# WHS G016 (interim) Guidelines for the Decontamination of Clinical/Biological Waste and Spill Management

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## 1. Decontamination - General Information

Decontamination is carried out to prevent the spread and escape of pathogens or other biological materials, which may cause disease in humans or animals or which could cause damage to the environment.   
  
All Clinical and Biological waste and waste containing Genetically Modified Organisms (GMOs) should be decontaminated prior to disposal.

This also includes wastes contaminated with, or potentially contaminated with Risk Group 1 and Risk Group 2 microorganisms (wastes contaminated with Risk Group 3 or 4 microorganisms are beyond the scope of these guidelines and should not be conducted at UNE, for queries on RG3 & 4 waste contact the biological safety officer or WHS@une.edu.au).

Waste in this category includes:

* **Laboratory and associated waste**

These are materials and items directly involved in specimen processing, examples include:

* + - all specimens used for laboratory testing;
    - cultures or suspensions of micro-organisms in tissue culture;
    - used Petri dishes;
    - culture bottles;
    - disposable equipment,
    - used gloves etc.
* **Human tissues** including materials or solutions that contain free-flowing or expressible blood.
* **Animal tissue** or carcasses that are contaminated or suspected to be contaminated by pathogenic organisms.
* **Other infectious or pathogenic material e.g. fungi, bacteria, viruses, prions and their cultures that could cause harm to humans, animals, plants or the environment**

## 2. Methods for the Decontamination of Clinical and Biological Waste

The two most common methods of decontamination are use of pressure steam sterilisers (autoclaves) and use of liquid chemicals (germicides, disinfectants).

### 2.1 Pressure steam sterilization (autoclaving)

**(See AS/NZS2243.3 Section 10.6 for more detail)**

#### 2.1.1 General

Autoclaves operate at high temperatures and pressures and are and are useful pieces of equipment for decontaminating microorganisms providing they are used correctly.  
  
Persons using an autoclave must be trained to ensure they understand:

* the correct preparation of items being autoclaved, to ensure effective sterilisation or decontamination (e.g. ensuring autoclave bags are used, effective steam penetration etc.)
* the correct loading of the autoclave which is essential to ensure sterilisation or decontamination of the load, which should be done according to the manufacturer’s instructions and taking into consideration the type of materials or waste being processed,
* the correct labelling and segregation of waste to ensure tracking and accountability, and
* the hazards associated with heat, steam and pressure,
* use of correct PPE, i.e. operators must be provided with protective clothing, including heat-insulating gloves, for use when unloading the autoclave. Use of a face shield and apron is also recommended.

#### 2.1.2 Considerations for choosing an appropriate autoclave

It is important to ensure the proper conditions for load sterilisation are produced in the autoclave chamber. To ensure effective sterilization or decontamination, steam must come into contact with the materials that need to be sterilized, as such air is removed from the chamber prior to the sterilization cycle commencing. This is achieved via two main methods which also relates to the type of autoclave being used:

1. Downward displacement – this is where air is displaced by steam.

To ensure steam can contact the materials to be sterilised, all the air must first be removed from the load and the autoclave chamber. This is achieved by using a vacuum pump before the sterilisation stage (pre-vacuum method). Downward displacement autoclaves are suitable for sterilization of media and fluids.

1. Pre-vacuum – where air is evacuated from the chamber prior to the sterilsation cycle. This type of autoclave is suitable for porous loads and large empty containers. It is less suitable for vessels containing fluids.

***Autoclaves for Infectious waste***

It is important to note, that when using autoclaves to sterilize clinical, pathogenic or infectious waste, or waste that may be perceived as infectious, the autoclave used must have the ability to sterilize the air (and any water) that is displaced or evacuated from the chamber prior to the sterilization cycle. This is achieved by either by trapping the air and sterilizing it along with the sterilization cycle, or by in-line 0.22um filters installed on all vents. Not all autoclaves are fitted with these functions, as many autoclaves are used in industry to sterilize ‘clean’ media.

Failure to use the correct autoclave when sterilizing infectious waste may mean that infectious aerosols are vented from the chamber into the room housing the autoclave, and could potentially expose nearby personnel to the infectious substance.

#### ****2.1.3 Sterilisation parameters****

***Preparing the waste***

As recommended by AS/NZS 2243.3 2010, autoclave bags should be carefully opened prior to commencement of the autoclave cycle, without disturbing the contents, to allow the penetration of steam and displacement of air. However you must ensure that with infectious material that a suitable autoclave is used (see section 2.1.2).  
  
***Transportation of waste***

Wastes being transported from a facility to the autoclave must be wholly contained within a primary sealed container (e.g. an autoclave bag) and the primary sealed container must be packed in a secondary sealed unbreakable container (e.g. unbreakable plastic container with a sealable lid or a garbage bin with a sealable lid). The secondary container must be able to be easily decontaminated. Transport routes should be planned to minimise possible exposure to the wastes by consideration of activity levels and population densities at various times of the day and places on the routes.   
  
***Spills***

Appropriate chemical disinfectants must be provided in the vicinity of the autoclave to assist in the clean-up of any spills outside the autoclave. Easy access to hand washing facilities, safety showers and eyewash facilities must also be provided.

***Sterilisation cycle***  
Parameters must be considered for each type of load. The minimum requirement is for all parts of the load to reach the correct temperature and contact time, which is:

1. 15 min at 121°and 103kPa; or
2. 3 min at 134°C and 203kPa

However, the duration normally needs to be extended depending on the size of the load and the density (or porosity) of the load – the larger and more dense loads requiring a longer time to achieve penetration to the center of the loads. It is recommended that for loads that are regularly processed, that a test-run be conducted involving a biological indicator. You may find that your regular load may need to be run for 30 minutes at 121°and 103kPa. The autoclave manufacturer should be able to help you to set the parameters required for regular autoclave cycles.

Furthermore, some biologicals, e.g. prions, are much more resilient and therefore require different parameters. See the section on [prion decontamination](#3).

#### ****2.1.4 Monitoring and verification of sterilisation cycles****

Autoclaves have a requirement under AS/NZS 2243.3 to undergo regular maintenance and calibration.

It is also important that each sterilisation cycle is verified, and to distinguish between waste that has been autoclaved and that which has not. This is achieved by:

* ***Visual indicators,*** e.g. sensitive tapes

Should be attached to each item being autoclaved. Such visual indicators are used to check that materials have been processed, but do not monitor the efficacy of the sterilisation procedure.

* ***Chemical indicators***

These progressively change colour with the time exposed at specific temperatures, and their use is recommended with each cycle, as they give an immediate indication of the efficacy of treatment.

* ***Biological indicators*** e.g. spore strips or tubes, or bacterial enzyme indicators

Should be used monthly to monitor the microbial killing power of the sterilisation process. They should be placed in several positions in a load, including those least likely to attain sterilisation conditions.

2.2 Chemical disinfection

Chemical disinfectants have a range of properties, and no single one is effective in all situations. A number of factors should be considered in choosing a chemical for decontamination. Micro-organisms vary in their resistance to chemical agents. Viruses containing lipids and vegetative forms of many fungi and bacteria are usually susceptible to chemical attack, whereas spores of micro-organisms tend to be quite resistant. Prions are extremely resistant to chemical disinfection. Refer to section on [prion decontamination](#3) for further detail.

To choose an appropriate disinfectant, account should be taken of the identity of the micro-organisms, their concentration and the degree of inactivation you wish to achieve. Physical factors such as whether a space or surface is to be decontaminated, the type of surface, and any interaction between the material and potential disinfectants will also be factors in the selection. Time available for disinfection and the time required for particular disinfectants to be effective need to be considered.   
  
The effectiveness of a disinfectant depends not only on the properties of the micro-organisms against which it is used, but also upon factors in the environment in which it is to be used. Factors that may affect the action of chemical disinfectants include the following:

* the concentration of the chemical in the disinfectant solution;
* the temperature;
* pH;
* Relative humidity of the environment;
* The presence of organic matter (which inactivates some chemicals);
* Duration of contact.

Some disinfectant solutions need to be regularly prepared as fresh solutions to avoid growth of micro-organisms in the solution and to ensure optimum activity of the disinfectant chemical. For optimum contact with the chemical it is advisable to clean surfaces and equipment first to reduce organic matter which might interfere with the disinfection process.  
  
Many disinfectants have toxic effects ranging from irritation of the skin and mucous membranes to carcinogenesis, and some have physical properties that make them dangerous to handle and use. These properties should be taken into consideration when selecting a disinfectant for a particular use. A Safety Data Sheet (SDS) should be available for all disinfectants to be used and should be read and understood by all persons with access to the disinfectants.

#### ****2.2.1 Chemical disinfectants for biological wastes****

**You should consult AS/NZS 2243.3 2010 Appendix F – Tables F1 and F2 to choose the correct chemical disinfectant for the biological that you need to disinfect against.** Table F1 provides recommended applications for chemical disinfectants in microbiological facilities. Table F2 provides a guide to the effectiveness of different classes of disinfectant against a range of microorganisms.  
  
The four most common chemicals that can be used for the decontamination of clinical and biological waste are as follows:

* **Alcohols e.g.** 80% v/v ethyl alcohol (ethanol) or 60-70% v/v isopropanol   
    
  These are most useful for cleaning surfaces (and may be used in disinfecting gloves/hands).

They are also active against vegetative bacteria and lipid containing viruses, but are inactive against spores. They are ineffective against Mycobacterium species and HIV dried on surfaces in the presence of sputum or serum.

Alcohols are unsuitable for application to proteinaceous material as they tend to coagulate and precipitate surface proteins which may then result in protection of the microorganisms present.

Alcohols are flammable, so they should not be used near flames or sparks. Because of their volatility, alcohol disinfectants should be used sparingly in biological safety cabinets and not with equipment that is likely to cause sparks. Alcohol disinfectants may be used from a dispensing bottle, but should never be sprayed as a mist.

They must be made up correctly, i.e. volume/volume not weight/volume and not weight/weight (as water and alcohol have different densities). Percentages greater than 70% are more likely to be flammable. Percentages less than 70% are less likely to be effective. Those greater than 90% (e.g. ‘absolute’) are less likely to be effective as they evaporate too quickly thus not meeting the minimum contact time required for disinfection.

* **Chlorine** e.g. sodium hypochlorite (bleaches)  
    
  **Chlorine** in the form of sodium hypochlorite or other chlorine releasing compounds is active against vegetative forms of bacteria and viruses and is the preferred disinfectant for HIV and hepatitis viruses. It is less effective against spores.

**It is the chlorine in these compounds that is the effective disinfecting agent. Some household ‘bleaches’ have *not been stabilized* and as such the ‘free-chlorine’ evaporates over time, making the solution less effective. This is also the case in diluted solutions. If using chlorine solution for laboratories, it is recommended that you purchase laboratory or hospital grade chlorine solution that has been stabilized.**

For effective disinfection, a pH range of 6-8 is optimum with a concentration of 5000‑1000ppm of free chlorine (0.5-1% sodium hypochlorite), with a contact time of at least 10 minutes. As the effective strength of chlorine solutions decreases on storage, working solutions should be freshly prepared daily.

Hypochlorite solutions are corrosive to stainless steel and other metal surfaces and tend to bleach and damage fabrics.

* **Quaternary ammonium compounds**   
    
  **A**re effective against Gram-positive and lipid-containing viruses e.g. herpes and influenza, but are less active against Gram-negative bacteria and non-lipid-containing viruses and are inactive against Mycobacterium species and bacterial spores.

These solutions are cationic detergents with powerful surface-active properties. They are inactivated by proteins, soap and anionic detergents, and are not recommended as general disinfectants. However they are most useful for cleaning of floors.

* **Chlorhexidine**   
    
  **Chlorhexidine** used as chlorhexidine gluconate in 70% alcohol - is a useful skin antiseptic that is active against HIV and Gram-positive organisms. Chlorhexidine is ineffective against non-lipid containing viruses and sporulating bacteria. It is not compatible with soap or anionic detergents. It is recommended that hand wash containing chlorhexidine is used in facilities where microorganisms are cultured or used.

In addition to the four chemicals described above, Iodine, Formaldehyde, Glutaraldehyde as well as Phenolic compounds, can also be used for decontaminating Clinical and Biological waste. These chemicals, however, have some major disadvantages over the four most commonly used chemicals. For further information on the use of these chemicals as disinfectants, refer to AS/NZS 2243.3 2010.

## 3. Decontamination method for Prion Contaminated Material

Prions are infectious agents that produce slow, progressive and fatal diseases of the central nervous system. Prions are the causative agents for a number of degenerative brain diseases, including scrapie (a fatal disease of sheep and goats), mad cow disease, Creutzfeldt Jacob disease (CJD) and Gertsmann-Straeussler-Scheinker (GSS) disease.  
  
Prions are resistant to most traditional methods of inactivation used for other microorganisms. Because of the difficulties in inactivating the infectivity, prions pose particular problems in the laboratory. Prions should only be handled in dedicated laboratories using dedicated equipment.  
  
***Sterilisation of articles or specimens potentially contaminated with prions***

Current recommendations for the sterilisation of articles or specimens that could be contaminated by prions are:

* At least 18 minutes at 134°C to 138°C in a pre-vacuum pressure steam steriliser or
* 1 hour at 132°C in a downward displacement pressure steam sterilizer, or
* 20 000 ppm (2% sodium hypochlorite) available chlorine for 1 hour with sodium hypochlorite as the chlorine releasing agent.

***Sterilisation of heat resistant instruments***

Current recommendations for decontaminating heat-resistant instruments that may be contaminated by prions involves a combination of pressure steam sterilising and chemical treatment. This can be either:

* Immerse in 1M sodium hydroxide **and** heat in a downward displacement autoclave at 121°C for 30 minutes, clean, rinse in water then subject to routine sterilisation (refer to pressure steam sterilising section); or
* Immerse in 1M sodium hydroxide or 20 000 ppm sodium hypochlorite for 1 hour, transfer instruments to water, heat in a downward displacement autoclave at 121°C for 1 hour, clean and subject to routine sterilisation (refer to pressure steam sterilising section).

Infectivity is strongly stabilised by drying or fixing, so contaminated material should be kept wet between the time of use and disinfection. Formalin-fixed and paraffin-embedded tissues, particularly of the brain, remain infectious for long periods, if not indefinitely. They should be assumed to remain infectious through the processes of embedding, sectioning, staining and mounting on slides. The most effective chemical treatment for decontaminating formalin‑fixed tissue is 96% formic acid for 1 hour.  
  
4. When and What to Decontaminate

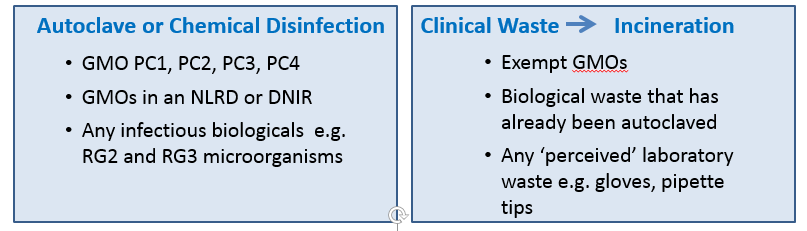
**Lab-benches, surfaces, equipment**

* + Your bench or workstation should be decontaminated before you start work and after you have finished your work. 80% v/v Ethanol is commonly used but you should use the correct disinfectant according to the organism used (i.e. bleach or Virkon may be more suitable see AS/NS 2243.3).
  + Equipment must be decontaminated before repair or maintenance and before it leaves the facility.

**Personnel / Workers**

* + Hands must be decontaminated after handling cultures and before using read/write areas in the lab, and before exiting the lab.
  + The options for decontaminating your hands are:
    - Wash your hands at a wash basin fitted with hands-free taps, and use handwash containing disinfectant such as chlorhexidine, OR
    - Hands-free dispenser containing a gel or emollient containing a disinfectant such as ethanol or isopropyl alcohol
  + Lab coats should be laundered regularly, e.g. on a weekly basis. If contaminated with infectious material or a GMO, then they must be immediately treated with bleach or autoclaved before laundering**.**

5. Summary of Biological Waste Streams



## 6. Spill Management

Spill kits should be available in all laboratories where clinical/biological material is stored or handled. Spill kits should also be available where clinical/biological waste is stored awaiting decontamination and disposal.

The contents of the spill kit should be appropriate for the type, nature and amount of material that could be spilled in an area. Spill kits should contain a means to collect and contain wastes, disinfectant or neutralising agent and personal protective equipment including gloves, laboratory coat/apron (can be disposable) and safety glasses. Materials used to clean up a spill (such as paper towel) should be decontaminated and disposed of according to the type of clinical/biological material cleaned up.

Refer to **AS2243.3:2010 Safety in laboratories – Part 3: Microbiological safety and containment, Section 9, Microbiological Spills** for more guidance on spill management. The following is a suggested spills procedure that may be adapted to suit your facility.

**Spill Clean-up Procedure**

1. Notify all persons within the facility of the spill
2. If spill is more than 1litre, place a DO NOT ENTER sign in the entrance door to the laboratory and alert the laboratory manager or supervisor immediately
3. Tell the laboratory manager/ supervisor of any hazardous information of the material involved in the spill.
4. For large volume or high risk spills do not enter the room for 30 minutes to allow aerosols to settle
5. Inspect people who were within the facility for visible signs of contamination
   1. If contamination is on lab coat only, change lab coats immediately, placing the contaminated lab coat in a biohazard bag for decontamination.
   2. If contamination is on hands and arms, gloves are to be removed and hands thoroughly washed with chlorhexidine handwash (e.g. Hibiclens or other) and dried.
   3. If contamination is through lab coat, and clothing, then lab manager/ supervisor/ safety coordinator is to be called immediately for further action.
6. Wear safety goggles and 2 pairs of gloves, and remove spill kit supplies from storage container (bio hazard bags, dustpan etc.).
7. If applicable, using the dust pan, pick up any contaminated sharp items (needles, broken glass etc.) and place them in a contaminated sharps container for disposal.
8. Cover the spill with absorbent material such as vermiculite or paper towel or absorbent pads.
9. Remove the absorbent material by using a dust pan and deposit in a biohazard bag along with the dust pan.
10. Spray the area with appropriate disinfectant (F10, Virkon, bleach or 70% ethanol) and allow at least 10 minutes contact time.
11. Remove any residual disinfectant with paper towels. Dispose towels in the biohazard bag.
12. Treat any contaminated equipment, and utensils.
    1. Spray with 70% ethanol and allow a 5 minute contact time.
    2. Remove contamination by wiping down with absorbent material and repeat steps a. and b.
    3. Dispose of used absorbent material in a biohazard bag.
13. Remove gloves only and dispose in the biohazard bag.
14. Wash your hands with Hibiclens / chlorhexidine handwash or similar and dry thoroughly.
15. Return spill kit, and report incident, making sure the spill kit is restocked.

## 5. References

AS/NZS 3816 - 1998: Australian/New Zealand Standard Management of clinical and related wastes  
  
Australian/New Zealand Standard 2243.3 2010 - Safety in Laboratories – Part 3: Microbiological safety and containment  
  
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