LABORATORY BIOSAFETY MANUAL
FOURTH EDITION
AND
ASSOCIATED MONOGRAPHS

DECONTAMINATION AND WASTE MANAGEMENT

World Health Organization

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(Laboratory biosafety manual, fourth edition and associated monographs)

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Glossary of terms

Available chlorine: A measure of the amount of chlorine available in hypochlorite compounds and other disinfectant chemicals, used as a source of chlorine, when compared with that of pure gaseous chlorine.

Biological agent: A microorganism, biological toxin, protein (prions) or human endoparasite either naturally occurring or genetically modified, which may have the potential to cause infection, allergy, toxicity or otherwise create a hazard to human health.

Biological indicator: Test system containing viable biological agents providing a specified resistance to a specific sterilization process.

Biological safety cabinet (BSC): An enclosed, ventilated working space designed to provide protection to the operator, the laboratory environment and/or the work materials for activities where there is an aerosol hazard. Containment is achieved by segregation of the work from the main area of the laboratory and/or through the use of controlled, directional airflow mechanisms. Exhaust air is passed through a high-efficiency particulate air (HEPA) filter before recirculating into the laboratory or into the building's heating, ventilation and air conditioning system. There are different classes (I, II and III) of BSCs that provide different levels of containment.

Biosafety: Containment principles, technologies and practices that are implemented to prevent unintentional exposure to biological agents or their inadvertent release.

Biosecurity: Principles, technologies and practices that are implemented for the protection, control and accountability of biological materials and/or the equipment, skills and data related to their handling. Biosecurity aims to prevent their unauthorized access, loss, theft, misuse, diversion or release.

Clean: Visually free of soil and below specified levels of analytes.

Cleaning: Reduction of viable biological agents to safe levels.

Cleaning agent: Physical or chemical substance or combination of entities, having activity to make an item clean.

Contamination: The introduction of undesired biological agents into tissues and specimens or onto surfaces.

Cross contamination: The process by which biological agents are unintentionally transferred from one substance or object to another, with potentially harmful effect.

Decimal reduction time, D-value, or D10-value: The time required to inactivate 90% of the cells present on a surface or item or to reduce the population of a biological agent by one tenth of its original number under the same conditions, that is a one-logarithm reduction.

Decontamination: Reduction of viable biological agents or other hazardous materials on a surface or object(s) to a pre-defined level by chemical and/or physical means.

Denaturation: Process by which complex molecules are forced to change their structure by chemical or physical means but maintain their composition. This process is often accompanied by a loss of function and can be reversible or irreversible.

Disinfectants: Agents capable of eliminating viable biological agents on surfaces or in liquid waste. These will have varying effectiveness depending on the properties of the chemical, its concentration, shelf life and contact time with the agent.

Disinfection: A process to eliminate viable biological agents from items or surfaces for further safe handling or use.

Dosimetry: Assessment of the ionizing radiation dose absorbed by an object.

Endospore: A cell which is formed by certain Gram-positive bacteria in unfavourable conditions for growth. An endospore is extremely resistant to heat and other harmful agents.

Fumigant: Chemical used for fumigation; also known as an airborne disinfectant.

Fumigation: Use of a poisonous gas or vapour to remove contamination of a biological agent from a surface, piece of equipment or area.

Heat disinfection: Disinfection achieved by the action of moist or dry heat.

Inactivation: Removal of the activity of biological agents by destroying or inhibiting reproductive or enzyme activity.

Load: Product, equipment or materials to be processed together within an operating cycle of, for example, an autoclave.

Pathogen: A biological agent capable of causing disease in humans, animals or plants.

Pressure vessel: Equipment designed and built to contain fluids under pressure, for example, autoclave, media preparation unit.

Primary containment device: A contained workspace designed to provide protection to its operator, the laboratory environment and/or the work materials for activities where there is an aerosol hazard. Protection is achieved by segregation of the work from the main area of the laboratory and/or through the use of controlled, directional airflow mechanisms. Primary containment devices include biological safety cabinets (BSCs), isolators, local exhaust ventilators and ventilated working spaces.

Relative humidity: Measure of water vapour in the air expressed as a percentage of the maximum the air is capable of holding at a given temperature.

Risk: A combination of the likelihood of an incident and the severity of the harm (consequences) if that incident were to occur.

Saturated steam: Water vapour in a state of equilibrium between its liquid and gas phases, as used for steam sterilization.

Soap: A water soluble cleaning compound used for cleaning skin and other materials. Note, soap does not necessarily inactivate biological agents.

Soil: Contamination with biological material including biological agents on a device or surface following its use.

Spore: See endospore.

Standard operating procedures (SOPs): A set of well-documented and validated stepwise instructions outlining how to perform laboratory practices and procedures in a safe, timely and reliable manner, in line with institutional policies, best practice and applicable national or international regulations.

Sterile: The state of having a complete absence of viable biological agents and spores.

Sterilization: A process that kills and/or removes all biological agents including spores.

Validation: Systematic and documented confirmation that the specified requirements are adequate to ensure the intended outcome or results. For example, in order to prove a material is decontaminated, laboratory personnel must validate the robustness of the decontamination method by measurement of the remaining biological agents against the detection limit by chemical, physical or biological indicators.

Verification: Confirmation that a given item (product, process or system) satisfies the specified requirements. For example, verification that the performance of an autoclave meets the standards specified by the manufacturer should be performed periodically.

Vegetative bacteria: A cell of a bacterium or unicellular alga that is actively growing rather than forming spores.

Executive summary

Disinfection, sterilization and waste management are key to safe handling of biological agents. It is therefore important to understand the basic mechanisms of the different methods of disinfection, sterilization and waste management that can be used in a laboratory. The specific decontamination requirements depend on the nature of the biological agents being handled. This monograph describes the methods for the management and final disposal of laboratory waste that is considered a biological hazard. The information can be used to develop standardized and more specific procedures on decontamination and waste management for a particular laboratory. The targeted readership for this monograph is personnel who perform the risk assessment, for example, laboratory managers or biosafety officers, as well as laboratory personnel and scientists who decontaminate laboratory items and workers who handle laboratory waste.

The information in this monograph on decontamination and waste management is designed to accompany and support the fourth edition of the WHO *Laboratory biosafety manual* (core document) and other associated monographs. The manual and the monographs adopt a risk- and evidence-based approach to biosafety, rather than a prescriptive approach, in order to ensure that laboratory facilities, safety equipment and work practices are locally relevant, proportionate to needs and sustainable. Emphasis is placed on the importance of a "safety culture" that incorporates risk assessment, good microbiological practice and procedure and standard operating procedures, relevant introductory, refresher and mentoring training of personnel, and prompt reporting of incidents and accidents followed by appropriate investigation and corrective actions. This new approach aims to facilitate laboratory design and ways of operating that ensure greater sustainability while maintaining adequate and appropriate control of biosafety.

The other associated monographs provide detailed information and help implement systems and strategies on the following specialized topics: risk assessment, laboratory design and maintenance, biological safety cabinets and other primary containment devices, personal protective equipment, biosafety programme management and outbreak preparedness and resilience.

The monograph includes a description of the methods of decontamination used in a microbiology laboratory including handwashing, and chemical, gaseous and heat disinfection. The different classes of chemical disinfectants and their constituents, their mechanisms of action and their advantages and disadvantages are discussed. Factors that can influence the effectiveness of disinfectants are also listed. The monograph includes an overview of fumigation methods, heat inactivation and how verification of the effectiveness of these treatments is carried out by means of indicators. The monograph also covers aspects of waste management as well as documentation and record-keeping which are an integral part of waste management. Considerations for removal of both liquid and solid waste products from the laboratory and their safe disposal are described. Methods for inactivation of specimens are also discussed.

SECTION 1

INTRODUCTION

This monograph supports and builds on information on decontamination and waste management set out in the fourth edition of the WHO *Laboratory biosafety manual (1)* (core document). It includes information on cleaning, chemical disinfection, gaseous disinfection, heat disinfection, sterilization, chemical and biological indicators, waste management and inactivation of biological specimens.

The other associated monographs provide detailed information and help implement systems and strategies on the following specialized topics: risk assessment *(2)*, laboratory design and maintenance *(3)*, biological safety cabinets and other primary containment devices *(4)*, personal protective equipment *(5)*, biosafety programme management *(6)* and outbreak preparedness and resilience *(7)*.

In the typical laboratory, management of waste potentially containing biological agents must include a validated means of decontamination which is determined by the risk assessment process. Decontamination is routinely achieved using a combination of chemical disinfectants and autoclaving, in some instances, supplemented by incineration. A basic knowledge of the laboratory personnel about cleaning, disinfection and sterilization is therefore crucial for biosafety in the laboratory. The following general principles apply to all known classes of biological agent. It is important to ensure that any disinfectants used – whether applied to surfaces or liquids – are validated as disinfectants against the biological agents typically being handled in the laboratory, and are used at the correct concentration, correct temperature and at least for the minimum contact time for which they have been validated. In addition, when choosing the appropriate disinfectant, it is important to consider some disinfectants can be inactivated by organic matter; this organic matter may include laboratory reagents such as liquid culture nutrient supplements.

It is also important to consider segregation (clean from contaminated materials, contaminated materials from personnel) and containment (for example, secure bagging and boxing of waste during handling and transfer of waste from the laboratory bench to the autoclave). In subsection 3.1 of the *Laboratory biosafety manual (1)*, fourth edition, good microbiological practice and procedure are described in detail.

In any busy laboratory, it is important to have a dedicated area for safely holding waste containing biological agents for a short time before its prompt processing and disposal. This area should ideally be separate from the work/analytical area of the

laboratory and away from areas where clean and sterile items are stored or processed. For more information on laboratory design, please refer to *Monograph: laboratory design and maintenance (3)*.

The generic information on decontamination and waste management given in this monograph can be used to develop both standard and more specific procedures to deal with pathogenic biological agents in a laboratory. Specific decontamination requirements will depend on the type of laboratory activity or procedure and the nature of the biological agents being handled. Laboratories should therefore determine appropriate waste management strategies based on the outcomes of the risk assessment, and that are applicable to local needs and conditions.

SECTION 2

METHODS OF DECONTAMINATION

Depending on the level of decontamination that needs to be achieved, processes such as cleaning, disinfection or sterilization can be used (Figure 2.1). The choice and use of a decontamination method, either chemical or physical, will depend on the application: decontamination of waste, surfaces, medical devices or specimens might be achieved with decontamination processes such as steam exposure or treatment with chemicals. The advantages and disadvantages of the different methods must be considered in order to select the most appropriate decontamination method.

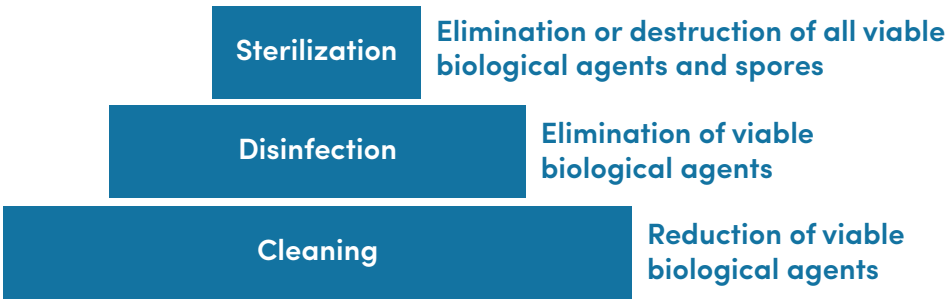

Figure 2.1 Levels of decontamination

Table 2.1 shows the methods of decontamination to control contamination with biological agents described in this monograph.
For a chemical method, four factors must be considered when selecting a disinfectant to use in the decontamination process: antimicrobial efficacy, safety, environmental impact and compatibility with the material of the surface and/or laboratory equipment being decontaminated. The ideal disinfectant does not yet exist and probably never will as it would have the following specifications:

- rapid and efficient action on biological agents,
- broad application range,
- effective at the lowest concentration possible,
- active at all temperatures,

Table 2.1 Physical and chemical methods of decontamination to control microbial contamination

CATEGORIES OF DECONTAMINATION		
CHEMICAL		PHYSICAL
GAS/VAPOUR	LIQUID	HEAT
Formaldehyde Hydrogen peroxide Chlorine dioxide	Phenols Peroxides Hypochlorites Chlorine dioxide Peracetic acid Formaldehyde Glutaraldehyde Quaternary ammonium compounds Alcohols	Autoclave Incineration Hot air oven Boiling

- low toxicity,
- not inactivated by organic matter,
- compatibility with all materials (for example, non-corrosive),
- stable, and
- degradable and environmentally friendly.

The correct sequence for successful disinfection is: (i) clean to remove dirt and organic matter, (ii) apply the disinfectant and (iii) after a pre-determined contact time, wipe with water to remove chemical residues, if necessary.

The following subsections describe a number of methods commonly used in the laboratory to treat contaminated waste (liquid or solid) or to remove biological agents from surfaces, equipment and materials to be discarded from the laboratory.

2.1 Cleaning and hand hygiene

2.1.1 Cleaning

Cleaning, in general, is the removal of any matter from an item that is not part of the item itself. Cleaning in the context of laboratory biosafety has two functions: i) it can remove dirt and organic matter from an item that would inactivate chemical disinfectants or impede them making contact with biological agents within the item and ii) it can remove a high proportion of biological agents, making reduction to safe levels by subsequent chemical disinfection more effective.

Cleaning should not be relied on as the only decontamination process. The need for cleaning before chemical disinfection is subjective: if an item looks physically clean, there is no need to clean it before chemical disinfection. A difficulty arises when areas cannot be seen, for example, inside opaque or hidden tubing. In these cases, risk assessments for the need to clean before disinfection should be made.

The cleaning can be carried out manually by scrubbing, if possible using warm water. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping preferably with warm water. Using a laboratory dishwasher is also a cleaning method. The addition of cleaning agents (surfactant that lowers surface tension, detergent) increases the effectiveness of cleaning. Examples of common cleaning agents include soda solution (3 kg sodium carbonate (Na_2CO_3) per 100 L of hot water), soap solution (3 kg soap per 100 L of hot water) or commercial preparations. Cleaning must be carried out in such a way as to keep the operator safe and prevent the spread of the disease or dispersion of contamination. Therefore, personnel must be trained and use PPE, proper techniques and adequate care.

2.1.2 Hand hygiene

While suitable gloves provide the wearer with a high degree of protection, they do not give complete protection and hands must be washed after gloves are removed. Gloves can develop holes, some that may be too small for the wearer to notice. Liquid contamination will pass through these holes by capillary action and spread out on the wearer's skin. Even with an excellent glove removal technique, as described in *Monograph: personal protective equipment (5)*, hands could become contaminated during the procedure. Therefore, hand hygiene must be performed after glove removal. If gloves have not been worn, handwashing after laboratory work or handling animals is essential. If hands are contaminated with biological agents in the laboratory, the contamination will be on the surface of the skin. As such, it can be easily removed by handwashing or readily inactivated with antimicrobial hand rubs.

There are two types of laboratory hand hygiene.

Handwashing

A short (about 20 seconds) but thorough handwash with soap and running water will efficiently remove laboratory-acquired contamination. A technique for effective handwashing is shown in Figure 2.2. There is no advantage to using antimicrobial soaps as the purpose of handwashing is to remove biological agents rather than inactivate or destroy them. Hands should be washed in running water, so a tap/faucet that mixes hot and cold water to a comfortable temperature should be used. A hands-free method (infrared-operated switch, or foot, knee or elbow operated tap/faucet) is an advantage. If taps/faucets need to be turned on and off by hand, a clean paper towel should be used to turn them off. Hands should be dried with single-use paper towels and the towels should properly discarded after use in a designated waste bin.

Alcohol hand rub

Alcohols (ethanol, propanol or isopropanol) at concentrations between 60% and 95% applied to the hands and rubbed to dryness can be effective in removing microbial contamination acquired during laboratory work *(8,9)*. The correct technique for using alcohol hand rubs can be found at the WHO website *(10)*. Alcohols are poor at penetrating proteins or protein-containing matter, so they should only be used on visibly clean hands. Alcohols have no activity against spores and poor activity against non-enveloped viruses; if hand contamination with these biological agents is likely, handwashing should be used instead of alcohol hand rubs.

2.2 Chemical disinfection

Biological agents will show different resistance or sensitivity to chemical products depending on the presence or absence of a cell wall skeleton, lipid membranes, layers of polysaccharides and peptidoglycan. Generally, higher concentrations of highly active disinfectants are required to inactivate spores than enveloped viruses. Typically, a solution of sodium hypochlorite (NaOCl) containing 1000 ppm (parts per million) available chlorine will be suitable for general surface disinfection, but stronger solutions (for example, 5000 ppm or 10 000 ppm) are recommended when dealing with heavy contamination, the presence of organic matter or disinfectant-resistant biological agents.

Figure 2.2 Hand hygiene – recommended procedure

2.2.1 Types of disinfectant

A number of classes of chemical disinfectants are available (Figure 2.3).

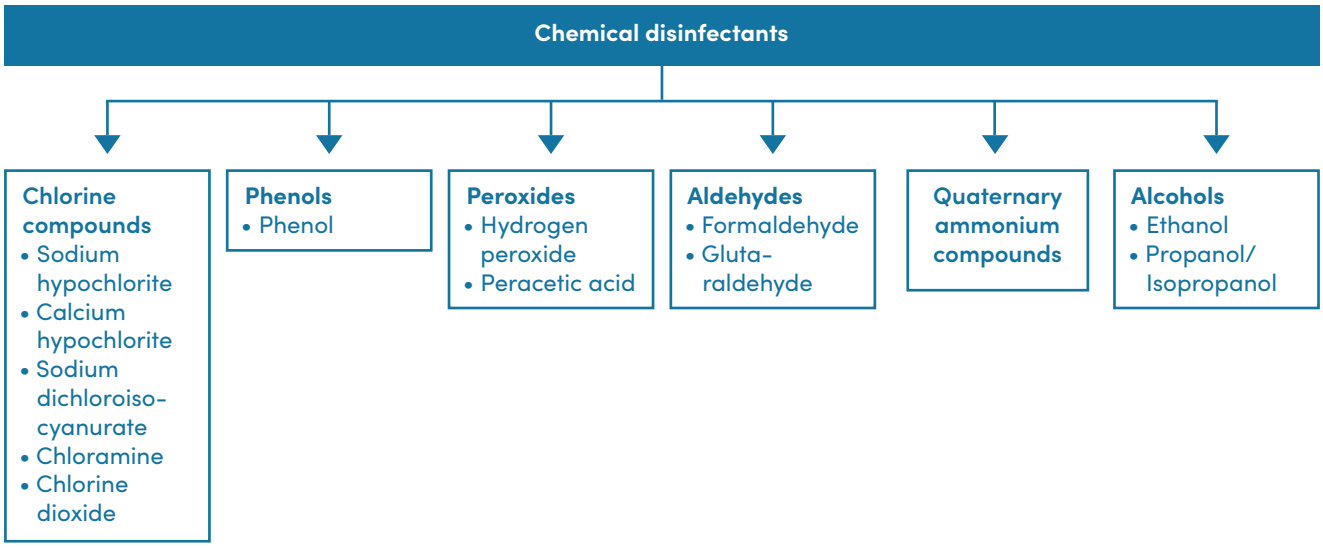

Figure 2.3 Types of chemical disinfectant

Chlorine compounds

Hypochlorites are chlorine-based disinfectants but the active component is oxygen loosely bound to chlorine; this oxygen is readily lost to become available to oxidize other compounds. These disinfectants are solutions with a variety of components in equilibrium but the most active chemical species are usually sodium hypochlorite (NaOCl), the hypochlorous ion (OCl^-) and hypochlorous acid (HOCl). The oxidizing capacity of hypochlorite solutions is expressed as either percentage of available chlorine, or parts per million of available chlorine (ppm av Cl). (This is a historical misnomer and really refers to oxygen bound to chlorine).

Hypochlorites are inactivated by organic matter. Even at apparently low levels of contamination ("clean conditions"), high concentrations are required, and the concentration needed increases as the amount of organic matter contamination increases ("dirty conditions"); see Table 2.2.

Hypochlorite solutions can be prepared from a number of different starting agents such as liquid bleach. This is cheap and readily available. However, there can be uncertainty about the available chlorine content of these solutions because liquid hypochlorites decay on storage; the speed of that decay depends on storage conditions, mainly temperature. Diluted hypochlorite solutions have a limited shelf-life, about one day, depending on exposure to heat and/or sunlight.

Granules or tablets of calcium hypochlorite ($Ca(ClO)_2$) generally contain about 70% available chlorine. Thus, solutions from tablets or granules containing, for example, 1.4 g/L calcium hypochlorite solution would contain about 1 g/L available chlorine, which equates to 1000 ppm av Cl.

Sodium dichloroisocyanurate (NaDCC) is a solid that is very stable in dry storage, even at high temperatures. It contains 60% available chlorine and forms an equilibrium that contains the active chemical species of hypochlorites. Once in solution, it is as unstable as other forms of liquid hypochlorite. Sodium dichloroisocyanurate is available in a variety of tablet forms which give a specific available chlorine concentration when dissolved in specified volumes of water.

Similar calculations can be done with chloramine, which contains about 25% available chlorine. Chloramines are thought to be more resistant to inactivation by organic matter than other sources of hypochlorite *(11)*.

Hypochlorites can cause severe corrosion of metals and irritation to exposed skin and mucous membranes. Their use should be avoided on metals unless the manufacturer states that the metals in their device are compatible with the concentration of hypochlorite used or if the occurrence of corrosion is not a problem, for example, the item is being disinfected before disposal.

Table 2.2 Recommended dilutions of compounds releasing chlorine

HYPOCHLORITE SOURCE (PERCENTAGE AVAILABLE CHLORINE)	**CLEAN CONDITIONS**[a] (AVAILABLE CHLORINE NEEDED FOR DISINFECTION: 1 G/L – 0.1% AVAILABLE CHLORINE – 1000 PPM AV CL)	**DIRTY CONDITIONS**[b] (AVAILABLE CHLORINE NEEDED FOR DISINFECTION: 5 G/L – 0.5% AVAILABLE CHLORINE – 5000 PPM AV CL)
Sodium hypochlorite solution (5% available chlorine)	20 mL	100 mL
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
Chloramine powder (25% available chlorine)	4 g/L	20 g/L

ppm av Cl = parts per million available chlorine.

[a] Low levels of contamination.

[b] High levels of contamination.

The frequency with which working solutions of hypochlorite should be changed depends on their starting strength, the frequency and nature of use (how much organic matter is added to them), and the ambient temperature. As a general guide, solutions that are used several times a day to decontaminate materials with high levels of organic matter should be changed at least daily, while those with less frequent use may last for as long as a week. The adequacy of hypochlorite solutions can be checked using starch–iodine papers to show they have not been inactivated. Starch–iodine papers identify the condition and relative strength of the oxidizing agent present in solutions.

Hypochlorite is relatively inexpensive and active against most species, including spores and prions *(12,13)*. The effective concentration and contact time are a function of the biological agent being decontaminated, with increased concentrations required for spores and prions. Sodium hypochlorite solutions are frequently stabilized in an alkaline solution to prolong their shelf-life and adjusted to pH 7 for use *(14)* for resistant species, such as *Bacillus anthracis* spores *(15)*.

Other factors such as the hardness of the diluent water, inorganic matter (for example, salts) or the presence of some detergents can also compromise the efficacy of some disinfectants (for example, quaternary ammonium compounds).

Given these different and sometimes poorly controllable factors, the quality assurance of chemical disinfection is usually not high. Chemical disinfection should only be used where no better means of decontamination (for example, autoclaving) can be applied.

Many chemical disinfectants can be harmful to humans and/or the environment. They should be selected, stored, handled, used and disposed of with care, following manufacturers' instructions and local legal/regulatory requirements.

When considering a disinfectant for use, the characteristics of its active ingredients will predict its likely activity. If a disinfectant is sold as a branded product, users should establish the active component(s). This can sometimes be difficult as brand names can cover a range of different products, each with different components or levels of those components. Users should know exactly what they are using and that what they are using will be effective as determined by risk assessment.

As noted in subsection 2.2.2 Factors affecting the effectiveness of disinfectants, dilute hypochlorite solutions have a limited shelf-life of about one day, depending on exposure to heat and/or sunlight. Hypochlorite is a non-specific oxidizer and if there are large quantities of other organic compounds in the solution being disinfected, additional quantities of hypochlorite will be needed.

Chlorine dioxide (ClO_2) has a similar microbiocidal mechanism to hypochlorite but is more effective. As it is more effective, it can be used at lower concentrations which are less corrosive than hypochlorite. Because chlorine dioxide is used at lower concentrations, it tends to be more easily inactivated by organic matter than hypochlorite solutions.

Chlorine dioxide solutions are made by mixing two components immediately before use. Once the components are mixed in the laboratory, the solution has a limited length of time it can be used (specified by the manufacturer).

Phenols

Phenols, also called phenolic compounds, are a class of chemical compounds consisting of a hydroxyl group (–OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol (C_6H_5OH). Phenol acts specifically on the cell membrane, denatures proteins and inactivates intracytoplasmic enzymes by forming unstable complexes. These interactions lead to leakage of bacterial elements or they induce lysis of the cell membrane. Phenolic compounds have greater stability than sodium hypochlorite, are less affected by higher concentrations of organic matter in the solution and are effective against vegetative bacteria, particularly Gram-positive species and enveloped viruses. However, they are not effective against spores and non-enveloped viruses. Despite these advantages, phenolic compounds can be toxic, are corrosive for the skin and are known to be respiratory irritants. Because of these major limitations, the use of phenolic compounds has decreased in recent years, although phenolic compounds can still be found in some household disinfectant and cleaning products, often in combination with quaternary ammoniums and alcohols.

Phenol-based disinfectants are still used as tuberculocidal agents to destroy mycobacteria. This is because these disinfectants are lipophilic molecules that are efficiently trapped by the many phospholipids found on the cell membrane of mycobacteria, thereby eliminating the bacterium.

Peroxides

Peroxides are widely used microbiocidal agents because of their strong oxidizing activity due to the presence of highly reactive hydroxyl radicals. They denature proteins and lipids of biological agents, leading to disorganization of the membrane. Swelling of the cell of the biological agent may take place when saturated with hydrogen ions, which attract water.

Hydrogen peroxide (H_2O_2) acts as an oxidizing agent by producing hydroxyl free radicals that attack essential cell components, including lipids, proteins and DNA. It has a wide range of bactericidal, viricidal and fungicidal activity, although activity is variable against bacterial spores and mycobacteria. However, the ability of bacteria to produce catalase can increase tolerance to hydrogen peroxide when low concentrations are used *(16)*. Hydrogen peroxide is considered environmentally friendly because it can rapidly degrade into the harmless products – water and oxygen. It is, however, a severe irritant to the skin, eyes and respiratory system.

Peracetic acid ($C_2H_4O_3$) is made by mixing acetic acid with hydrogen peroxide and a strong acid catalyst. It is a stronger disinfectant than hydrogen peroxide. It denatures proteins, disrupts cell wall permeability and oxidizes sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites.

As a result, peracetic acid is highly sporicidal, bactericidal, viricidal and fungicidal at low concentrations (< 0.3%). Peracetic acid also decomposes to safe by-products (acetic acid and oxygen) and has the added advantages of not being decomposed by peroxidases, unlike hydrogen peroxide, and remaining active in the presence of organic loads *(17)*. It is available from the main chemical suppliers and is therefore relatively cheap, and it can be diluted at the point of use. However, as with hydrogen peroxide, peracetic acid is a severe irritant to the skin, eyes and respiratory system.

Aldehydes

Formaldehyde (CH_2O) is available as formalin, a stabilized solution of about 37% formaldehyde, or paraformaldehyde, a solid polymer that is heated and reacts with air to form formaldehyde gas. Formaldehyde causes irritation of the skin, eyes, nose and throat. High levels of exposure may cause some types of cancer *(18,19)*; because of this, some countries are considering restricting its future use. Its laboratory use should not expose people to contact with formaldehyde or its vapour. Formaldehyde should only be used for specific processes that require it, such as tissue fixation, but not for general disinfection. It should not be used to wipe surfaces or equipment. It should only be used to fumigate spaces (see subsection 2.3 Gaseous disinfection) if the vapour can be completely contained, properly inactivated and exhausted safely after fumigation. When formaldehyde is used, it should be in controlled conditions, such as in sealable rooms or cabinets, which limit people's exposure in accordance with national safety and environmental requirements.

Glutaraldehyde ($C_5H_8O_2$) is normally used as a solution that is buffered (activated) to alkaline pH just before use. Before activation, glutaraldehyde is a stable solution but after activation, it has a limited shelf-life. The advantages of glutaraldehyde are its wide microbiocidal spectrum and low corrosiveness. The disadvantage of glutaraldehyde is that it is toxic and acts as a chemical sensitizer (can cause allergic reaction after exposure). If used, appropriate PPE must be used in order to limit potential exposure to either the liquid or the vapour form.

Quaternary ammonium compounds

Quaternary ammonium compounds and similar compounds, such as triamines, are a varied family of molecules, some of which can be used as disinfectants. They work by their surfactant activity disrupting the structure of biological agents. Quaternary ammonium compounds are more stable than hypochlorites, less irritating to the respiratory tract and less toxic than phenolic compounds. However, they are affected by high levels of organic materials and anything with a large surface area that could bind the disinfectant, such as fabrics.

They have activity against bacteria in non-spore forms and enveloped (lipid-containing) viruses. They are effective against a smaller range of biological agents than either hypochlorites or phenolic compounds, and have limited effectiveness against non-enveloped viruses, most *Mycobacterium* species and spores. However, for extended use in solutions containing mostly enveloped viruses, quaternary ammonium compounds may be the best disinfectant to use.

They are non-corrosive but more expensive than sodium hypochlorite. If being considered for laboratory use, both potential inactivation and the range of biological agents they would be expected to act against need to be considered in the risk assessment.

Alcohols

Alcohols used for laboratory disinfection are either ethanol (usually denatured by the addition of methylated spirits, making it unsuitable for consumption), propanol (propan-1-ol) or isopropanol (propan-2-ol). The normal concentration for use is 70%, although, depending on which alcohol is used, anywhere between 60% and 90% can be effective *(20)*. The activity of the three alcohols is broadly similar; they are effective against a wide range of bacteria in non-spore form and enveloped (lipid-containing) viruses. They have variable activity against non-enveloped viruses and no activity against bacteria spores. While alcohols are not inactivated by organic matter, their activity is unreliable in the presence of proteins; they can coagulate proteins, forming a barrier against their further penetration to layers inside. Alcohols evaporate quickly which make them convenient to use as surface disinfectants. However, their quick evaporation also reduces the exposure time and therefore their effectiveness.

2.2.2 Factors affecting the effectiveness of disinfectants

Many different factors can affect the effectiveness of chemical disinfection. The most important ones are shown in Table 2.3. These factors must be considered in the risk assessment for selecting the best decontamination process.

Other factors such as the hardness of the diluent water, inorganic matter (for example, salts) or the presence of some detergents can also compromise the efficacy of some disinfectants (for example, quaternary ammonium compounds).

Given these different and sometimes poorly controllable factors, the quality assurance of chemical disinfection is usually not high. Chemical disinfection should only be used where no better means of decontamination (for example, autoclaving) can be applied.

Many chemical disinfectants can be harmful to humans and/or the environment. They should be selected, stored, handled, used and disposed of with care, following manufacturers' instructions and local legal/regulatory requirements.

When considering a disinfectant for use, the characteristics of its active ingredients will predict its likely activity. If a disinfectant is sold as a branded product, users should establish the active component(s). This can sometimes be difficult as brand names can cover a range of different products, each with different components or levels of those components. Users should know exactly what they are using and that what they are using will be effective as determined by risk assessment.

Table 2.3 Factors that can affect the effectiveness of chemical disinfection

FACTOR	REASON
Concentration	As illustrated with sodium hypochlorite, the amount of active compound available in a disinfectant is critical: too little active compound and decontamination is not achieved.
Organic matter	Disinfectants will react with living and non-living organic matter alike. If organic matter is present, it can inactivate the disinfectant before all the viable biological agents present have been eliminated. Solid organic matter can also inhibit the penetration of the disinfectant so it cannot reach its target. Specific organic materials are often defined in standard methods to indicate "clean" (low levels of contamination) and "dirty" (high levels of contamination) situations.
pH	Many chemical agents for decontamination are active only within a specific pH range. The manufacturer's information must be considered to keep the disinfectant within this range.
Contact time	Chemical disinfection is not immediate. Generally, the longer the disinfectant is in contact with the contamination from biological agents, the greater the microbial decontamination. Once the disinfectant dries, the disinfectant molecules can no longer migrate into their target. Rapid evaporation of a disinfectant applied to a surface can compromise efficient disinfection. Contact times used in test conditions should reflect those used in practice.
Contact	If objects to be disinfected are floating on the surface of a disinfectant, if air bubbles (for example, in tubing (hollow lumens)) prevent contact between the disinfectant and its target, or if application of a disinfectant to a surface does not give complete coverage, the disinfectant cannot be fully effective.
Range of microbiocidal activity	Not all disinfectants decontaminate all biological agents. Vegetative bacteria, fungi (including fungal spores), enveloped (lipid-containing or lipophilic) viruses and non-encysted protozoa tend to be readily susceptible to a wide range of disinfectants. Mycobacteria, non-enveloped viruses and encysted protozoa are less susceptible. Bacterial spores are resistant to some disinfectants and have variable sensitivity to others.
Temperature	In general, the higher the temperature, the more effective the disinfectant will be, the lower the temperature, the less effective the disinfectant action. This can be important in laboratory disinfection if refrigerators or cold rooms require disinfection, or where thermal treatment is also used, such as washer disinfectors used in some laboratories.
Shelf-life/stability	Chemical compounds may degrade over time, thus reducing the efficiency of the decontamination product. The degradation rate is often accelerated when the product is exposed to air or when the product is diluted. Typically, diluted sodium hypochlorite solutions become rapidly inefficient and work should be done with freshly prepared dilutions to achieve the desired effect.

2.3 Gaseous disinfection

In a limited number of situations – mainly in laboratories with maximum containment measures – the risk assessment determines that gaseous disinfectants (Figure 2.4), also known as fumigants, are required to decontaminate the laboratory space, furniture and/or equipment.

In situations where the laboratory has widespread contamination in difficult to access areas or where equipment needs to be taken out of a contaminated area or disinfected before maintenance, then gaseous disinfectants may be needed. Rooms and equipment can be decontaminated by fumigation with formaldehyde gas generated by heating paraformaldehyde or boiling formalin solutions.

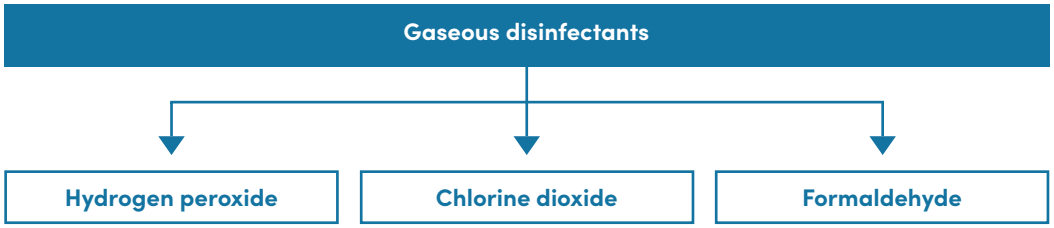

Figure 2.4 Types of gaseous disinfectants

This is a hazardous process that requires specially trained personnel and should be the last resort when risk assessment concludes that surface disinfection is impractical or inefficient. Ideally, a treated room should be sealable for fumigation but, at the very least, all openings in the room (windows, doors) should be sealed with gas impermeable tape before the formaldehyde gas is generated. Fumigation should be conducted at an ambient temperature of at least 20 °C and a relative humidity of more than 70%.

With all methods of fumigation, the area must be properly ventilated after fumigation before any personnel are allowed to enter. In most cases, the recognized/measured limits for workplace exposure to gaseous disinfectants are low. Therefore, in emergency situations, appropriate respiratory protective equipment, fit tested and with the correct filter fitment, must be worn by anyone entering the room before it has been ventilated. For more information on respiratory protective equipment, refer to *Monograph: personal protective equipment (5)*.

After a period of room aeration, a calibrated hand-held monitor or built-in device should be used wherever possible to check fumigant levels before re-entering the room. Ammonia, normally from sublimation of ammonium bicarbonate, can be used to neutralize formaldehyde fumigations before release of the fumigant. However, this step does not guarantee complete removal of the fumigant or negate the need to wear respiratory protective equipment when re-entering a room that is not fully aerated; toxic residual chemicals may still be present in the air. After formaldehyde fumigation, the walls and ceiling of the room may have to be wiped down with ammonia to neutralize any remaining paraformaldehyde.

Commercial fumigation systems using hydrogen peroxide or chlorine dioxide are also available. These systems have several advantages over formaldehyde in terms of safety, environmental protection, controllability of the fumigation process and speed but they are more expensive. Information on the use and effectiveness of commercial systems is widely available from the scientific literature.

2.3.1 Gaseous decontamination of BSC

Gaseous decontamination may be required on occasions such as when a BSC needs to be serviced *(21)*. These occasions should be specified in risk assessments and gaseous decontamination should not be carried out on a regular basis. To decontaminate a BSC, equipment is available that independently generates and circulates gaseous formaldehyde or vapour hydrogen peroxide. These procedures should only be carried out by suitably trained personnel. Gaseous decontamination should not be used on BSCs that recirculate air into the laboratory. More regular decontamination of BSCs should be carried out by wiping surfaces using validated surface disinfectants.

2.4 Heat disinfection

Heat is the most common physical method used for the decontamination of biological agents. Both dry and moist heat can be used (Figure 2.5). The contact of the biological agents with water is essential for steam sterilization, that is, the steam must reach all the surfaces or materials to be sterilized or disinfected. Moist heat is most effective when used in autoclaving. Autoclaving performed on pre-cleaned items in a well-maintained autoclave with a validated programmable cycle and interlocked doors is the gold standard for disinfection of solid wastes. However, for small items and loads, pressure cookers can also be used. Boiling does not necessarily remove all biological agents, but it may be used as the minimum processing for disinfection where other methods (chemical disinfection or decontamination and autoclaving) are not applicable or available.

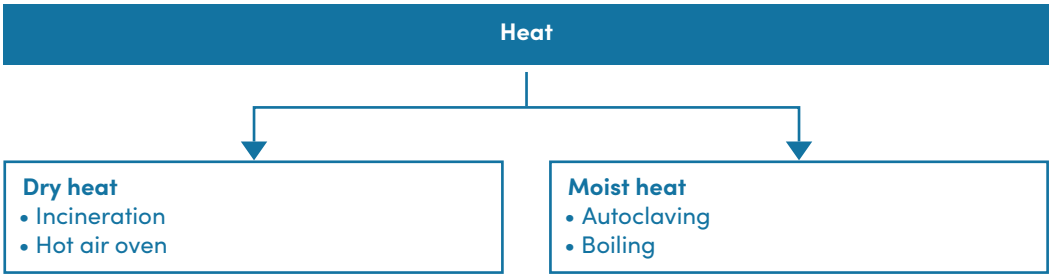

Figure 2.5 Methods of heat disinfection

Although dry heat can also be used for sterilization, much higher temperatures and longer periods are necessary. For example, dry heat in a hot air oven, which is totally non-corrosive, can be used to process many items that can withstand temperatures of 160 °C or more for 2–4 hours. Burning or incineration (see subsection 2.4.2 Incineration) is also a form of dry heat.

2.4.1 Autoclaving

Saturated steam under pressure (autoclaving) is the most effective and reliable means of decontaminating/sterilizing laboratory materials and wastes. This is because, once placed in the autoclave, treated items cannot be removed until the system has completed the cycle and the pressure and temperature within the chamber have returned to safe levels.

Autoclaves need to be able to process a wide range of materials and different types of loads which generally require different operating cycles (for example, porous loads, fluids, wrapped materials). Different cycles exist for solid and liquid material. In the risk assessment process autoclaves should be selected based on criteria defined by the user such as intended use, and type and amount of waste.

It is essential for effective decontamination by steam that all of the trapped air inside the material being autoclaved is removed and that sufficient time for decontamination and adequate means for steam penetration are provided. If these conditions are met, then autoclaving is a most reliable means of decontamination. There are two ways to remove air, passive and active.

Passive: Steam is either produced inside the autoclave at the bottom of the chamber or comes in at the top of the chamber and air is forced out of the chamber. This is the simpler and cheaper method, but it is only suitable for loads in which air removal is not impeded by fabrics or glassware.

Active: The chamber is subjected to successive pressure changes to draw air from the chamber. This is required for loads such as fabrics, glassware and other equipment where trapped air cannot reliably be removed by passive methods. The more difficult air is to remove, the more pulses will be required. This method is preferred for the decontamination of laboratory waste.

The various types of autoclave are described in Table 2.4.

Autoclave cycles

Autoclaving relies on two factors to decontaminate biological agents: time and temperature/pressure. These factors can be manipulated into different cycle procedures to sterilize various types of load, for example, bags, cages, animal bedding and other material. However, the cycle requirements for every load type can vary substantially.

As a general principle, materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags or containers need to be open enough to allow the steam to reach their contents. Biological or chemical indicators (see subsection 2.6 Biological and chemical indicators) are used to validate the autoclaving process and ensure effective decontamination of biological agents.

Table 2.4 Types of autoclave

TYPE OF AUTOCLAVE	CHARACTERISTICS
Gravity displacement autoclave	These autoclaves have a heating element fully or partially submerged in a pool of water at the bottom of the autoclave chamber. As the water in the pool is heated, it begins to evaporate, forming steam and compressing the air inside the chamber. Since steam is lighter than air, as the chamber fills with steam most of the air in the chamber is pushed to the bottom of the chamber and escapes through the fill hole, which is connected to a temperature-sensitive diaphragm that closes once it is sufficiently heated. Once the diaphragm closes pressure builds up inside the autoclave chamber.
Positive-pressure displacement autoclave	These autoclaves create the steam in a separate internal unit, called a steam generator. Once the amount of steam needed to displace air in the chamber is produced, a valve opens and a pressurized burst of steam enters the autoclave chamber. This system results in a higher percentage of air from the chamber being removed than with a gravity displacement autoclave, which decreases autoclave cycle times.
Fuel-heated pressure cooker autoclaves **Upward displacement autoclave (pressure cooker)**	These devices should only be used if a gravity displacement or vacuum-assisted autoclave is not available. They are loaded from the top and heated by gas, electricity or other types of fuel. 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 pre-set 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 lower before the lid is opened.
Pre-vacuum autoclaves **Negative-pressure or vacuum displacement autoclaves**	These autoclaves have a separate internal steam generator, as well as a vacuum pump. After the autoclave chamber is closed, the vacuum pump removes all air from the chamber, and steam is injected into the chamber. These autoclaves are able to provide among the highest sterility levels as long as air is removed from and steam enters all parts of the load, including hollow items and bagged items. Any bagged waste must have an opening at one end which is not tightly tied to allow air removal and steam entry. The air is removed through a valve which, based on a risk assessment, may be fitted with a HEPA filter.

HEPA = high-efficiency particulate air.

After a thorough risk assessment and validation, the following cycle will usually provide sterilization of correctly loaded autoclaves:

- 3 min holding time at 134 °C
- 10 min holding time at 126 °C
- 15 min holding time at 121 °C
- 25 min holding time at 115 °C.

Safety precautions

The following general safety precautions must be taken when using steam autoclaves.

- Operation and maintenance of autoclaves must be assigned to trained, competent individuals.
- Operating instructions for the autoclave must be available. Sterilization programmes with application area (for example, solids, liquids) and the parameter conditions to be maintained (temperature, pressure, time) must be defined.
- A loading plan (with information on the contents, number, volume and mass of the items/material to be sterilized) should also be available.
- A maintenance programme must be developed, including regular visual inspection of the chamber, door seals, gauges and controls, and the inspections must be done by qualified personnel.
- A regular verification process must be developed to ensure that the autoclave is functioning as designed, including the regular use of biological indicators and, for pre-vacuum autoclaves, Bowie-Dick and vacuum leak tests that confirm the correct removal of air in such autoclaves (see subsection 2.6.2 Chemical indicators).
- A reliable steam source must be used to provide appropriately saturated steam. The steam supply must be clean, to ensure materials are sterile after use, and free of chemicals which may inhibit the function of the autoclave or may damage the pipes or chamber of the autoclave.
- Materials placed in the autoclave must be in containers that readily allow removal of air and permit good steam penetration.
- The chamber of the autoclave must be loosely packed so that steam can penetrate evenly.
- Hazardous chemicals (for example, bleach, mercury or radioactive material) must never be treated in an autoclave.
- Operators must wear appropriate PPE including suitable gloves that provide thermal protection, protective clothing and eye and face protection when opening an autoclave, even when the temperature has fallen to levels considered safe for opening the autoclave door.
- Care should be taken to ensure that the relief valves and drains of autoclaves do not become blocked by paper, plastic or other materials included in the waste or materials for decontamination.

2.4.2 Incineration

Incineration is useful for disposing of animal carcasses as well as anatomical and other laboratory waste, with or without prior decontamination (see Section 2 Methods of decontamination). Incineration of infectious materials is an alternative to autoclaving only if the waste transport process to the incinerator is done in a controlled manner with trained personnel, suitable transport container and an SOP for the transport container loading procedure. Mobile and transportable incinerators are available for emergency and temporary facilities. All national and environmental legislation on incineration must be followed.

Effective incineration requires an efficient means of temperature control and a way to ensure complete combustion of all flammable materials. Many incinerators and other methods to burn waste, especially those with a single combustion chamber, may not be suitable 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 biological agents, toxic chemicals and smoke *(22)*. For incinerators with secondary chambers, 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 to the incinerator in leak-proof containers. Incinerator personnel should receive proper instructions about loading, manual handling and temperature control. It should also be noted that the efficient operation of an incinerator depends greatly on the type of materials (for example, organic material, plastic and paper/cardboard) in the waste being treated. If the wastes are transported in reusable containers, decontamination of the transport containers needs to be considered as well.

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

Burn pits and furnaces

Burn pits are a traditional way of open burning using a shallow depression that is sealed with clay, cement or concrete and items for incineration are thoroughly combusted to ash. Furnaces work in a similar way and may be an effective means to burn a range of waste. These methods can potentially expose operators at the pits to harmful combustion products and extremely high temperatures.

Disposal of incineration products (ashes)

The incineration process can concentrate potentially hazardous chemicals (for example, toxic metals and phosphate from carcasses) and incineration ash must be disposed of in compliance with national/local regulations. Autoclaved waste may be disposed of by off-site incineration or in licensed landfill sites.

2.5 Sterilization

Sterilization is used when a complete elimination of any biological agent, including spores and prions, is necessary; for example, for medical items and waste if the risk assessment indicates the need for very strict decontamination procedures.

Sterilization can be achieved using several decontamination methods such as autoclaving, certain chemical disinfectants and gaseous disinfection combined with a strict SOP, and irradiation. Important as the selected method is, the validation and adherence to the SOP to ensure the most efficient decontamination is equally important. To monitor the effectiveness of the sterilization process, biological indicators are used (Table 2.5).

2.6 Biological and chemical indicators

Indicators are routinely used to check and/or monitor the effectiveness of decontamination processes (cleaning, disinfection or sterilization). They include chemical, biological and sometimes mechanical/physical indicators.

2.6.1 Biological indicators

Biological indicators consist of a standardized population of microorganisms that provide a defined resistance to a specific sterilization process (Table 2.5). Non-pathogenic bacterial endospores are commonly used as test organisms as they are highly resistant to sterilization processes and easy to detect when cultured. If the spores are not decontaminated by the sterilization process, they will germinate and grow and eventually release dipicolinic acid that can be detected by a pH indicator dye present in the growth medium. With a longer incubation period, turbidity of the medium will also indicate bacterial growth. Various endospore-forming species will show different resistance patterns to the usual sterilization processes. *Geobacillus stearothermophilus* is used to test the efficacy of fumigation or autoclave treatments, whereas *B. subtilis*, *B. atrophaeus* or *B. pumilus* is preferred when verifying the completeness of dry heat or irradiation (Table 2.5).

Table 2.5 Sterilization processes and appropriate biological indicators

STERILIZATION PROCESS	BIOLOGICAL INDICATOR
Formaldehyde	*Geobacillus stearothermophilus*
Hydrogen peroxide	*G. stearothermophilus*
Moist heat	*G. stearothermophilus*
Dry heat	*Bacillus atrophaeus, B. subtilis*
Ionizing radiation	*B. pumilus*

2.6.2 Chemical indicators

Chemical indicators are widely used as they give an instant result. They check for specific direct parameters that are essential for disinfection or sterilization. These parameters include verification that a minimum concentration of a disinfectant has been used or that a specific condition has been reached in the autoclave. Chemical indicators also check for indirect variables that are important to the efficacy of the process; for example, the Bowie-Dick test that confirms the correct removal of air in pre-vacuum autoclaves.

There are six classes chemical indicators (Table 2.6). Depending on the parameters that need to be checked (for example, exposure control, autoclave performance, pack control monitoring, cycle monitoring), one or several indicators might be selected to gain insight into the decontamination process.

Table 2.6 Six classes of chemical indicators for decontamination processes

	TYPE 1	TYPE 2	TYPE 3	TYPE 4	TYPE 5	TYPE 6
Indicator type	Process indicators, "through-put indicators"	Indicators for the use in specific tests, "speciality indicators"	Single-variable indicators	Multivariable indicators	Integrating indicators	Emulgating indicators
What they indicate	Exposure of item to be sterilized to minimal process conditions: used to differentiate exposed from unexposed items	That a specific process is obtained which is linked to the sterilization process, for example air removal from a pre-vacuum steam sterilizer	Change in exposure to one parameter, for example temperature, time, concentration of a biocide	Change in exposure to at least two parameters, for example: -time and temperature for steam sterilization -time and concentration for ethylene oxide sterilization	Change in exposure to all critical parameters for a given process	Are specific for specified sterilization cycles The response of class six emulgating indicators does not necessarily correlate with a biological indicator
Use	Exposure control	Sterilizer performance	Pack control monitoring Exposure control	Pack control monitoring	Pack control monitoring Cycle monitoring tool	Pack control monitoring
Example	Autoclave tape	Bowie-Dick test, for example Dart® daily air removal test	Temperature tube with chemical pellet that melts at a specific temperature	Paper strips printed with a chemical indicator		

Specific indicators exist to monitor exposure to chemical disinfectants (formaldehyde, hydrogen peroxide and chlorine dioxide), to heat (steam and dry heat) or to monitor pressure variations.

SECTION 3

WASTE MANAGEMENT AND DECONTAMINATION OF WASTE PRODUCTS

3.1 Considerations for waste management

During laboratory activities, different contaminated materials and liquids will be generated (Table 3.1). Some of the materials such as glassware, equipment, devices or laboratory clothing may be reused or recycled. However, a large part of those materials will be disposed of as waste. The overriding principle is that all contaminated materials or liquids leaving the laboratory should either be treated onsite to allow further safe handling or packed and transported safely to another treatment site. Decontamination can be done chemically, by autoclaving or by incineration, but the method and the protocol must be based on a risk assessment and be properly validated.

Decontamination and final disposal are closely interrelated. Decontamination procedures and waste management are part of the risk assessment. More detailed information and relevant templates can be found in *Monograph: risk assessment (2)*.

Table 3.1 Examples of waste generated in laboratories

SHARPS	CONTAMINATED WASTE	CHEMICAL WASTE	NON-HAZARDOUS OR GENERAL WASTE
Needles, broken glass, Petri dishes, slides and cover slips, broken pipettes, syringes, scalpels	Blood and body fluids, microbiological cultures and stocks, tissue, infected animal carcasses, tubes and containers contaminated with blood or body fluids, liquid effluent	Fixatives; formaldehyde, xylene, toluene, methanol, methylene chloride and other solvents; broken laboratory thermometers	Uncontaminated packaging, paper, plastic containers

The following elements need to be considered when conducting a risk assessment for waste management:

- facilities and decontamination methods available,
- type and volume of waste (objects, materials, liquids),
- method of decontamination,
- segregation categories (uncontaminated, contaminated, sharps, glass),
- packaging, labelling and transport,
- presence of radioactive material,
- presence of chemicals, and
- recycling and reuse requirements.

All personnel handling contaminated materials need to be specially trained and must use appropriate PPE.

3.1.1 Contaminated waste treated onsite: segregation and storage

Contaminated and decontaminated materials including waste should be clearly distinguishable, labelled (by using different colour coding systems or by using the biohazard symbol) and stored or disposed of separately from each other.

The following elements need to be considered for segregation and storage:

- nature of the waste, for example, liquids, solids, general waste, infectious waste, chemical waste, sharps (contaminated or not) and perishables;
- volume of the waste to process;
- place where waste decontamination takes place (for example, in the laboratory itself, onsite, off-site);
- potential necessity to store waste before decontamination processing;
- kind and type of packaging for storing the waste (for example, bags, boxes, bins, tins, buckets; liquid-proof, puncture-proof, heat-resistant, chemicals-resistant, sealable);

- use of a coherent and consistent identification system:
  - colour codes for various waste categories
  - unambiguous informative labels
  - appropriate hazard symbols (for example, biohazard, radioactive, flammable);
- constraints for transferring material internally and for transporting it off-site;
- access to a restricted storage area before off-site transport;
- duration and conditions of storage (for example, short- or long-term storage, temperature control and ventilation requirements);
- possibility for regular cleaning and disinfection of the storage area.

Biohazard bags leaving the laboratory should be transported and stored in a safe way, by using a secondary container or a trolley or any other means that prevents contamination of the floor and walls of the storage site. Proper packaging methods ensure the safety of all personnel, from those at the laboratory to those at the site of decontamination, even if damage occurs during transit. For example, sharps should be collected in puncture-proof and impermeable containers that are difficult to break open after closure. A first-aid kit is essential and should be readily accessible. Depending on the temperature and the length of time the waste has to be stored, cooling may be required before treatment or disposal.

After decontamination treatment, it must be clearly visible to all personnel handling the waste that the items (for example, bins, bags, containers) that underwent a decontamination process do not represent an infectious risk anymore. For example, the biohazard symbol should be crossed out, removed or hidden (autoclaved bags may be put into a second non-transparent bag). Often, decontaminated waste is considered municipal waste and is transported and disposed of as such. However, sharps and other special waste (chemical, radioactive) must be disposed of as special waste in designated waste containers.

3.1.2 Contaminated waste treated off-site: handling and transportation

In many laboratories, infectious waste cannot be decontaminated where it is produced. It must therefore be collected and transported to an off-site facility for treatment and final disposal. Off-site treatment may also be needed when unusual amounts of contaminated waste accumulate (for example, after a major technical breakdown in the onsite decontamination systems or a disease outbreak) or when an unexpected source of contamination is discovered and material needs to be decontaminated before final disposal.

Ideally, the waste should be packed in United Nations (UN) certified containers and transported by a licensed contractor. The compliance of the contractor with the current regulations and guidelines should be checked (as part of the risk assessment process). The following information must be available to those transporting contaminated waste:

- waste classes, waste type,
- waste producer (for example, institution, laboratory),
- pick-up date,
- destination,
- driver's name and licence,
- number of containers and estimated volume, and
- a receipt of load received from a responsible person at the pick-up area.

When waste is decontaminated off-site, it must be packaged, labelled and transported according to national and international regulations. See subsection 3.1.4 Legislative, regulatory and policy considerations for additional guidance on applicable regulations. Packages containing solid waste should withstand a drop of one metre minimum without breaking open. Liquids should be ideally kept in small containers (up to 10 L) because this reduces the volume of liquid spread around in case of leakage, and small containers are much more easily handled. Secondary liquid retention containers (trays, tanks or large canisters) must be used. The transport vehicle should be equipped to carry waste in a closed or covered container which is securely fastened in place. The driver must be instructed on the procedure in case of an accident or incident during transportation on public roads. A spill kit containing absorbent materials, an effective disinfectant, heavy-duty reusable gloves, mask, apron, goggles, and a leak-proof waste disposal container should be in the vehicle.

3.1.3 Documentation for waste management

Keeping clear records of the wastes stored, their treatment methods and disposal dates is important to ensure good control of waste management. The following documentation should be gathered:

- list of the personnel authorized to handle the waste including their training records,
- SOP for waste handling (including internal transport, short-term storage and decontamination),
- validation records for decontamination,
- off-site transport records and final disposal records,

- database (paper or electronic) of the relevant material safety data sheets, and
- contingency plan, including for spill management emergency procedures.

In case of an emergency, certain information must be made available.

- WHAT is disposed of (for example, pipette tips, bottles, chemicals, animals)?
- WHAT special needs have to be considered?
- WHERE/HOW is different waste stored (for example, room temperature)?
- WHO is disposing of the waste and who trains the personnel?
- WHEN is training done (for example, how often – on demand, continuously)?
- WHO is responsible in case of an emergency?

Decontamination of waste products off-site uses the same chemical and physical methods described in the preceding subsection. The rigour of the conditions must be based on the risk assessment, but the end goal is decontamination, not strictly sterilization. Different methods are used for liquid and solid waste products (see subsection 3.2 Decontamination of liquid waste and subsection 3.3 Decontamination of solid waste).

3.1.4 Legislative, regulatory and policy considerations

The decontamination of biohazardous waste containing pathogenic biological agents generated within a laboratory may occur in the laboratory (for example, treatment of bacterial culture with bleach, autoclaving), in a designated decontamination area outside the laboratory (for example, centralized autoclaving area for the facility), or in an off-site facility by a third-party service for biohazardous waste management or disposal (for example, incineration, steam sterilization). It is the responsibility of the individual or the laboratory producing waste to ensure that it is safely and effectively decontaminated before release or removal from the laboratory, or that it is safely transported for off-site decontamination.

Biohazardous laboratory waste, human blood and bodily fluids are no longer considered biohazardous once they have been effectively decontaminated. However, they may still be considered hazardous waste if they contain hazards other than biological agents (for example, chemicals, sharps, radioactive material). There are international standards (for example, ISO 23907-1 Sharps injury protection) that apply to sharps waste that should be reflected in the waste management programme (for example, procedures for segregation of various materials). In addition, while human anatomical waste that has been effectively decontaminated is no longer a biohazard, sociocultural, religious, and aesthetic norms and practices generally influence the regulation of their disposal.

Additional waste management and waste treatment considerations or requirements may be specified by international, national, provincial or municipal authorities and organizations and should be consulted when establishing and implementing a waste management programme *(23,24)*. Laws, regulations, policies and guidelines that govern waste decontamination and disposal in a given jurisdiction may reflect regional differences, variations in local capacity and socioeconomic conditions. Guidelines and manuals, codes of professional conduct and advice shared between experienced personnel may also be available through professional organizations or institutions to supplement mandatory requirements.

Features of a waste management programme that are built on domestic and international requirements may include:

- definition of biohazardous waste,
- categories or types of waste,
- legal obligations of the producer of biohazardous waste for safe handling and disposal,
- record-keeping and reporting requirements, and
- need for authorization (for example, permits or licences) for waste treatment and handling systems.

International considerations

WHO's *Safe management of wastes from health-care activities: a practical guide*, second edition, provides recommendations for handling hazardous waste that can be considered in the waste management programme *(25)*.

International standards for certain waste decontamination methods also exist. For example, while there are no microbial standards for stack emissions (flue gases) from an incinerator, incinerators must comply with international requirements regarding the environment, for example, the Stockholm Convention on Persistent Organic Pollutants *(26)*. There are stringent international, and sometimes domestic, requirements on the efficiency of destruction and removal processes and the concentrations of substances that can be released into the atmosphere. Compliance with these requirements must be demonstrated for the incinerator used for hazardous waste. If third-party biohazardous waste management or disposal services are used, they may be requested by the laboratory producing the waste and contracting out the disposal to demonstrate how their process complies with international regulations.

Domestic considerations

In addition to international requirements, the waste management programme must take into consideration any applicable local laws, regulations, policies and guidelines. In some countries, local jurisdiction has site-specific requirements for waste storage to prevent the community from being exposed to hazardous waste. For example, to prevent access by scavengers and pests, local authorities may require that containers of biohazardous waste be stored in a secure location until transportation for off-site disposal.

Regulations on biohazardous waste can vary greatly from one region to another. For example, biohazardous waste entry into the general waste disposal system may not be permitted in some countries, even if the waste has been thoroughly and effectively decontaminated (for example, by disinfection or autoclaving). In such cases, the waste may have to undergo a decontamination process that handles the remaining hazards in the waste (for example, chemicals, radioactive material, sharps, anatomical waste).

It is also important to respect local guidelines that reflect regional differences, variations in local capacity and socioeconomic conditions. For example, it is important for the waste management programme to consider whether the local climate warrants additional procedures for waste storage (for example, storing waste in a cool location or on an elevated platform if there is a risk of flooding, or decontaminating waste as it is produced to avoid the need for storage).

Transportation of waste

When waste is decontaminated off-site, it must be packaged, labelled and transported according to national and international regulations. Whether transported within a region or exported for decontamination or disposal in another country, the transportation of biohazardous waste is governed by domestic and international requirements that are usually based on the UN Recommendations on the transport of dangerous goods: model regulations *(27)*.

Where there are no national regulations, responsible municipal authorities may refer to the UN recommendations. The transportation by air of biohazardous waste must comply with the International Civil Aviation Organization's Technical instructions for the safe transport of dangerous goods by air and the International Air Transport Association's (IATA) IATA dangerous goods regulations, both of which are based on the UN recommendations *(28,29)*.

In addition to international conventions and agreements, the movement of hazardous waste across an international border may be subject to regulation by the importing country, the exporting country or both *(30–32)*.

3.2 Decontamination of liquid waste

Disposal of liquid waste is an important issue for research facilities. Many methods are available, all with advantages and disadvantages that need to be evaluated in a risk assessment. The choice of method is driven by the chemical composition of the waste, the biological agents to be decontaminated and the initial and ongoing cost of each method. Methods for decontamination of liquid waste are outlined in the following subsections.

3.2.1 Sewer system

Based on the risk assessment, for low-risk specimens handled in a research or clinical facility attached to a technologically advanced municipal sewer system, direct emptying of liquid waste containers into the sanitary sewer may be an appropriate method of disposal. Although not technically a decontamination process, sanitary sewer systems attached to a waste treatment plant will adequately disinfect liquid waste before discharge into the environment. For biological agents or laboratory procedures that need heightened control measures or maximum containment measures (as determined by the risk assessment), this is not an acceptable option. In fact, even for less hazardous biological agents, this method may not be allowed under local or national regulations. Putting liquid biological waste in the sewage system mixed with chemicals (for example, ethanol, formaldehyde or guanidine hydrochloride) may also be prohibited. In all circumstance, strict adherence to local and national regulations is required.

3.2.2 Chemical disinfection

Most research laboratories prohibit the direct disposal of biological agents without any treatment; therefore, chemical disinfection has become a standard means of decontaminating solutions before disposal. The risk assessment helps to identify the most appropriate method for chemical disinfection of liquid waste.

The activity of the chemical disinfectant against the biological agent being handled in the laboratory is the first factor to consider when selecting a chemical disinfectant. Generally, pre-defined biological agents will be used for research, but a wider range of biological agents can be found in clinical specimens. Spores and prions require a more rigorous decontamination process before disposal. The organic load (amount of organic matter mixed with the biological agents) must be considered because most chemical disinfectants, including hypochlorites, are inactivated by organic matter.

The stability of ready-to-use disinfectants is important to consider as it affects how often the disinfectant needs to be changed. Consideration should also be given to the toxicity and corrosiveness of the disinfectant and the irritants in it. In addition, costs and shelf-life may need to be considered for sustainability reasons.

The most common chemicals used for decontamination of liquid waste are sodium hypochlorite, phenols and quaternary ammonium compounds.

Before the chemical disinfection of any solution, the other chemicals in the liquid must be taken into account in order to avoid the adverse effects of mixing incompatible chemicals. Guanidine thiocyanate is a common chaotropic agent (able to break hydrogen bonds) and is used to denature cells before nucleic acid isolation and sequencing or polymerase chain reaction. Mixing of a solution containing guanidine thiocyanate and sodium hypochlorite generates a toxic gas mixture of hydrochloric acid and hydrogen cyanide. The combination of formaldehyde solutions and sodium hypochlorite generates a mix of toxic gases, including hydrochloric acid, chlorine and formic acid. The combination of ethanol and solutions containing sodium hypochlorite generates chloroform.

3.2.3 Autoclaving

Autoclaving liquid wastes may be a preferred method of sterilization because the treated liquids can generally then be disposed of through the sewer system without concern of contaminating the environment with chemical disinfectants or their by-products. As noted in subsection 2.4.1 Autoclaving, the steam must come into direct contact with the solution to be disinfected, so the containers holding the liquid waste should not be sealed. The waste containers should be in a secondary containment receptacle, but this receptacle should not be taller than the waste containers in order to allow the replacement of air with steam. Either gravity displacement or pre-vacuum cycles may be used, but the autoclave should have a specific liquid cycle which slowly reduces the temperature and pressure after completion of a cycle in order to allow the waste liquids to slowly cool. The use of a cycle meant for dry goods will result in flash boiling of the waste liquids when their temperature exceeds the boiling point for the residual pressure in the autoclave. This will lead to an overflow of the waste containers and spreading of waste liquids into the secondary containment receptacle or onto the autoclave floor.

Autoclaving liquids that have been previously treated with a chemical disinfectant can also be hazardous. Solutions containing significant amounts of hypochlorite, ethanol or formaldehyde should not be autoclaved because they will become volatile and their concentration may exceed acceptable levels in the autoclave air when it is opened. Autoclaved solids that liquefy at temperatures found in an autoclave (for example, agar) require careful handling. If not fully contained in a secondary pan, these materials after liquefaction can drip from the bags they were placed in, collect around the drip pan and drain, and block the drain completely when the autoclave cools, resulting in a difficult and costly repair.

3.3 Decontamination of solid waste

For treatment of potentially infectious solid waste, chemical disinfection is rarely a preferred option because ensuring contact of the chemical with all surfaces is difficult, if not impossible. For most solids, autoclaving or incineration is the preferred method. For large animal carcasses, incineration or alkaline digestion, with rendering (see subsection 3.3.4 Rendering), is possible. Commercial systems that combine heating, shredding or mixing and/or alkaline digestion are available.

3.3.1 Autoclaving

Autoclaving solid wastes, as noted in subsection 2.4.1 Autoclaving, is a reliable way to sterilize biological agents in solid waste. However, a key concern is the need to ensure that all parts of the waste are exposed to the steam; pockets of trapped air act as insulation and may allow parts of the waste to remain potentially infectious. Ensuring this exposure is more difficult with irregular packaging of waste materials and if the solid waste contains liquids. Completely dry items such as gloves and gowning can trap pockets of dry air and therefore disinfection will not be achieved. Therefore, waste or materials placed in the autoclave must be in containers that readily allow removal of air and permit good steam/heat penetration. It is essential to validate operating conditions through the use of test specimens of dry waste and placement of biological indicators in this test waste. The need for additional water in the solid waste must be determined experimentally; some research has indicated the addition of more water may assist in disinfection under specific conditions *(33)*. In order to reach disinfecting temperatures throughout the solid waste, it is likely that successful disinfection will require longer cycles than are normally used for sterilizing materials. Large and bulky material, large animal carcasses, sealed heat-resistant containers and other waste that impedes the transfer of heat must be avoided.

Autoclave decontamination of animal carcasses is a particular challenge. Carcasses may be stored frozen until a sufficient number have been collected for decontamination. Regardless of the method of carcass thawing, the additional time needed to completely thaw all materials must be added to any cycle or processing time.

Moreover, experimental validation must be done in advance and on a routine basis to demonstrate that the appropriate temperature (usually 121 °C) is reached throughout all carcasses *(34)*. Ongoing demonstration of decontamination is necessary Successful decontamination may be verified either: by placing biological indicators in strategically-placed test specimens of waste interspersed in actual waste containers; or by using indicators attached to rods which can be inserted and retrieved without disturbing the contents of the waste containers.

3.3.2 Incineration

Incineration is a means to eliminate all known biological agents in solid waste, including spores and prions; ideally, incineration is performed in a technologically advanced incinerator. As noted in subsection 2.4.2 Incineration, correct temperature and length of time in the primary chamber are essential to ensure complete combustion of the solid waste and decontamination of any biological agent. In addition, incinerator operators need to be trained on the safe handling of the waste before loading it into the incinerator. If reusable containers are to be used, the means for disinfecting these containers needs to be established before starting incineration.

Aside from biological agents, two additional materials need to be considered when incinerating solid waste: plastic and soda lime glass. Most plastic used in research laboratories burns hotter than paper waste and can overheat an incinerator if the amount put in the incinerator is more than that recommended by the incinerator manufacturer. Soda lime glass melts at about 550 °C and will coat the refractory brick and reduce its lifespan; therefore, minimizing the incorporation of soda lime glass in the solid waste is important. The ash from soda lime glass incineration requires special handling and disposal as it may be enriched with heavy metals and phosphate.

3.3.3 Alkaline digestion

Alkaline digestion uses elevated temperature and pressure in the presence of alkali (1 N and above) to break down most cellular materials found in carcasses into soluble forms. The process can reduce carcasses to a soluble fraction and a calcium-rich solid residue. Alkaline digestion is effective in decontaminating nearly all known biological agents. Digestion at 150 °C in the presence of 1 N sodium hydroxide (NaOH) or potassium hydroxide (KOH) has been shown to make prions non-infectious *(35)*. The process requires a substantial amount of energy and time (several hours) and should only be used in institutions with a large number of carcasses (several kilograms a week).

3.3.4 Rendering

Rendering is another method to decontaminate animal carcasses, using steam at about 130 °C in a pressure vessel to break down carcasses into fat, protein and bone. The method is traditionally used with larger animals and is effective in eliminating most biological agents. However, rendering is ineffective against prions and should not be used for animals infected with prions.

SECTION 4

METHODS OF INACTIVATION

Decontamination processes are designed to make waste safe to dispose of, or to remove or destroy biological agents on laboratory surfaces. Inactivation processes are used in laboratories to make safe any material containing biological agents so it can be handled in a laboratory with less rigorous controls. Often the materials being inactivated contain high concentrations of the biological agents and may need a very effective method of treatment to ensure complete inactivation. Processes may also be needed to keep proteins, nucleic acids, and other biochemical compounds and structures stable for future analysis. Therefore, such processes need validation. There may be several reasons to remove infectious material from an area with higher risk control measures for later processing. The most common reason is the extraction of nucleic acids for nucleic acid amplification techniques such as polymerase chain reaction. For laboratory biosafety, the inactivation of biological agents reduces the risk for later work and makes it possible to work under the core requirements in most cases. The advantages of working under the core requirements include: a faster workflow, no need to decontaminate devices that are affected by chemical disinfectants, and cheaper maintenance of the workspace. Depending on the risk assessment, two different inactivation methods might be necessary to inactivate the biological agent.

The use of controls or biological indicators is recommended with inactivation methods. However, often validation of the inactivation method is only performed with the actual biological agent, and controls are used to monitor the efficacy of other processes combined with the inactivation method, such as the extraction of nucleic acids or cross contamination of specimens.

4.1 Inactivation of specimens

Inactivation of specimens is a pre-analytical treatment to remove/inactivate biological agents. There are also other reasons for inactivation of a specimen such as fixation and stopping reactions. Therefore, the choice of the inactivation method depends on the risk assessment and on the later experimental steps that will be taken.

In the risk assessment, liquid media separated from the specimen need to be considered because decontamination is usually different from that used to inactivate the specimen.

If disinfectants are used for decontamination purposes (spills), a positive control in the experiment could ensure that the specimens did not come in contact with the disinfectant. For most inactivation procedures, no control for the efficacy of the inactivation of the biological agent is done, but a control must be used in the initial validation of the inactivation procedure to demonstrate efficacy of the chosen method.

4.1.1 Nucleic acid extraction

When genetic techniques are used, a nucleic acid extraction step is often required to release the nucleic acid from the cell or virion so the nucleic acid is available for amplification. Efficient extraction is needed so as to give an accurate estimation of the amount of the biological agent. However, complete inactivation may be required for biosafety and biosecurity purposes. Inactivation of the biological agent will be particularly important if it is known to have a low infectious dose. For the purposes of polymerase chain reaction, inactivation is often done using lytic solutions that contain protein denaturants such as guanidine salts, or using direct heat, or both.

4.1.2 Thermal inactivation of slides

Microscope slides are frequently heated in clinical laboratories to bond or adhere the specimen to the glass before staining. For formalin-fixed paraffin-embedded tissue specimens, it is done to remove excess paraffin. The heat treatment is not an inactivation process, biological agents are already inactivated with the formalin fixation. Only tissue sections that may contain prions must be considered potentially contaminated even if further treated on a slide warmer or similar heating device.

For a number of clinical processes, including Gram staining for a variety of bacteria and preparing sputum specimens from patients potentially infected with *M. tuberculosis* for Ziehl–Neelsen staining, a key step is drying the specimen on a slide using a slide warmer. However, heat treatment may fail to completely inactivate *M. tuberculosis* unless 5% phenol is added to the smear *(36)*.

Although heat inactivation is likely to reduce the number of biological agents on a slide, it should not be assumed that heating to dryness is sufficient to inactivate all biological agents on a slide. Inactivation of all biological agents can only be assumed if: heating has been validated experimentally; the heating surface has been evaluated to ensure that the heat is applied evenly across the work surface; and a means to verify the temperature is available.

4.1.3 Formaldehyde treatment of tissue

Formaldehyde is widely used for the fixation of pathological and anatomical specimens. Even though this treatment is mainly to preserve the histological features of the tissue, formaldehyde also reliably inactivates biological agents (as mentioned in subsections 2.2.1 Types of disinfectant and 2.3 Gaseous disinfection).

Formaldehyde is used for tissue fixation as formalin, often as buffered formalin solution with 4% formaldehyde. Formaldehyde penetrates the tissue at about 1 mm per hour *(37)*; therefore, the incubation time of the tissue in formalin depends on the thickness of the specimen. Usually, no control is used for the formaldehyde fixation process as it is impractical and its use is based on validation of the SOP.

After the estimated incubation time, a cautious cut into the specimen can indicate, by observing the colour of the tissue, if longer incubation is necessary before safe handling of the specimen. The tissue is then processed into a formalin-fixed paraffin-embedded tissue specimen for microscopy and molecular analysis methods. Formaldehyde causes cross-linking of amino groups of proteins and nucleic acids, which results in formalin artefacts such as denatured proteins and damaged DNA. Therefore, later analyses are limited and special protocols (such as antigen-retrieval for antibody-based detection methods) are necessary *(38)*.

Formaldehyde is an irritant and carcinogen *(18,19)* and its use for analytical and medical purposes (as a fixative for histology or for embalming deceased people) is under evaluation.

4.1.4 Ionizing radiation

Ionizing radiation as a sterilization agent has significant advantages: this radiation is capable of inactivating biological agents through a closed package – direct physical contact with the biological agent is not needed – and it inactivates without leaving behind any residue (such as a chemicals) from the process. Since ionizing radiation easily penetrates all matter, it is capable of inactivating any type of biological agent, including spores. It is, however, generally expensive, requires specialized facilities and takes more time than most treatments.

The use of ionizing radiation is restricted to two roles: (i) inactivation of small quantities of biological agents for later structural or immunological analyses or for use as a vaccine; or (ii) sterilization of bulky items/materials, often medical supplies or devices that could be damaged by exposure to steam or chemical disinfectants.

Three commercial sources for ionizing radiation are available: gamma irradiators, X-ray machines and electron beam accelerators, also known as e-beams. They all inactivate biological agents by the generation of free radicals as the radiation interacts with matter. These free radicals create nucleic acid strand breaks and adducts, which are generally considered the critical impairments for inactivation. In general, radiation sensitivity is directly related to the size of the genetic material, with D10-values ranging from 0.2 kilogray (kGy) for *S.* Typhimurium to 13.0 kGy for foot and mouth virus *(39)*.

Gamma irradiators

Gamma irradiators generate high-energy photons through the decay of radioactive elements; cobalt-60 (60Co) or caesium-137 (137Cs) are the typical sources of gamma radiation. Cobalt irradiators have a shorter half-life (5.3 years versus 30 years for 137Cs) and therefore need to be recharged with fresh cobalt more often. However, cobalt irradiators provide more powerful gamma photons (two gamma rays of 1.17 and 1.33 mega electron volts (MeV) photon versus a 660 kilo electron volts (keV) photon for 137Cs), which allows decontamination of a greater mass.

The half-life of the radioactive element must be considered to ensure proper exposure of the material to be inactivated. Moreover, cobalt sources are metal and, for room-sized commercial irradiators used for sterilization of large quantities, can be submerged in a water pool to provide shielding; caesium sources are usually provided as a powered salt, so they cannot generally be used around water.

Theft of gamma radiation sources are a concern to national authorities because they could be used to spread radioactive contamination. Obtaining a gamma irradiator has become difficult and many national authorities are trying to replace them with X-ray machines.

X-ray machines

X-ray machines generate ionizing radiation by accelerating electrons into a metal target. The resulting interaction between the electrons and the target, which either knock out orbital electrons or alter the direction of the accelerated electrons, leads to the creation of X-ray photons. Unlike gamma irradiators, X-ray machines can be turned off and are thus inherently safer devices. However, the photon energy from an X-ray machine is generally much lower than a gamma irradiator. The lower energy may result in a lower dose rate, which means a longer exposure time is needed with an X-ray machine than a gamma irradiator.

The penetrating power of X-ray or gamma photons requires shielding – often concrete or steel – around the device and the material being irradiated. This shielding is needed to reduce the potential radiation exposure to below any regulatory limit set by a national authority. The national authority may also set exposure limits for personnel working with gamma irradiators or X-ray machines to ensure that they are not over-exposed to radiation, which may require the use of personal dosimeters to track the exposure of personnel.

E-beam machines

E-beam machines with energies of up to 10 MeV are commercially available. Similar to X-ray machines, removing the electrical power stops the production of electrons which makes them safer than gamma irradiators. Apart from the considerable cost, the main drawback of e-beams is the relatively short penetration of electrons in water or in materials of similar density (approximately 4 cm for a 6 MeV beam) *(40)*. The electrons interact with matter in the target directly to break atomic bonds and generate secondary ionizing particles, which result in inactivating the critical target (generally nucleic acids). In addition, materials with high atomic number, such as metals, must be kept out of the beam to prevent the formation of high-energy X-rays. Provided the use only of glass or plastic containers is strictly adhered to, an e-beam unit can be self-contained and may need no additional shielding beyond that provided by the manufacturer.

For each type of irradiator, the penetrating power of the radiation must be carefully calculated (radiation dose per min per cm of material) and a survival curve drawn for the organism in the same physical state as routinely used (for example, liquid, frozen, lyophilized). For vegetative bacteria, oxygen status can influence survival considerably and it must be similar in the validation studies and in routine use.

Adequate dosimetry (assessment of the ionizing radiation dose absorbed by an object) is also essential when using radiation as the inactivation method. Dosimetry can be a challenge because, for example, a dose at which there is a one in a million chance of a virus particle surviving in a solution containing 106 Ebola virus particles/mL can be in the order of 30 kGy *(41)*, far higher than the range of most chemical dosimeters or biological indicators, such as *B. pumilus* spores. Specialized dosimeters, using techniques such as alanine free radical dosimetry *(42)* or fluorescent film dosimeters *(43)* must be used.

Failure to remain within the limits established in the validation studies for total volume, oxygen concentration, concentration of the biological agent to be inactivated, physical state of the material (solid, liquid) and the state of the organism (vegetative versus spore) may result in organisms surviving the ionizing radiation procedure, with the potential for the release of contaminated materials.

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