

## 14. Disinfection and sterilization

A basic knowledge of disinfection and sterilization is crucial for biosafety in the laboratory. Since heavily soiled items cannot promptly be disinfected or sterilized, it is equally important to understand the fundamentals of cleaning prior to disinfection (precleaning). In this regard, the following general principles apply to all known classes of microbial pathogens.

Specific decontamination requirements will depend on the type of experimental work and the nature of the infectious agent(s) being handled. The generic information given here can be used to develop both standardized and more specific procedures to deal with biohazard(s) involved in a particular laboratory.

Contact times for disinfectants are specific for each material and manufacturer. Therefore, all recommendations for use of disinfectants should follow manufacturers' specifications.

### Definitions

Many different terms are used for disinfection and sterilization. The following are among the more common in biosafety:

**Antimicrobial** – An agent that kills microorganisms or suppresses their growth and multiplication.

**Antiseptic** – A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

**Biocide** – A general term for any agent that kills organisms.

**Chemical germicide** – A chemical or a mixture of chemicals used to kill microorganisms.

**Decontamination** – Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

**Disinfectant** – A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

**Disinfection** – A physical or chemical means of killing microorganisms, but not necessarily spores.

**Microbicide** – A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “chemical germicide” or “antimicrobial”.

**Sporocide** – A chemical or mixture of chemicals used to kill microorganisms and spores.

**Sterilization** – A process that kills and/or removes all classes of microorganisms and spores.

### **Cleaning laboratory materials**

Cleaning is the removal of dirt, organic matter and stains. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing a soap or detergent. Dirt, soil and organic matter can shield microorganisms and can interfere with the killing action of decontaminants (antiseptics, chemical germicides and disinfectants).

Precleaning is essential to achieve proper disinfection or sterilization. Many germicidal products claim activity only on precleaned items. Precleaning must be carried out with care to avoid exposure to infectious agents.

Materials chemically compatible with the germicides to be applied later must be used. It is quite common to use the same chemical germicide for precleaning and disinfection.

### **Chemical germicides**

Many types of chemicals can be used as disinfectants and/or antiseptics. As there is an ever-increasing number and variety of commercial products, formulations must be carefully selected for specific needs.

The germicidal activity of many chemicals is faster and better at higher temperatures. At the same time, higher temperatures can accelerate their evaporation and also degrade them. Particular care is needed in the use and storage of such chemicals in tropical regions, where their shelf-life may be reduced because of high ambient temperatures.

Many germicides can be harmful to humans or the environment. They should be selected, stored, handled, used and disposed of with care, following manufacturers' instructions. For personal safety, gloves, aprons and eye protection are recommended when preparing dilutions of chemical germicides.

Chemical germicides are generally not required for regular cleaning of floors, walls, equipment and furniture. However, their use may be appropriate in certain cases of outbreak control.

Proper use of chemical germicides will contribute to workplace safety while reducing the risk from infectious agents. As far as possible, the number of germicidal chemicals to be used should be limited for economic reasons, inventory control and to limit environmental pollution.

Commonly used classes of chemical germicides are described below, with generic information on their applications and safety profiles. Unless otherwise indicated, the germicide concentrations are given in weight/volume (w/v). Table 12 summarizes the recommended dilutions of chlorine-releasing compounds.

**Table 12. Recommended dilutions of chlorine-releasing compounds**

	"CLEAN" CONDITIONS <sup>a</sup>	"DIRTY" CONDITIONS <sup>b</sup>
Available chlorine required	0.1% (1 g/l)	0.5% (5 g/l)
Sodium hypochlorite solution (5% available chlorine)	20 ml/l	100 ml/l
Calcium hypochlorite (70% available chlorine)	1.4 g/l	7.0 g/l
Sodium dichloroisocyanurate powder (60% available chlorine)	1.7 g/l	8.5 g/l
Sodium dichloroisocyanurate tablets (1.5 g available chlorine per tablet)	1 tablet per litre	4 tablets per litre
Chloramine (25% available chlorine) <sup>c</sup>	20 g/l	20 g/l

<sup>a</sup> After removal of bulk material.

<sup>b</sup> For flooding, e.g. on blood or before removal of bulk material.

<sup>c</sup> See text.

### Chlorine (sodium hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine.

Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. The frequency with which working solutions of bleach should be changed depends on their starting strength, the type (e.g. with or without a lid) and size of their containers, the frequency and nature of use, and ambient conditions. As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week.

A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine. A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite solutions, as domestic bleach, contain 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively. Industrial solutions of bleach have a sodium hypochlorite concentration of nearly 120 g/l and must be diluted accordingly to obtain the levels indicated above.

Granules or tablets of calcium hypochlorite (Ca(ClO)<sub>2</sub>) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4 g/l and 7.0 g/l, will then contain 1.0 g/l and 5 g/l available chlorine, respectively.

Bleach is not recommended as an antiseptic, but may be used as a general-purpose

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disinfectant and for soaking contaminated metal-free materials. In emergencies, bleach can also be used to disinfect water for drinking, with a final concentration of 1–2 mg/l available chlorine.

Chlorine gas is highly toxic. Bleach must therefore be stored and used in well-ventilated areas only. Also, bleach must not be mixed with acids to prevent the rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, in particular bleach, should be avoided.

### **Sodium dichloroisocyanurate**

Sodium dichloroisocyanurate (NaDCC) in powder form contains 60% available chlorine. Solutions prepared with NaDCC powder at 1.7 g/l and 8.5 g/l will contain 1 g/l or 5 g/l available chlorine, respectively. Tablets of NaDCC generally contain the equivalent of 1.5 g available chlorine per tablet. One or four tablets dissolved in 1 l of water will give approximately the required concentrations of 1 g/l or 5 g/l, respectively. NaDCC as powder or tablets is easy and safe to store. Solid NaDCC can be applied on spills of blood or other biohazardous liquids and left for at least 10 min before removal. Further cleaning of the affected area can then take place.

### **Chloramines**

Chloramines are available as powders containing about 25% available chlorine. Chloramines release chlorine at a slower rate than hypochlorites. Higher initial concentrations are therefore required for efficiencies equivalent to those of hypochlorites. On the other hand, chloramine solutions are not inactivated by organic matter to the same extent as hypochlorite solutions, and concentrations of 20 g/l are recommended for both “clean” and “dirty” situations.

Chloramine solutions are virtually odour-free. However, items soaked in them must be thoroughly rinsed to remove any residue of the bulking agents added to chloramine-T (sodium tosylchloramide) powders.

### **Chlorine dioxide**

Chlorine dioxide ( $\text{ClO}_2$ ) is a strong and fast-acting germicide, disinfectant agent and oxidizer, often reported to be active at concentrations levels lower than those needed by chlorine as bleach. Chlorine dioxide is unstable as a gas and will undergo decomposition into chlorine gas ( $\text{Cl}_2$ ), oxygen gas ( $\text{O}_2$ ), giving off heat. However, chlorine dioxide is soluble in water and stable in an aqueous solution. Chlorine dioxide can be obtained in two ways: (1) on-site generation by mixing of two separate components, hydrochloric acid (HCl) and sodium chlorite ( $\text{NaClO}_2$ ); and (2) ordering its stabilized form, which is then activated on-site when required.

Of the oxidizing biocides, chlorine dioxide is the most selective oxidant. Ozone and chlorine are much more reactive than chlorine dioxide, and they will be consumed by most organic compounds. Chlorine dioxide, however, reacts only with reduced sulfur

compounds, secondary and tertiary amines, and some other highly reduced and reactive organic compounds. A more stable residue can therefore be achieved with chlorine dioxide at much lower doses than when using either chlorine or ozone. Generated properly, chlorine dioxide can be used more effectively than ozone or chlorine in cases of higher organic loading because of its selectivity.

### **Formaldehyde**

Formaldehyde (HCHO) is a gas that kills all microorganisms and spores at temperatures above 20 °C. However, it is not active against prions.

Formaldehyde is relatively slow-acting and needs a relative humidity level of about 70%. It is marketed as the solid polymer, paraformaldehyde, in flakes or tablets, or as formalin, a solution of the gas in water of about 370 g/l (37%), containing methanol (100 ml/l) as a stabilizer. Both formulations are heated to liberate the gas, which is used for decontamination and disinfection of enclosed volumes such as safety cabinets and rooms (see section on Local environmental decontamination in this chapter). Formaldehyde (5% formalin in water) may be used as a liquid disinfectant.

Formaldehyde is a suspected carcinogen. It is a dangerous, irritant gas that has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well-ventilated area. National chemical safety regulations must be followed.

### **Glutaraldehyde**

Like formaldehyde, glutaraldehyde ( $\text{OHC}(\text{CH}_2)_3\text{CHO}$ ) is also active against vegetative bacteria, spores, fungi and lipid- and nonlipid-containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to kill bacterial spores.

Glutaraldehyde is generally supplied as a solution with a concentration of about 20 g/l (2%) and some products may need to be “activated” (made alkaline) before use by the addition of a bicarbonate compound supplied with the product. The activated solution can be reused for 1–4 weeks depending on the formulation and type and frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions under use. Glutaraldehyde solutions should be discarded if they become turbid.

Glutaraldehyde is toxic and an irritant to skin and mucous membranes, and contact with it must be avoided. It must be used in a fume-hood or in well-ventilated areas. It is not recommended as a spray or solution for the decontamination of environmental surfaces. National chemical safety regulations must be followed.

### **Phenolic compounds**

Phenolic compounds, a broad group of agents, were among the earliest germicides. However, more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid-containing viruses and, when properly formulated, also show

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activity against mycobacteria. They are not active against spores and their activity against nonlipid viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces, and some (e.g. triclosan and chloroxylenol) are among the more commonly used antiseptics.

Triclosan is common in products for hand-washing. It is active mainly against vegetative bacteria and safe for skin and mucous membranes. However, in laboratory-based studies, bacteria made resistant to low concentrations of triclosan also show resistance to certain types of antibiotics. The significance of this finding in the field remains unknown.

Some phenolic compounds are sensitive to and may be inactivated by water hardness and therefore must be diluted with distilled or deionized water.

Phenolic compounds are not recommended for use on food contact surfaces and in areas with young children. They may be absorbed by rubber and can also penetrate the skin. National chemical safety regulations must be followed.

### **Quaternary ammonium compounds**

Many types of quaternary ammonium compounds are used as mixtures and often in combination with other germicides, such as alcohols. They have good activity against some vegetative bacteria and lipid-containing viruses. Certain types (e.g. benzalkonium chloride) are used as antiseptics.

The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness and anionic detergents. Care is therefore needed in selecting agents for precleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Owing to low biodegradability, these compounds may also accumulate in the environment.

### **Alcohols**

Ethanol (ethyl alcohol,  $C_2H_5OH$ ) and 2-propanol (isopropyl alcohol,  $(CH_3)_2CHOH$ ) have similar disinfectant properties. They are active against vegetative bacteria, fungi and lipid-containing viruses but not against spores. Their action on nonlipid viruses is variable. For highest effectiveness they should be used at concentrations of approximately 70% (v/v) in water: higher or lower concentrations may not be as germicidal. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items.

Mixtures with other agents are more effective than alcohol alone, e.g. 70% (v/v) alcohol with 100 g/l formaldehyde, and alcohol containing 2 g/l available chlorine. A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and biosafety cabinets, and to soak small pieces of surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or not possible. However, it must be remembered

that ethanol is ineffective against spores and may not kill all types of nonlipid viruses.

Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. Bottles with alcohol-containing solutions must be clearly labelled to avoid autoclaving.

### **Iodine and iodophors**

The action of these disinfectants is similar to that of chlorine, although they may be slightly less inhibited by organic matter. Iodine can stain fabrics and environmental surfaces and is generally unsuitable for use as a disinfectant. On the other hand, iodophors and tinctures of iodine are good antiseptics. Polyvidone-iodine is a reliable and safe surgical scrub and preoperative skin antiseptic. Antiseptics based on iodine are generally unsuitable for use on medical/dental devices. Iodine should not be used on aluminium or copper.

Iodine can be toxic. Organic iodine-based products must be stored at 4–10 °C to avoid the growth of potentially harmful bacteria in them.

### **Hydrogen peroxide and peracids**

Like chlorine, hydrogen peroxide ( $H_2O_2$ ) and peracids are strong oxidants and can be potent broad-spectrum germicides. They are also safer than chlorine to humans and the environment.

Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30% aqueous solution to be diluted to 5–10 times its volume with sterilized water. However, such 3–6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content, to accelerate its germicidal action and to make it less corrosive.

Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or peracetic acid ( $CH_3COOOH$ ) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment.

Hydrogen peroxide and peracids can be corrosive to metals such as aluminium, copper, brass and zinc, and can also decolorize fabrics, hair, skin and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes. They should always be stored away from heat and protected from light.

### **Local environmental decontamination**

Decontamination of the laboratory space, its furniture and its equipment requires a combination of liquid and gaseous disinfectants. Surfaces can be decontaminated using

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a solution of sodium hypochlorite (NaOCl); a solution containing 1 g/l available chlorine may be suitable for general environmental sanitation, but stronger solutions (5 g/l) are recommended when dealing with high-risk situations. For environmental decontamination, formulated solutions containing 3% hydrogen peroxide ( $H_2O_2$ ) make suitable substitutes for bleach solutions.

Rooms and equipment can be decontaminated by fumigation with formaldehyde gas generated by heating paraformaldehyde or boiling formalin. This is a highly dangerous process that requires specially trained personnel. All openings in the room (i.e. windows, doors, etc.) should be sealed with masking tape or similar before the gas is generated. Fumigation should be conducted at an ambient temperature of at least 21 °C and a relative humidity of 70%. (See also section on Decontamination of biological safety cabinets in this chapter.)

After fumigation the area must be ventilated thoroughly before personnel are allowed to enter. Appropriate respirators must be worn by anyone entering the room before it has been ventilated. Gaseous ammonium bicarbonate can be used to neutralize the formaldehyde.

Fumigation of smaller spaces with hydrogen peroxide vapour is also effective but requires specialized equipment to generate the vapour.

### **Decontamination of biological safety cabinets**

To decontaminate Class I and Class II cabinets, equipment that independently generates, circulates and neutralizes formaldehyde gas is available. Alternatively, the appropriate amount of paraformaldehyde (final concentration of 0.8% paraformaldehyde in air) should be placed in a frying pan on an electric hot plate. Another frying pan, containing 10% more ammonium bicarbonate than paraformaldehyde, on a second hot plate is also placed inside the cabinet. The hot plate leads are plugged in outside the cabinet, so that operation of the pans can be controlled from the outside by plugging and unplugging the hot plates as necessary. If the relative humidity is below 70%, an open container of hot water should also be placed inside the cabinet before the front closure is sealed in place with strong tape (e.g. duct tape). Heavy gauge plastic sheeting is taped over the front opening and exhaust port to make sure that the gas cannot seep into the room. Penetration of the electric leads passing through the front closure must also be sealed with duct tape.

The plate for the paraformaldehyde pan is plugged in. It is unplugged when all the paraformaldehyde has vaporized. The cabinet is left undisturbed for at least 6 h. The plate for the second pan is then plugged in and the ammonium bicarbonate is allowed to vaporize. This plate is then unplugged and the cabinet blower is switched on for two intervals of approximately 2 s each to allow the ammonium bicarbonate gas to circulate. The cabinet should be left undisturbed for 30 min before the front closure (or plastic sheeting) and the exhaust port sheeting are removed. The cabinet surfaces should be wiped down to remove residues before use.



### **Hand-washing/hand decontamination**

Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 10 s, rinsed in clean water and dried using a clean paper or cloth towel (if available, warm-air hand-dryers may be used).

Foot- or elbow-operated faucets are recommended. Where not fitted, a paper/cloth towel should be used to turn off the faucet handles to avoid recontaminating washed hands.

As mentioned above, alcohol-based hand-rubs may be used to decontaminate lightly soiled hands when proper hand-washing is not available.

### **Heat disinfection and sterilization**

Heat is the most common among the physical agents used for the decontamination of pathogens. “Dry” heat, which is totally non-corrosive, is used to process many items of laboratory ware which can withstand temperatures of 160 °C or higher for 2–4 h. Burning or incineration (see below) is also a form of dry heat. “Moist” heat is most effective when used in the form of autoclaving.

Boiling does not necessarily kill all microorganisms and/or pathogens, but it may be used as the minimum processing for disinfection where other methods (chemical disinfection or decontamination, autoclaving) are not applicable or available.

Sterilized items must be handled and stored such that they remain uncontaminated until used.

### **Autoclaving**

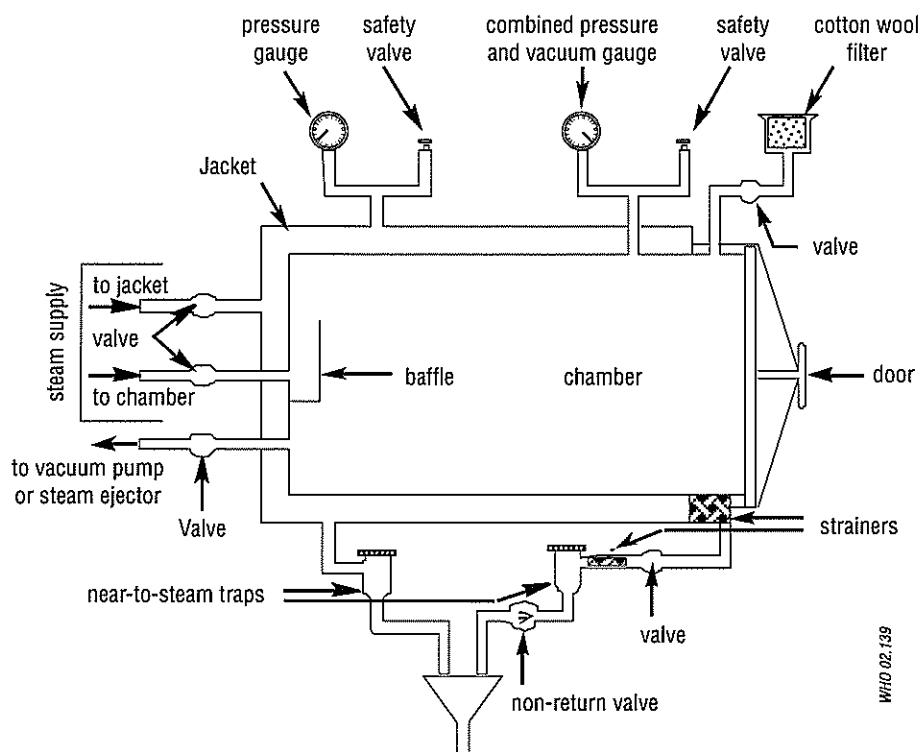
Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves:

1. 3 min holding time at 134 °C
2. 10 min holding time at 126 °C
3. 15 min holding time at 121 °C
4. 25 min holding time at 115 °C.

Examples of different autoclaves include the following.

*Gravity displacement autoclaves.* Figure 10 shows the general construction of a gravity-displacement autoclave. Steam enters the chamber under pressure and displaces the heavier air downwards and through the valve in the chamber drain, fitted with a HEPA filter.

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**Figure 10. Gravity displacement autoclave**

*Pre-vacuum autoclaves.* These machines allow the removal of air from the chamber before steam is admitted. The exhaust air is evacuated through a valve fitted with a HEPA filter. At the end of the cycle, the steam is automatically exhausted. These autoclaves can operate at 134 °C and the sterilization cycle can therefore be reduced to 3 min. They are ideal for porous loads, but cannot be used to process liquids because of the vacuum.

*Fuel-heated pressure cooker autoclaves.* These should be used only if a gravity displacement autoclave is not available. They are loaded from the top and heated by gas, electricity or other types of fuels. Steam is generated by heating water in the base of the vessel, and air is displaced upwards through a relief vent. When all the air has been removed, the valve on the relief vent is closed and the heat reduced. The pressure and temperature rise until the safety valve operates at a preset level. This is the start of the holding time. At the end of the cycle the heat is turned off and the temperature allowed to fall to 80 °C or below before the lid is opened.

### *Loading autoclaves*

Materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags should allow the steam to reach their contents.

*Precautions in the use of autoclaves*

The following rules can minimize the hazards inherent in operating pressurized vessels.

1. Responsibility for operation and routine care should be assigned to trained individuals.
2. A preventive maintenance programme should include regular inspection of the chamber, door seals and all gauges and controls by qualified personnel.
3. The steam should be saturated and free from chemicals (e.g. corrosion inhibitors) that could contaminate the items being sterilized.
4. All materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration; the chamber should be loosely packed so that steam will reach the load evenly.
5. For autoclaves without an interlocking safety device that prevents the door being opened when the chamber is pressurized, the main steam valve should be closed and the temperature allowed to fall below 80 °C before the door is opened.
6. Slow exhaust settings should be used when autoclaving liquids, as they may boil over when removed due to superheating.
7. Operators should wear suitable gloves and visors for protection when opening the autoclave, even when the temperature has fallen below 80 °C.
8. In any routine monitoring of autoclave performance, biological indicators or thermocouples should be placed at the centre of each load. Regular monitoring with thermocouples and recording devices in a "worst case" load is highly desirable to determine proper operating cycles.
9. The drain screen filter of the chamber (if available) should be removed and cleaned daily.
10. Care should be taken to ensure that the relief valves of pressure cooker autoclaves do not become blocked by paper, etc. in the load.

**Incineration**

Incineration is useful for disposing of animal carcasses as well as anatomical and other laboratory waste, with or without prior decontamination (see Chapter 3). Incineration of infectious materials is an alternative to autoclaving only if the incinerator is under laboratory control.

Proper incineration requires an efficient means of temperature control and a secondary burning chamber. Many incinerators, especially those with a single combustion chamber, are unsatisfactory for dealing with infectious materials, animal carcasses and plastics. Such materials may not be completely destroyed and the effluent from the chimney may pollute the atmosphere with microorganisms, toxic chemicals and smoke. However, there are many satisfactory configurations for combustion chambers. Ideally the temperature in the primary chamber should be at least 800 °C and that in the secondary chamber at least 1000 °C.

Materials for incineration, even with prior decontamination, should be transported

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to the incinerator in bags, preferably plastic. Incinerator attendants should receive proper instructions about loading and temperature control. It should also be noted that the efficient operation of an incinerator depends heavily on the right mix of materials in the waste being treated.

There are ongoing concerns regarding the possible negative environmental effects of existing or proposed incinerators, and efforts continue to make incinerators more environmentally friendly and energy-efficient.

##### **Disposal**

The disposal of laboratory and medical waste is subject to various regional, national and international regulations, and the latest versions of such relevant documents must be consulted before designing and implementing a programme for handling, transportation and disposal of biohazardous waste. In general, ash from incinerators may be handled as normal domestic waste and removed by local authorities. Autoclaved waste may be disposed of by off-site incineration or in licensed landfill sites (see Chapter 3).

For further information see references (13) and (29–39).