



PART IV

Good microbiological techniques

12. Laboratory techniques

Human error, poor laboratory techniques and misuse of equipment cause the majority of laboratory injuries and work-related infections. This chapter provides a compendium of technical methods that are designed to avoid or minimize the most commonly reported problems of this nature.

Safe handling of specimens in the laboratory

Improper collection, transport and handling of specimens in the laboratory carry a risk of infection to the personnel involved.

Specimen containers

Specimen containers may be of glass or preferably plastic. They should be robust and should not leak when the cap or stopper is correctly applied. No material should remain on the outside of the container. Containers should be correctly labelled to facilitate identification. Specimen request or specification forms should not be wrapped around the containers but placed in separate, preferably waterproof envelopes.

Transport of specimens within the facility

To avoid accidental leakage or spillage, secondary containers, such as boxes, should be used, fitted with racks so that the specimen containers remain upright. The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated.

Receipt of specimens

Laboratories that receive large numbers of specimens should designate a particular room or area for this purpose.

Opening packages

Personnel who receive and unpack specimens should be aware of the potential health hazards involved, and should be trained to adopt standard precautions (2), particularly when dealing with broken or leaking containers. Primary specimen containers should be opened in a biological safety cabinet. Disinfectants should be available.

Use of pipettes and pipetting aids

1. A pipetting aid must always be used. Pipetting by mouth must be prohibited.
2. All pipettes should have cotton plugs to reduce contamination of pipetting devices.
3. Air should never be blown through a liquid containing infectious agents.
4. Infectious materials should not be mixed by alternate suction and expulsion through a pipette.
5. Liquids should not be forcibly expelled from pipettes.
6. Mark-to-mark pipettes are preferable to other types as they do not require expulsion of the last drop.
7. Contaminated pipettes should be completely submerged in a suitable disinfectant contained in an unbreakable container. They should be left in the disinfectant for the appropriate length of time before disposal.
8. A discard container for pipettes should be placed within the biological safety cabinet, not outside it.
9. Syringes fitted with hypodermic needles must not be used for pipetting.
10. Devices for opening septum-capped bottles that allow pipettes to be used and avoid the use of hypodermic needles and syringes should be used.
11. To avoid dispersion of infectious material dropped from a pipette, an absorbent material should be placed on the working surface; this should be disposed of as infectious waste after use.

Avoiding the dispersal of infectious materials

1. In order to avoid the premature shedding of their loads, microbiological transfer loops should have a diameter of 2–3 mm and be completely closed. The shanks should be not more than 6 cm in length to minimize vibration.
2. The risk of spatter of infectious material in an open Bunsen burner flame should be avoided by using an enclosed electric microincinerator to sterilize transfer loops. Disposable transfer loops, which do not need to be resterilized, are preferable.
3. Care should be taken when drying sputum samples, to avoid creating aerosols.
4. Discarded specimens and cultures for autoclaving and/or disposal should be placed in leakproof containers, e.g. laboratory discard bags. Tops should be secured (e.g. with autoclave tape) prior to disposal into waste containers.
5. Working areas must be decontaminated with a suitable disinfectant at the end of each work period.

For further information see reference (12).

Use of biological safety cabinets

1. The use and limitations of biological safety cabinets should be explained to all potential users (see Chapter 10), with reference to national standards and relevant literature. Written protocols or safety or operations manuals should be issued to staff. In particular, it must be made clear that the cabinet will not protect the operator from spillage, breakage or poor technique.

2. The cabinet must not be used unless it is working properly.
3. The glass viewing panel must not be opened when the cabinet is in use.
4. Apparatus and materials in the cabinet must be kept to a minimum. Air circulation at the rear plenum must not be blocked.
5. Bunsen burners must not be used in the cabinet. The heat produced will distort the airflow and may damage the filters. An electric microincinerator is permissible but sterile disposable transfer loops are better.
6. All work must be carried out in the middle or rear part of the working surface and be visible through the viewing panel.
7. Traffic behind the operator should be minimized.
8. The operator should not disturb the airflow by repeated removal and reintroduction of his or her arms.
9. Air grills must not be blocked with notes, pipettes or other materials, as this will disrupt the airflow causing potential contamination of the material and exposure of the operator.
10. The surface of the biological safety cabinet should be wiped using an appropriate disinfectant after work is completed and at the end of the day.
11. The cabinet fan should be run for at least 5 min before beginning work and after completion of work in the cabinet.
12. Paperwork should never be placed inside biological safety cabinets.

For further information about biological safety cabinets see Chapter 10.

Avoiding ingestion of infectious materials and contact with skin and eyes

1. Large particles and droplets ($> 5 \mu\text{m}$ in diameter) released during microbiological manipulations settle rapidly on bench surfaces and on the hands of the operator. Disposable gloves should be worn. Laboratory workers should avoid touching their mouth, eyes and face.
2. Food and drink must not be consumed or stored in the laboratory.
3. No articles should be placed in the mouth – pens, pencils, chewing gum – in the laboratory.
4. Cosmetics should not be applied in the laboratory.
5. The face, eyes and mouth should be shielded or otherwise protected during any operation that may result in the splashing of potentially infectious materials.

Avoiding injection of infectious materials

1. Accidental inoculation resulting from injury with broken or chipped glassware can be avoided through careful practices and procedures. Glassware should be replaced with plastic ware whenever possible.
2. Accidental injection may result from sharps injuries e.g. with hypodermic needles (needle-sticks), glass Pasteur pipettes, or broken glass.
3. Needle-stick injuries can be reduced by: (a) minimizing the use of syringes and needles (e.g. simple devices are available for opening septum-stoppered bottles so

- that pipettes can be used instead of syringes and needles; or (b) using engineered sharp safety devices when syringes and needles are necessary.
4. Needles should never be recapped. Disposable articles should be discarded into puncture-proof/puncture-resistant containers fitted with covers.
 5. Plastic Pasteur pipettes should replace those made of glass.

Separation of serum

1. Only properly trained staff should be employed for this work.
2. Gloves and eye and mucous membrane protection should be worn.
3. Splashes and aerosols can only be avoided or minimized by good laboratory technique. Blood and serum should be pipetted carefully, not poured. Pipetting by mouth must be forbidden.
4. After use, pipettes should be completely submerged in suitable disinfectant. They should remain in the disinfectant for the appropriate time before disposal or washing and sterilization for reuse.
5. Discarded specimen tubes containing blood clots, etc. (with caps replaced) should be placed in suitable leakproof containers for autoclaving and/or incineration.
6. Suitable disinfectants should be available for clean-up of splashes and spillages (see Chapter 14).

Use of centrifuges

1. Satisfactory mechanical performance is a prerequisite of microbiological safety in the use of laboratory centrifuges.
2. Centrifuges should be operated according to the manufacturer's instructions.
3. Centrifuges should be placed at such a level that workers can see into the bowl to place trunnions and buckets correctly.
4. Centrifuge tubes and specimen containers for use in the centrifuge should be made of thick-walled glass or preferably of plastic and should be inspected for defects before use.
5. Tubes and specimen containers should always be securely capped (screw-capped if possible) for centrifugation.
6. The buckets must be loaded, equilibrated, sealed and opened in a biological safety cabinet.
7. Buckets and trunnions should be paired by weight and, with tubes in place, correctly balanced.
8. The amount of space that should be left between the level of the fluid and the rim of the centrifuge tube should be given in manufacturer's instructions.
9. Distilled water or alcohol (propanol, 70%) should be used for balancing empty buckets. Saline or hypochlorite solutions should not be used as they corrode metals.
10. Sealable centrifuge buckets (safety cups) must be used for microorganisms in Risk Groups 3 and 4.
11. When using angle-head centrifuge rotors, care must be taken to ensure that the tube is not overloaded as it might leak.

12. The interior of the centrifuge bowl should be inspected daily for staining or soiling at the level of the rotor. If staining or soiling are evident then the centrifugation protocols should be re-evaluated.
13. Centrifuge rotors and buckets should be inspected daily for signs of corrosion and for hair-line cracks.
14. Buckets, rotors and centrifuge bowls should be decontaminated after each use.
15. After use, buckets should be stored in an inverted position to drain the balancing fluid.
16. Infectious airborne particles may be ejected when centrifuges are used. These particles travel at speeds too high to be retained by the cabinet airflow if the centrifuge is placed in a traditional open-fronted Class I or Class II biological safety cabinet. Enclosing centrifuges in Class III safety cabinets prevents emitted aerosols from dispersing widely. However, good centrifuge technique and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.

Use of homogenizers, shakers, blenders and sonicators

1. Domestic (kitchen) homogenizers should not be used in laboratories as they may leak or release aerosols. Laboratory blenders and stomachers are safer.
2. Caps and cups or bottles should be in good condition and free from flaws or distortion. Caps should be well-fitting and gaskets should be in good condition.
3. Pressure builds up in the vessel during the operation of homogenizers, shakers and sonicators. Aerosols containing infectious materials may escape from between the cap and the vessel. Plastic, in particular, polytetrafluoroethylene (PTFE) vessels are recommended because glass may break, releasing infectious material and possibly wounding the operator.
4. When in use, homogenizers, shakers and sonicators should be covered by a strong transparent plastic casing. This should be disinfected after use. Where possible, these machines should be operated, under their plastic covers, in a biological safety cabinet.
5. At the end of the operation the containers should be opened in a biological safety cabinet.
6. Hearing protection should be provided for people using sonicators.

Use of tissue grinders

1. Glass grinders should be held in absorbent material in a gloved hand. Plastic (PTFE) grinders are safer.
2. Tissue grinders should be operated and opened in a biological safety cabinet.

Care and use of refrigerators and freezers

1. Refrigerators, deep-freezers and solid carbon dioxide (dry-ice) chests should be defrosted and cleaned periodically, and any ampoules, tubes, etc. that have broken during storage removed. Face protection and heavy duty rubber gloves should be worn during cleaning. After cleaning, the inner surfaces of the cabinet should be disinfected.

2. All containers stored in refrigerators, etc. should be clearly labelled with the scientific name of the contents, the date stored and the name of the individual who stored them. Unlabelled and obsolete materials should be autoclaved and discarded.
3. An inventory must be maintained of the freezer's contents.
4. Flammable solutions must not be stored in a refrigerator unless it is explosion-proof. Notices to this effect should be placed on refrigerator doors.

Opening of ampoules containing lyophilized infectious materials

Care should be taken when ampoules of freeze-dried materials are opened, as the contents may be under reduced pressure and the sudden inrush of air may disperse some of the materials into the atmosphere. Ampoules should always be opened in a biological safety cabinet. The following procedures are recommended for opening ampoules.

1. First decontaminate the outer surface of the ampoule.
2. Make a file mark on the tube near to the middle of the cotton or cellulose plug, if present.
3. Hold the ampoule in alcohol-soaked cotton to protect hands before breaking it at a file scratch.
4. Remove the top gently and treat as contaminated material.
5. If the plug is still above the contents of the ampoule, remove it with sterile forceps.
6. Add liquid for resuspension slowly to the ampoule to avoid frothing.

Storage of ampoules containing infectious materials

Ampoules containing infectious materials should never be immersed in liquid nitrogen because cracked or imperfectly sealed ampoules may break or explode on removal. If very low temperatures are required, ampoules should be stored only in the gaseous phase above the liquid nitrogen. Otherwise, infectious materials should be stored in mechanical deep-freeze cabinets or on dry ice. Laboratory workers should wear eye and hand protection when removing ampoules from cold storage.

The outer surfaces of ampoules stored in these ways should be disinfected when the ampoules are removed from storage.

Standard precautions with blood and other body fluids, tissues and excreta

Standard precautions (which include “universal precautions” (19)) are designed to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources of infection (2).

Collection, labelling and transport of specimens

1. Standard precautions (2) should always be followed; gloves should be worn for all procedures.
2. Blood should be collected from patients and animals by trained staff.
3. For phlebotomies, conventional needle and syringe systems should be replaced by

single-use safety vacuum devices that allow the collection of blood directly into stoppered transport and/or culture tubes, automatically disabling the needle after use.

4. The tubes should be placed in adequate containers for transport to the laboratory (see Chapter 15 for transport requirements) and within the laboratory facility (see section on Transport of specimens within the facility in this chapter). Request forms should be placed in separate waterproof bags or envelopes.
5. Reception staff should **not** open these bags.

Opening specimen tubes and sampling contents

1. Specimen tubes should be opened in a biological safety cabinet.
2. Gloves must be worn. Eye and mucous membrane protection is also recommended (goggles or face shields).
3. Protective clothing should be supplemented with a plastic apron.
4. The stopper should be grasped through a piece of paper or gauze to prevent splashing.

Glass and “sharps”

1. Plastics should replace glass wherever possible. Only laboratory grade (borosilicate) glass should be used, and any article that is chipped or cracked should be discarded.
2. Hypodermic needles must not be used as pipettes (see also section on Avoiding injection of infectious materials in this chapter).

Films and smears for microscopy

Fixing and staining of blood, sputum and faecal samples for microscopy do not necessarily kill all organisms or viruses on the smears. These items should be handled with forceps, stored appropriately, and decontaminated and/or autoclaved before disposal.

Automated equipment (sonicators, vortex mixers)

1. Equipment should be of the closed type to avoid dispersion of droplets and aerosols.
2. Effluents should be collected in closed vessels for further autoclaving and/or disposal.
3. Equipment should be disinfected at the end of each session, following manufacturers' instructions.

Tissues

1. Formalin fixatives should be used.
2. Frozen sectioning should be avoided. When necessary, the cryostat should be shielded and the operator should wear a safety face shield. For decontamination, the temperature of the instrument should be raised to at least 20 °C.

Decontamination

Hypochlorites and high-level disinfectants are recommended for decontamination. Freshly prepared hypochlorite solutions should contain available chlorine at 1 g/l for general use and 5 g/l for blood spillages. Glutaraldehyde may be used for decontaminating surfaces (see Chapter 14).

Precautions with materials that may contain prions

Prions (also referred to as “slow viruses”) are associated with the transmissible spongiform encephalopathies (TSEs), notably Creutzfeldt-Jakob disease (CJD; including the new variant form), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia and kuru in humans; scrapie in sheep and goats; bovine spongiform encephalopathy (BSE) in cattle; and other transmissible encephalopathies of deer, elk and mink. Although CJD has been transmitted to humans, there appear to be no proven cases of laboratory-associated infections with any of these agents. Nevertheless, it is prudent to observe certain precautions in the handling of material from infected or potentially infected humans and animals.

The selection of a biosafety level for work with materials associated with TSEs will depend on the nature of the agent and the samples to be studied, and should be undertaken in consultation with national authorities. The highest concentrations of prions are found in central nervous system tissue. Animal studies suggest that it is likely that high concentrations of prions are also found in the spleen, thymus, lymph nodes and lung. Recent studies indicate that prions in lingual and skeletal muscle tissue may also present a potential infection risk (20–23).

As complete inactivation of prions is difficult to achieve, it is important to stress the use of disposable instruments whenever possible, and to use a disposable protective covering for the work surface of the biological safety cabinet.

The main precaution to be taken is to avoid ingestion of contaminated materials or puncture of the laboratory worker’s skin. The following additional precautions should be taken, as the agents are not killed by the normal processes of laboratory disinfection and sterilization.

1. The use of dedicated equipment, i.e. equipment not shared with other laboratories, is highly recommended.
2. Disposable laboratory protective clothing (gowns and aprons) and gloves must be worn (steel mesh gloves between rubber gloves for pathologists).
3. Use of disposable plastic ware, which can be treated and discarded as dry waste, is highly recommended.
4. Tissue processors should not be used because of the problems of disinfection. Jars and beakers (plastic) should be used instead.
5. All manipulations must be conducted in biological safety cabinets.
6. Great care should be exercised to avoid aerosol production, ingestion, and cuts and punctures of the skin.

7. Formalin-fixed tissues should be regarded as still infectious, even after prolonged exposure to formalin.
8. Histological samples containing prions are substantially inactivated after exposure to 96% formic acid for 1 h (24), (25).
9. Bench waste, including disposable gloves, gowns and aprons, should be autoclaved using a porous load steam sterilizer at 134–137 °C for a single cycle of 18 min, or six successive cycles of 3 min each, followed by incineration.
10. Non-disposable instruments, including steel mesh gloves, must be collected for decontamination.
11. Infectious liquid waste contaminated with prions should be treated with sodium hypochlorite containing available chlorine at 20 g/l (2%) (final concentration) for 1 h.
12. Paraformaldehyde vaporization procedures do not diminish prion titres and prions are resistant to ultraviolet irradiation. However, the cabinets must continue to be decontaminated by standard methods (i.e. formaldehyde gas) to inactivate other agents that may be present.
13. Prion-contaminated biological safety cabinets and other surfaces can be decontaminated with sodium hypochlorite containing available chlorine at 20 g/l (2%) for 1 h.
14. High-efficiency particulate air (HEPA) filters should be incinerated at a minimum temperature of 1000 °C after removal. Recommended additional steps prior to incineration include:
 - a. spraying of the exposed face of the filter with lacquer hairspray prior to removal,
 - b. “bagging” of filters during removal, and
 - c. removal of the HEPA filter from the working chamber so that the inaccessible plenum of the cabinet is not contaminated.
15. Instruments should be soaked in sodium hypochlorite containing available chlorine at 20 g/l (2%) for 1 h and then rinsed well in water before autoclaving.
16. Instruments that cannot be autoclaved can be cleaned by repeated wetting with sodium hypochlorite containing available chlorine at 20 g/l (2%) over a 1-h period. Appropriate washing to remove residual sodium hypochlorite is required.

For further information on the handling of unconventional agents see references (12), (26) and (27).

13. Contingency plans and emergency procedures

Every laboratory that works with infective microorganisms should institute safety precautions appropriate to the hazard of the organisms and the animals being handled.

A written contingency plan for dealing with laboratory and animal facility accidents is a necessity in any facility that works with or stores Risk Group 3 or 4 microorganisms (containment laboratory – Biosafety Level 3 and maximum containment laboratory – Biosafety Level 4). National and/or local health authorities should be involved in the development of the emergency preparedness plan.

Contingency plan

The contingency plan should provide operational procedures for:

1. Precautions against natural disasters, e.g. fire, flood, earthquake and explosion
2. Biohazard risk assessment
3. Incident-exposure management and decontamination
4. Emergency evacuation of people and animals from the premises
5. Emergency medical treatment of exposed and injured persons
6. Medical surveillance of exposed persons
7. Clinical management of exposed persons
8. Epidemiological investigation
9. Post-incident continuation of operations.

In the development of this plan the following items should be considered for inclusion:

1. Identification of high-risk organisms
2. Location of high-risk areas, e.g. laboratories, storage areas, animal facilities
3. Identification of at-risk personnel and populations
4. Identification of responsible personnel and their duties, e.g. biosafety officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists, and fire and police services
5. Lists of treatment and isolation facilities that can receive exposed or infected persons
6. Transport of exposed or infected persons
7. Lists of sources of immune serum, vaccines, drugs, special equipment and supplies
8. Provision of emergency equipment, e.g. protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies.

Emergency procedures for microbiological laboratories

Puncture wounds, cuts and abrasions

The affected individual should remove protective clothing, wash the hands and any affected area(s), apply an appropriate skin disinfectant, and seek medical attention as necessary. The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

Ingestion of potentially infectious material

Protective clothing should be removed and medical attention sought. Identification of the material ingested and circumstances of the incident should be reported, and appropriate and complete medical records kept.

Potentially infectious aerosol release (outside a biological safety cabinet)

All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice. The laboratory supervisor and the biosafety officer should be informed at once. No one should enter the room for an appropriate amount of time (e.g. 1 h), to allow aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entrance should be delayed (e.g. for 24 h).

Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed, supervised by the biosafety officer. Appropriate protective clothing and respiratory protection should be worn.

Broken containers and spilled infectious substances

Broken containers contaminated with infectious substances and spilled infectious substances should be covered with a cloth or paper towels. Disinfectant should then be poured over these and left for the appropriate amount of time. The cloth or paper towels and the broken material can then be cleared away; glass fragments should be handled with forceps. The contaminated area should then be swabbed with disinfectant. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant. Cloths, paper towels and swabs used for cleaning up should be placed in a contaminated-waste container. Gloves should be worn for all these procedures.

If laboratory forms or other printed or written matter are contaminated, the information should be copied onto another form and the original discarded into the contaminated-waste container.

Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets

If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 min) to allow settling. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately

and left closed (e.g. for 30 min). In both instances, the biosafety officer should be informed.

Strong (e.g. thick rubber) gloves, covered if necessary with suitable disposable gloves, should be worn for all subsequent operations. Forceps, or cotton held in the forceps, should be used to retrieve glass debris.

All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a noncorrosive disinfectant known to be active against the organisms concerned (see Chapter 14). Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.

The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried. All materials used in the clean-up should be treated as infectious waste.

Breakage of tubes inside sealable buckets (safety cups)

All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved. Alternatively, the safety cup may be chemically disinfected.

Fire and natural disasters

Fire and other services should be involved in the development of emergency preparedness plans. They should be told in advance which rooms contain potentially infectious materials. It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents.

After a natural disaster, local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings. They should enter only when accompanied by a trained laboratory worker. Infectious materials should be collected in leakproof boxes or strong disposable bags.

Salvage or final disposal should be determined by biosafety staff on the basis of local ordinances.

Emergency services: whom to contact

The telephone numbers and addresses of the following should be prominently displayed in the facility:

1. The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)
2. Director of the institution or laboratory
3. Laboratory supervisor
4. Biosafety officer
5. Fire services
6. Hospitals/ambulance services/medical staff (names of individual clinics, departments, and/or medical staff, if possible)

7. Police
8. Medical officer
9. Responsible technician
10. Water, gas and electricity services.

Emergency equipment

The following emergency equipment must be available:

1. First-aid kit, including universal and special antidotes
2. Appropriate fire extinguishers, fire blankets

The following are also suggested but may be varied according to local circumstances:

1. Full protective clothing (one-piece coveralls, gloves and head covering – for incidents involving microorganisms in Risk Groups 3 and 4)
2. Full-face respirators with appropriate chemical and particulate filter canisters
3. Room disinfection apparatus, e.g. sprays and formaldehyde vaporizers
4. Stretcher
5. Tools, e.g. hammers, axes, spanners, screwdrivers, ladders, ropes
6. Hazard area demarcation equipment and notices.

For further information see references (12) and (28).

14. Disinfection and sterilization

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

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

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

Definitions

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

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

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

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

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

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

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

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

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

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

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

Cleaning laboratory materials

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

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

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

Chemical germicides

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

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

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

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

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

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

Table 12. Recommended dilutions of chlorine-releasing compounds

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

^a After removal of bulk material.

^b For flooding, e.g. on blood or before removal of bulk material.

^c See text.

Chlorine (sodium hypochlorite)

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

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

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

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

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

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

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

Sodium dichloroisocyanurate

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

Chloramines

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

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

Chlorine dioxide

Chlorine dioxide (ClO_2) is a strong and fast-acting germicide, disinfectant agent and oxidizer, often reported to be active at concentrations levels lower than those needed by chlorine as bleach. Chlorine dioxide is unstable as a gas and will undergo decomposition into chlorine gas (Cl_2), oxygen gas (O_2), giving off heat. However, chlorine dioxide is soluble in water and stable in an aqueous solution. Chlorine dioxide can be obtained in two ways: (1) on-site generation by mixing of two separate components, hydrochloric acid (HCl) and sodium chlorite (NaClO_2); and (2) ordering its stabilized form, which is then activated on-site when required.

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

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

Formaldehyde

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

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

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

Glutaraldehyde

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

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

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

Phenolic compounds

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

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

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

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

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

Quaternary ammonium compounds

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

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

Alcohols

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

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

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

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

Iodine and iodophors

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

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

Hydrogen peroxide and peracids

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

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

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

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

Local environmental decontamination

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

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

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

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

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

Decontamination of biological safety cabinets

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

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

Hand-washing/hand decontamination

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

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

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

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

Heat disinfection and sterilization

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

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

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

Autoclaving

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

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

Examples of different autoclaves include the following.

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

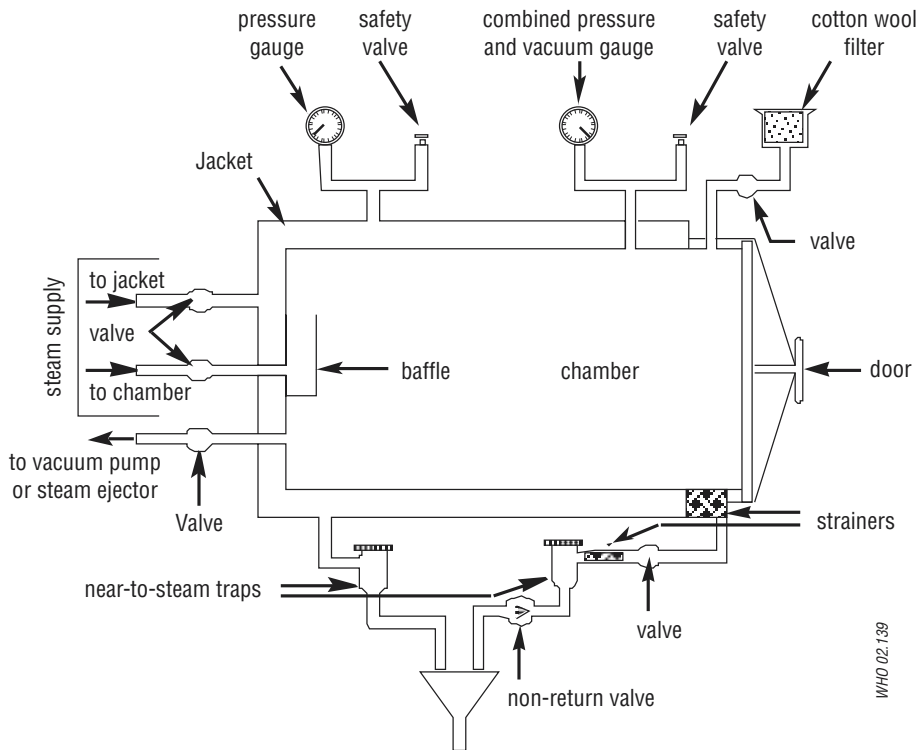


Figure 10. **Gravity displacement autoclave**

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

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

Loading autoclaves

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

Precautions in the use of autoclaves

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

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

Incineration

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

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

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

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

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

Disposal

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

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

15. Introduction to the transport of infectious substances

Transport of infectious and potentially infectious materials is subject to strict national and international regulations. These regulations describe the proper use of packaging materials, as well as other shipping requirements.

Laboratory personnel must ship infectious substances according to applicable transport regulations. Compliance with the rules will:

1. Reduce the likelihood that packages will be damaged and leak, and thereby
2. Reduce the exposures resulting in possible infections
3. Improve the efficiency of package delivery.

International transport regulations

The regulations for the transport of infectious materials (by any mode of transport) are based upon the United Nations Model *Regulations on the Transport of Dangerous Goods* (40). These recommendations are developed by the United Nations Committee of Experts on the Transport of Dangerous Goods (UNCETDG). To become legally binding, the United Nations Model Regulations have to be introduced into national regulations and international modal regulations by the competent authorities (e.g. the *Technical Instructions for the Safe Transport of Dangerous Goods by Air* (41) of the International Civil Aviation Organization (ICAO) for air transport and the *European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR)* (42).

The International Air Transport Association (IATA) issues *Infectious Substances Shipping Guidelines* (43) every year. IATA guidelines must follow ICAO's *Technical Instructions* as a minimal standard, but may impose additional restrictions. IATA guidelines must be followed if a shipment is carried by members of IATA.

Since the United Nations *Model Regulations on the Transport of Dangerous Goods* is a dynamic set of recommendations subject to biennial amendments, the reader is referred to the latest issuances of national and international modal regulations for applicable regulatory texts.

WHO serves in an advisory capacity to UNCETDG. Major changes to the transport regulations pertaining to the transport of infectious substances were introduced into the 13th edition (2003) of the United Nations *Model Regulations* (40). Guidance on the background to adopted amendments is available from WHO (44).

International modal regulations are not intended to supersede any local or national requirements. However, in situations where national requirements do not exist, international modal regulations should be followed.

It is important to note that international transport of infectious substances is also dependent on and subject to national import/export regulations.

The basic triple packaging system

The triple packaging system, the choice for the transport of infectious and potentially infectious substances, is exemplified in Figure 11. This packaging system consists of three layers: the primary receptacle, the secondary packaging and the outer packaging.

The primary receptacle containing the specimen must be watertight, leakproof and appropriately labelled as to content. The primary receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage or leakage.

A second watertight, leakproof packaging is used to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in a single secondary packaging. Volume and/or weight limits for packaged infectious substances are included in certain regulatory texts.

The third layer protects the secondary packaging from physical damage while in transit. Specimen data forms, letters and other types of information that identify or describe the specimen and identify the shipper and receiver, and any other documentation required, must also be provided according to latest regulations.

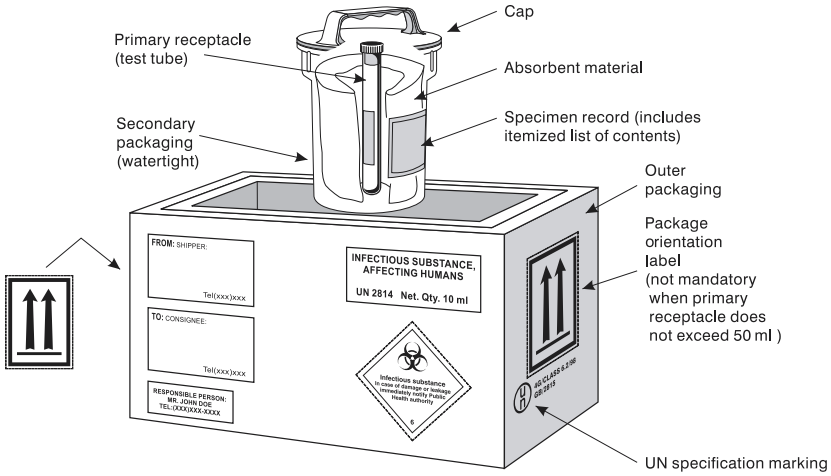
The United Nations *Model Regulations* prescribe the use of two different triple packaging systems. The basic triple packaging system applies for the transport of a variety of infectious substances; however, high-risk organisms must be shipped according to more stringent requirements. For further details about the use of the different packagings according to the materials to be transported, it is advisable to consult national and/or international modal regulations for applicable regulatory texts.

Spill clean-up procedure

In the event of a spill of infectious or potentially infectious material, the following spill clean-up procedure should be used.

1. Wear gloves and protective clothing, including face and eye protection if indicated.
2. Cover the spill with cloth or paper towels to contain it.
3. Pour an appropriate disinfectant over the paper towels and the immediately surrounding area (generally, 5% bleach solutions are appropriate; but for spills on aircraft, quaternary ammonium disinfectants should be used).
4. Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the centre.
5. After the appropriate amount of time (e.g. 30 min), clear away the materials. If there is broken glass or other sharps involved, use a dustpan or a piece of stiff cardboard to collect the material and deposit it into a puncture-resistant container for disposal.

Packing and labelling of Category A infectious substances



Packing and labelling of Category B infectious substances

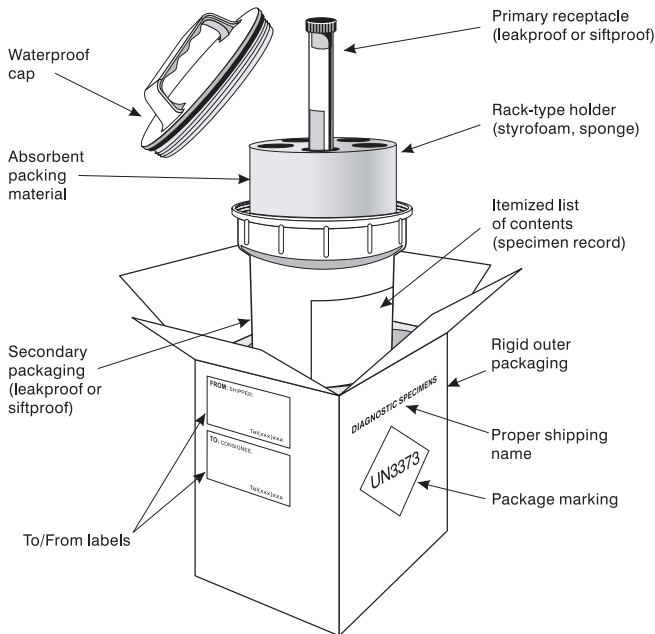


Figure 11. **Examples of triple packaging systems**
(graphics kindly provided by IATA, Montreal, Canada)

6. Clean and disinfect the area of the spillage (if necessary, repeat steps 2–5).
7. Dispose of contaminated materials into a leakproof, puncture-resistant waste disposal container.
8. After successful disinfection, inform the competent authority that the site has now been decontaminated