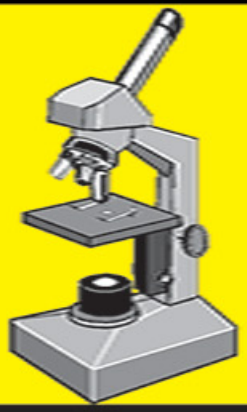
Textbook of Medical Laboratory Technology



Ramnik Sood

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CHAPTER 2

Sterilisation

The terms *sterilisation* and *disinfection* are used to indicate the treatment of material so as to destroy or otherwise eliminate any living organisms present. However, the term sterilisation is used where physical methods are used and disinfection is used where chemical agents are made use of.

METHODS COMMONLY USED FOR STERILISATION

The methods used commonly in practice are:

1. *Killing organisms by heat:* Heat may be dry or moist
2. *Destroying organisms by employing chemical antiseptics,* e.g. lysol, phenol, perchloride of mercury, etc.
3. *Removing organisms mechanically by filtration:* e.g. Seitz, unglazed porcelain.

Sterilisation by Heat

Adequate heat is the most certain and rapid method for sterilisation. The time needed for sterilisation is inversely related to the temperature of exposure—the higher the temperature, the shorter the time needed. High temperature kills bacteria by coagulating their proteins. Different types of bacteria show considerable differences in heat susceptibility. In general, vegetative forms are destroyed at lower temperatures, whereas high temperatures are needed for sporing organisms.

Dry Heat

This is the preferred method for sterilising glassware, e.g. of glass syringes and of materials such as oils, jellies and powders which are impervious to steam. Dry heat requires a much higher temperature or a much longer time at the same temperature than does moist heat. Dry heat can be used in the following ways:

1. *Flaming:* The articles are passed through the Bunsen flame, without letting them become red hot. It is used for scalpels, needles, mouths of culture tubes, glass slides, coverslips and points of forceps. Only the surfaces actually touched by the flame are sterilised.

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2. *Red heat:* Platinum loops, inoculating wires and needles are heated in the bunsen flame until red hot.

3. *Hot air oven:* These are electrically heated and thermostatically controlled. The oven itself is a double-walled steel chamber with a stout door. The top or side contains a ventilator which is left open during sterilisation to disperse any moisture or volatile matter. Air circulates within the oven by convection currents. Suitable sterilising times in the hot air oven are 3 hours at 140°C, 1 hour at 160°C and 30 minutes at 180°C. All dry glassware, such as test tubes, petri-dishes, flasks, pipettes and throat swabs, etc. are made sterile by using hot air oven.

This method is not suitable for sterilizing culture media, liquids, rubber connections, glass to metal fitting and fabrics, e.g., masks, towels or gowns.

Moist Heat

a. *A temperature of 60-65°C* kills most vegetative bacteria (made use of in pasteurisation of milk and preparation of vaccines).
b. *Boiling* is frequently used for sterilizing syringes etc. but is not adequate as many spores withstand this temperature.
c. *Steam* is the most effective technique of moist heat sterilisation. Steam may be employed in three ways.
   1. *Steam at 100°C:* The apparatus used commonly is called Koch's steamer. It has a vertical metal cylinder with a conical lid. It is fitted with a thermometer and has a small opening for escape of steam.
      Sterilisation by free steam can be done in two ways.
      - *Prolonged exposure* for 1½ hours, used for broth or nutrient agar.
      - *Intermittent heat or tyndallisation:* It involves exposure for 20 minutes on three successive days and is used to sterilize sugars and gelatin which decompose on higher temperatures.
      - *Principle:* Spores would germinate after first steaming and destroyed on the next, three steamings would eliminate all spores and their vegetative forms.
   2. *Low temperature steam:* This method is employed for sterilizing materials (blankets, polyethene tubing, etc.) which would be damaged at higher temperatures.
   3. *Steam at temperatures above 100°C (Autoclaving)*: Autoclaves are made of strong metal jackets; strong enough to withstand high pressures required (Fig. 2.1). The autoclave door is hermetically sealed. It has a safety valve set to blow off at a predetermined pressure. The principle is that water boils when its vapour pressure is equal to the pressure of the surrounding atmosphere. If the pressure is raised inside a closed vessel, the temperature at which water boils will rise above 100°C. At 15 lbs. pressure water boils at 120°C.
      Following are the measures that must be taken care of during autoclaving:
      a. The steam must be saturated.
      b. There must be complete discharge of air from the sterilizing chamber.
      c. The autoclave must be loaded in such a way that all the materials to be sterilized can be adequately penetrated by steam.
      d. The duration of autoclaving would depend on the pressure inside and hence on the steam temperature.

Method

1. Fill boiler with water to a point just below the basket bottom.
2. Place articles within the basket, bottles should not be more than 3/4 full and should have loosely screwed on caps.
3. Close the lid and tighten the screws.
4. Open outlet valve and adjust safety valve to the required pressure.

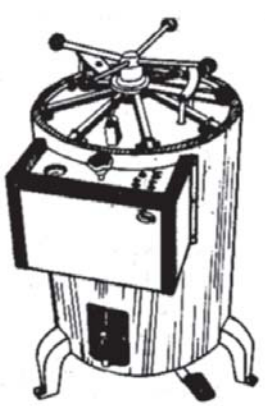

Fig. 2.1: Vertical autoclave

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5. Turn on the heat source and when steam flows smoothly, close the ventcock and let the internal pressure rise. See that all air has been expelled from the cylinder.
6. Let pressure rise to the required level and maintain at that level for the required period of time.
7. Switch off the heat source and let the pressure meter register zero. Open the ventcock and the lid slowly.

(If the autoclave pressure is taken down very quickly—the fluid-filled bottles may burst).

Timings
10 lbs. Pressure for 10 minutes—culture media.
15 lbs. Pressure for 20 minutes—infected material.
20 lbs. Pressure for 30 minutes—rubber gloves

4. *Inspisation:* Used to sterilize serum containing media e.g., Loeffler's for diphtheria and Dorset's, or Lowenstein's media for TB. The inspissator consists of a double-walled copper box, with water flowing between the 2 walls; the temperature is controlled between 75-80°C thermostatically. Sterilisation is done for 2-3 hours on each of 3 successive days. A higher temperature may cause bubbling of the surface of the media.

Cold

Not used clinically
Cold shock: A sudden drop of temperature (e.g. 45-15°C) without actual freezing causes irregular contraction of cytoplasmic organelles leading to disorganization of cellular structures (95% drop in *E. coli* viable number is reported by using this method).

Freezing: This helps by (1) formation of ice crystals outside the cell by the withdrawal of water from the cell interior, increases the intracellular salt concentration—protein denaturation and (2) formation of ice within the cell.

Ultraviolet Radiation

Ultraviolet (UV) rays of wavelength 2400 to 2800 Angstrom units are most effective. Low pressure mercury vapour type lamps can be used to produce UV rays. Take care that the UV rays do not directly enter the eyes. Gram-negative bacteria are destroyed more rapidly than gram-positive bacteria, spores are highly resistant and susceptibility of viruses is variable.

Ionising Radiations

Cathode rays and gamma rays are the most effective and are being increasingly used to sterilise disposable items. These radiations have considerable disinfectant action.

Filtration

It may be used for the preparation from cultures of cell-free bacterial products, e.g. toxins and enzymes, to free virus containing fluids from bacteria and for the sterilisation of media or media ingredients which would be damaged by heating. For this purpose, filters with pores sufficiently small to hold back bacteria must be employed. Filtration is usually carried out under negative pressure, the fluid being sucked through the filter into a receiving flask, which is connected to an exhaust pump. During filtration, the filter surface may adsorb material carrying an opposite charge—the material adsorbed may be the one desired in the filtrate.

Seitz filter: This employs filter that consists of a flat disc or asbestos material of special composition and is inserted into metal holders which ensure a tight joint (Fig. 2.2). The disc is used only once. The discs are available in three grades, viz., (1) clarifying (2) normal and (3) special. This method is good for obtaining bacteria-free filtrates. The large pore filters are good for filtration of serum for making media.

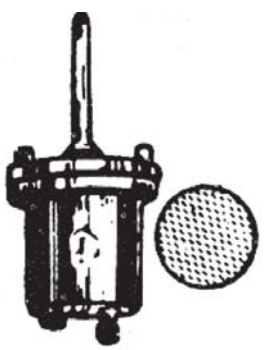

Fig. 2.2: Seitz filter

Colloidion or Gradocol membranes: These are virtually free of any adsorptive effects and have replaced Seitz filters considerably. These, too, are replaced after a single use.

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Berkefeld and Mandler filters: These are made from diatomaceous earth. Grades available are V—coarse, N—medium and W—fine.

Chamberland and Doulton filters— These are made of unglazed porcelain. Available in various grades:

L/a—coarse clarifying.
L/a L2 and L3—medium.
L5 to L15—very fine.

Sintered glass: It is made of a pad of finely ground glass fused into a glass cup.

Sand and paper pulp filter: They used for removing large particles and clearing emulsions, etc.

Chemical Sterilisation

Chemical agents can exert bactericidal or bacteriostatic effect. The bactericidal agents in lower concentrations exert bacteriostatic effect. The bactericidal effect is probably because of enzyme inactivation either by protein denaturation, oxidation or by a combination of the antibacterial agent with specific groups of enzyme proteins.

Chemicals Used

Various chemicals used are:

1. *Chloroform (volatile antiseptic):* Used in preservation of serum for culture media at 0.25% concentration. Can be removed by heating to 56°C).
2. *Phenol group:* Cresol, lysol (strong antiseptics) are mainly employed for surgical instruments, discarded routine cultures, and pipettes, slides, etc., and disinfecting hands. Phenol 0.5% is used for preserving sera and vaccines.
3. *Metallic salts:* Perchloride of mercury in 1:1000 strength solution.
4. *Glycerol:* A 50% solution is used for preservation of certain viruses. Glycerol also kills contaminating organisms.
5. *Formalin:* It is the only method for sterilising perspex or polythene and for killing cultures on plates. Can also be used for fumigation purposes.
6. *Sulphur:* Burning in air form sulphur dioxide (SO_2) for fumigation.
7. *Halogens:* Chlorine and iodine. Chlorine for water disinfection and iodine for skin.
8. *Acids and alkalies:* Most bacteria grow in pH range of 5 to 9 and many grow at pH 7. Strong acids and alkalies can be used to disinfect contaminated materials.
9. *Alcohols:* These are used for disinfecting skin before injecting and before operations. Alcohols act by protein denaturation.
10. Two groups of dyes (a) the aniline dyes and (b) the acridines have been widely used as skin and wound disinfectants.
11. Quaternary ammonium compounds are active against both gram-positive and gram-negative species but are not effective against spores and *Mycobacteria*. They are used for sterilising food utensils in restaurants and hotels and for disinfecting blankets in hospitals.
12. Glutaraldehyde (Cidex) affects even spores and *Mycobacteria*. It is employed as 2% solution and is recommended for sterilising cystoscopes, etc.
13. Ethylene oxide gas is being widely used to sterilise disposable plastic syringes, petridishes, etc.

GLASSWARE PREPARATION FOR USE

1. *Selection:* Grade A glassware needs no testing. Grade B is satisfactory for most routine work. Others should be heat resistant, have a low coefficient of expansion and be free from soluble metals and free alkali; in addition, they should be mechanically strong.
2. *Cleaning Glassware*

   New: Look for cracks if any. Soak in 2% HCl for overnight to neutralize any alkali present. Wash in running water. Boil in synthetic detergent for 30 minutes, rinse in tap water and finally in distilled water.

   Used glassware should be rinsed immediately after use. Boil in a detergent for 30 minutes and clean thoroughly with a brush, rinse in tap water and finally in distilled water. Dry them in the oven with temperature not exceeding 80°C.

Dichromate Cleaning Solution

Dissolve 25 gm potassium dichromate in 25 ml of water. Add 50 ml conc. sulphuric acid (slowly, always add acid to water and not (*vice-versa*), cool, store it in a stoppered bottle; discard when it starts turning green.

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Petridishes
- Autoclave to remove infected material
- Wash in soapy water
- Rinse in running water, let dry
- Rinse in methylated spirit, let dry
- Sterilize in hot air oven.

Pipettes
- Soak in chromic acid solution overnight
- Wash in running water
- Rinse in distilled water
- Dry on suction pump using methylated spirit, or ether, or methylated acetone
- To sterilize—plug mouth piece with nonabsorbent cotton wool, wrap in kraft paper and hot air sterilize (160°C for 1 hour).

Test tubes
- Autoclave to remove infected material
- Boil in detergent solution for 30 minutes
- Clean with a brush
- Rinse in running water, rinse in distilled water and place them in a wire basket upside down and dry in an oven
- Cotton plug them and sterilize in the hot air oven.

Pasteur pipettes
- Soak in 3% lysol for 1 hour
- Wash as before (as for test tubes).

Screw-capped bottles
- Wash the liners separately
- Dry quickly to avoid corrosion
- Sterilize metal caps and their bottles in hot air oven
- Rubber liners—autoclaved.

Glass slides

New
- Boil in a detergent for 30 minutes
- Place in dichromate for overnight
- Wash in running water
- Keep in methylated spirit
- For using, take them out with a forceps and hold them only by the edges.

Used
- As for used glassware.

Infected slides
- Should be autoclaved and then cleaned as mentioned earlier (never use slides used for examining acid-fast bacilli for the same purpose).

Infected Glassware

Contaminated material—may be disposed in paper or cardboard wrappers and incinerated.

Autoclave the glassware that has been contaminated. Having autoclaved, wash and prepare in the usual way.

Syringes

Complete bacterial sterility can be achieved either by sterilising them in the hot air oven or in an autoclave. Keep injection syringes separate from blood withdrawing syringes. Fresh syringes (sterilised) should be used for withdrawing blood for each patient. Before re-using them, clean them properly and then sterilise them. Needles used should be sharp and not with blunted ends.

Choice of Syringes and Needles

All glass syringes are preferred over glass and metal ones. Preferrably keep size 5 ml or more syringes for withdrawing blood. Needles should be of size less than 21 (SWG). A needle with a smaller diameter would cause lysis of blood when used for blood withdrawing. Withdrawing needles should be at least an inch long.

New syringes These are washed in the usual way. Dried with acetone. Wrap the plunger and the barrel in a paper and sterilise in hot air oven.

Used syringes Immediately after use, wash them thoroughly with cold water (hot water will coagulate proteins and will make the syringes difficult to clean). Clean them thoroughly in a detergent, brush the barrel properly, rinse in tap water and then in distilled water. Rinse in acetone and let dry. Sterilise in hot air oven as mentioned above.

Infected syringes These should be washed at first with cold 2% lysol solution and then clean as above. Syringes infected with highly virulent material should at first be autoclaved. The syringes should be placed in the cold oven and be heated at 160°C for 90 minutes. Syringes not used for 3 months should be resterilised before use.

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Glass barrel and metal plunger syringes The only precaution to be taken here is that metal corrosion should be avoided and the barrel and the plunger should be sterilised separately (kept in a wrapper) by autoclaving them.

Needles

- Should first be rinsed in cold water.
- Clean the mounts with a cotton-wool swab.
- Wash again, rinse in acetone.
- Pass a styllete through the hole to remove any plugs if present.
  (It is important to discard all needles with blunted tips, a hand lens can be used to examine needle tips).
- Serum hepatitis and HIV can be transmitted through using imperfecty cleaned and sterilised needles.
- The needle should be sterilised in hot air oven.

Disinfection of syringes by boiling In an emergency, syringes can be fairly effectively sterilised by boiling them in distilled water for at least 5 minutes after having cleaned them in the usual way.

Disposable Sterile Syringes

This is the world and time of disposables. In the interest of the patient every laboratory should ideally use disposable syringes only.

As far as possible use disposable, sterilised plasticware instead of glassware.

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Textbook of Medical Laboratory Technology

Are you a lab techonolgy student or working as one?

Are you a medical undergraduate or working for post graduation in lab technology or clinical sciences?

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Do you want the methodologies of investigations alongwith their interpretations?

Do you want to thoroughly understand the principles behind various investigations and ways to trouble shoot the associated problems and difficulties?

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A book that deals with current/latest medical laboratory technologies and provides you with an in-depth knowledge of simple to sophisticated investigative systems.

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JAYPEE BROTHERS
MEDICAL PUBLISHERS (P) LTD
EMCA House, 23/23B Ansari Road, Daryaganj
New Delhi 110 002, India