUTOCLAVING

Most common method for sterilizing surgical instruments at high temperature, pressure and humidity used. Sterilization is done by steam under pressure in an autoclave. Water boils and it is vapor pressure equal that of surrounding atmosphere. When pressure increase inside a closed vessel, temperature increase saturated steam has better penetrating power. When steam come into contact with a cooler surface it condense into water and given up it is latent heat to the surface.

Autoclave temperature is 121c° for 15 to 45 mints. Autoclave sterilize surgical instruments, syringes, needles, linen (gown) masks, swabs.

RADIATIONS

They are two types:

- Non-ionizing radiation
- Ionizing radiation

NON IONIZING RADIATION

In this type we have ultraviolet and infrared rays. They cause damage to cell by formation of thymine dimer in DNA of animals. Thymine dimer leads to inhibition of replication. They do not affect spores of bacteria.

IONIZING RADIATION:

They are most powerful and form hydroxyl free radicals due to hydrolysis of water, which damage cells. They have more penetrating power e.g. beta rays, X-rays and gamma rays.

Infrared radiation and ultraviolet radiation used for pre-packed items such as syringes, catheters entryways Operation Theater and labs,

(II) CHEMICAL METHODS OF STERILIZATION AND DISINFECTION

Chemicals are also used for sterilization heat sensitive materials like biological materials, fiber optics electronics and many plastic items can be sterilized using chemical sterilants.

ALCOHOLS

Ethyl alcohol and isopropyl alcohol most commonly used act by denaturing bacterial protein and dehydration of cell. Used mainly as skin antiseptic, disinfection of clinical thermometer to be effective use at 60 to 70% concentration in water.

Ethyl alcohol is effective against fungal spore, cabinets and incubators.

ALDEHYDES

FORMALDEHYDE

10% aqueous solution of formalin is commonly used to bind with nucleic acid and proteins. Used to sterilize bacterial vaccines to prepare. Used for killing of bacterial culture and suspensions

GLUTARALDEHYDE

Sterilization of cystoscopes, endoscopes, and bronchoscopes. To sterilize endotracheal tube, face masks, rubber anaesthetic tubes and metal instrument.

PHENOLS

Phenol 1% has bactericidal action causes cell membrane damage releasing cell contents and causing lysis.

HALOGENS (CHLORINE)

In the form of bleaching powder (sodium hypo chloride and chloramine) are used in water supplies and swimming pools. Bleach powder is used to sterilize HIV infected material.

OXIDISING AGENTS

(A)HYDROGEN PEROXIDE (H_2O_2)

It kills all organisms including spores at higher concentration. Liberate free hydroxyl radical on decomposition of H_2O_2. It use disinfection of contact lenses, surgical prosthesis and plastic implants (3-6% concentration is effective)

(B)PARACETIC ACID

High level of disinfectant used in sterilization procedure

TISSUE PROCESSING

Tissues from the body are taken for diagnosis of disease ,processed in histology laboratory to prepare microscopic slides that are viewed under microscope by pathologist.

GROSS EXAMINATION

Tissues removed from body for diagnosis arrive in the pathology department and are examined. Gross examination consist of describing the specimen and take gross – section (that containing or suspected tumor cells) for tissue processing to make ready for microscopy.

3. CLEARING

Clearing consist of removal of dehydrator with the substance that will be miscible with the embedding medium (paraffin). The commonest clearing agent is xylene. Other clearing agents include toluene, chloroform, and methyl salicylate.

4. EMBEDDING

Finally the tissue is infiltrated with the embedding agent, almost and always paraffin. Tissue is surrounded by paraffin wax. This will provide external support to the tissue. It provides hardness to the tissue for proper section cutting.

5. SECTIONING

Once the tissues have been embedded, they must be cut into the sections that can be placed into slide. This done with Microtome. Thickness of section cut is 3-5μm. Once the sections are cut, they are floated in warm water bath. That helps remove wrinkles. Then they are picked up on microscopic slides. The glass Slide is then placed in warm oven for about 15 mints to help the section to adhere to the slide. Or take the slide coated with Mayer's egg albumin which helps in adhesion of tissue to slide.

6. STAINING

The embedded process must be reversed in order to get the paraffin wax out of the tissue to allow water soluble dyes to penetrate the section

7. DEPARAFFINIZATION

Therefore before any staining can be done, the slides are deparaffinized by hydration step. First dip the slide in xylene jar to completely remove of paraffin wax. Now we will use alcohol in descending (higher to lower) 90%, 70%, 50%, and 30% and then distilled water. After hydration, stain the section. The routine stain is Hematoxylin and eosin (H/E). Other stains are referred to special stains because they are employed in specific situation according to diagnosis need.

8.COVERSLIPPING

After staining again remove water for long term storage of slide. For that again will do dehydration step (30%, 50%, 70%, 90%, and 100%) and then clearing with xylene. After that mount the slide with DPX (dibutylphthalate polystyrene xylene). Mounting will help the tissue to preserve long storage without any harm to tissue. Finally observe the slide under the microscopes.

TOOLS AND INSTRUMENTS INVOLVED IN TISSUE PROCESSING

TISSUE PROCESSOR

A tissue processor is used to prepare tissue sample for analysis by fixing, staining, dehydrating or calcifying them. The processors are mostly single unit devices that can accommodate a variety of processing techniques to suit the different needs of the laboratory, therefore improving the efficiency of tissue processing.

4. L-MOULDS (leuckhart's embedding mould)

- Made of brass
- L shaped
- To facilitate change of block size
- Tissue kept on flat glass
- Embedded in paraffin wax

5. MICROTOME

small.Microtome is an instrument which is used to cut extremely thin sections of tissues after embedding in paraffin wax. This instrument is used in histopathology laboratory.

6. SLIDE WARMER

Slide warmer is an equipment used to warm slides to a specified temperature for safe fixing, drying, and staining of samples. Products include heating blocks, convection ovens, and bench style slide heaters that very in temperature range and slide holding capacity.

7. FLOATING BATH

A floatation bath or water bath is the intermediate step between cutting paraffin sections and placing them on slides. Simply sticking paraffin ribbons on slides will not work! A worm water bath allows tissue to relax and smooth out prior and remove wrinkles and folds to being mounted on a glass slide. The warm also make the paraffin stick to the glass slides. Water baths are filled with water, heated to a temperature 5-10°c below the melting point of paraffin, and the water bath is usually kept at 40 -50°c. This is an optimal temperature range for various types of paraffin's.

8. TISSUE CASSETTES

Cassettes are disposable plastic cassettes hold tissue specimens during the embedding process, as well as in a storage file. Molded from a special high density polymer, these cassettes keep specimens safely submerged in liquid and are totally resistant to the chemical action of histological solvents.