University of Otago

Laboratory Biohazard Waste Disposal Guidelines

Approved by the IBSC on the 25th January, 2011

1. Scope

These guidelines are intended to cover biohazardous wastes originating in laboratory areas at the University of Otago, including (but not restricted to) those laboratories that are registered as part of the University of Otago Containment and Transitional Facility for Microorganisms and Uncleared Biological Products.

Biohazardous wastes originating from such areas must be decontaminated in accordance with the requirements of the following standard;

> **AS/NZS2243.3:2002** (Safety in laboratories, Part 3: Microbiological aspects and containment facilities).

The purpose of these guidelines is to provide guidance on managing the treatment and/or disposal of biohazardous wastes in a manner that is safe and complies with the requirements of the above standard, but these guidelines **DO NOT COVER** the disposal of the following;

i) Human cadavers or body parts.

ii) Biological wastes that are not biohazardous and which are not specifically required to be treated as such by any New Zealand legislation, Standard or project approval.

iii) Any wastes originating from specific facilities or projects which are subject to disposal requirements other than those specified in AS/NZS2243.3:2002 (for example, disposal requirements specified in an Import Health Standard or as containment controls in an ERMA/IBSC project approval).[1]

[1] The Sector Manager responsible for such facilities or projects must ensure disposal procedures meet the requirements of any applicable standards or project approval controls.

2. Definition

A biohazard is any material that may contain an infectious agent or other hazardous biological material that presents a potential risk to the health of humans, animals or the environment[2]. In practice, this means that all of the following wastes must be treated as biohazardous;

i) Any waste that may be contaminated with pathogenic microorganisms, including; human blood and body fluids, cultures of pathogenic microorganisms and carcasses of infected laboratory animals.

ii) Any waste contaminated with organisms or biological materials that have not been approved for release outside of a Containment or Transitional Facility (including the University of Otago Containment and Transitional Facility for Microorganisms and Uncleared Biological Products), including; any genetically modified organisms, imported uncleared biologicals, animal cell cultures, unwanted organisms, carcasses of laboratory animals infected with genetically modified organisms or which contain genetically modified cells or tissue (e.g. as a graft or implant) and any other 'risk goods'.

iii) Any obviously laboratory-related waste that originates from a PC2 laboratory, particularly disposable lab-ware such as used gloves, pipette tips, petri dishes, plastic syringes and tubes, irrespective of whether the waste is known to be contaminated or not (unless the waste is known to be non-biohazardous, but is contaminated with a hazardous substance or radioactive material and is subject to other specific disposal requirements for these hazardous wastes)[3]. Note that there are specific requirements for the disposal of non-hazardous laboratory-related waste from PC1 laboratories and these are detailed in Section 6.5.

[2] Note that certain wastes are specifically required to be treated as biohazardous under various New Zealand standards. Examples that commonly occur at the University of Otago are listed in Appendix 1.

[3] Note that this requirement is to minimise the risk of any material contaminated with a biohazard from a PC2 laboratory being accidentally disposed into the general waste stream. In addition, cleaners are trained not to touch or empty waste bins that contain obviously laboratory-related material (this is again to minimise the risk of contaminated waste being accidentally disposed of into the general waste stream, as well as to protect the health and safety of cleaning personnel).

3. Responsibilities

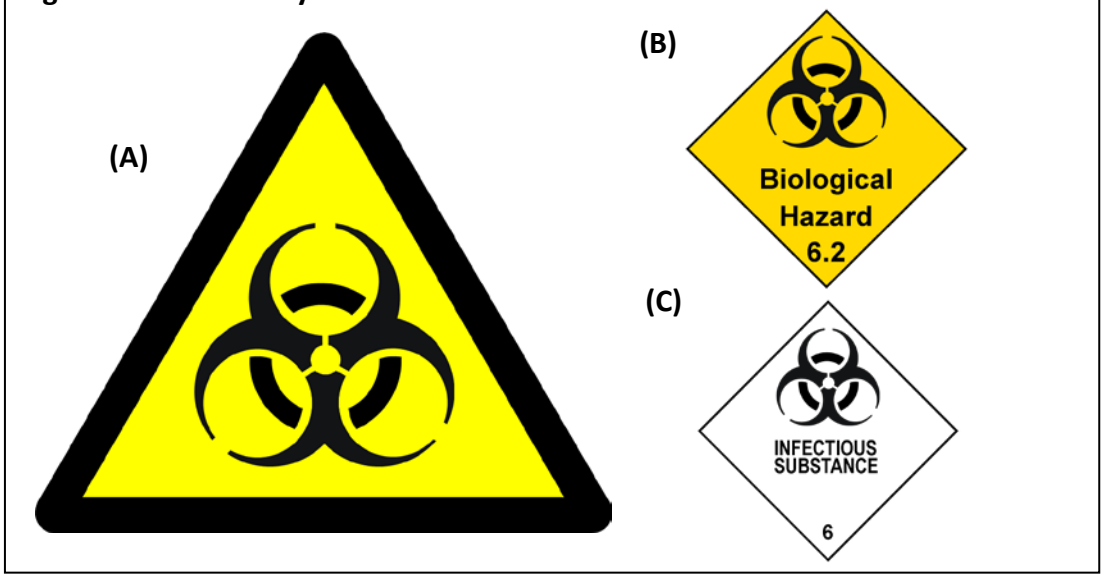
Departments which generate biohazardous wastes MUST have disposal procedures in place which meet the requirements of these guidelines and anyone working with biohazardous material must receive training in these procedures. Anyone generating a biohazardous waste is responsible for disposing of this in accordance with the procedures that apply in their area. Responsibility for ensuring appropriate procedures are in place lies with either the Sector Manager, for wastes originating from laboratories that are part of a MAF-audited Containment or Transitional Facility, or with the Departmental Laboratory Manager (DLM), for wastes originating from PC2 Clinical Laboratories where human blood and body fluids are handled.

4. Labelling

All containers of biohazardous waste (including bins used to contain biohazard bags) MUST be clearly labelled with a Biohazard warning symbol (Figure 1, page 5). Note that (A) is the biohazard warning symbol specified for internal facility use in both AS/NZ2243.3:2002 and NZS4304:2002, while (B) is a MAF approved warning symbol (and is required to be used on door signs of PC1 and PC2 laboratories). Either (A) or (B) may be used for labelling fridges, freezers, bins and other containers or areas that contain biohazardous materials.

Symbol (C) is the official UN Transport of Dangerous Goods pictogram for Infectious Substances (Class 6.2) but in general this should not be used for internal facility use, with the exception of items that are specifically designed for the transport of biohazardous materials (including biohazard bags and bins).

Figure 1. Biohazard symbols



5. Approved external contractor

Some biohazard waste disposal procedures require waste to be removed for decontamination by an '*approved external contractor*'. Currently, the approved external contractor for all biohazardous wastes is Interwaste Ltd. For any queries relating to the services provided by Interwaste Ltd (including pricing) please contact the Custodial Services Manager at Property Services.

6. Segregation and disposal

The appropriate disposal procedure for biohazardous waste depends on the type of waste and as a result all biohazardous waste must be segregated into one of the following types;

i) Sharps, e.g. contaminated needles, scalpel blades, microscope slides, blood tubes.
➔ Refer SECTION 6.1

ii) Solid, e.g. contaminated gloves, paper towels, agar, plasticware.
➔Refer SECTION 6.2

iii) Liquid, e.g. cultures, trap waste, used media.
➔Refer SECTION 6.3

iv) Animal (vertebrate) carcasses
➔Refer SECTION 6.4

v) Non-hazardous laboratory-related waste from PC1 laboratories
➔Refer SECTION 6.5

vi) All other biohazardous wastes
➔Refer SECTION 12

Wastes that contain hazardous substances (i.e. substances with a HSNO classification) or are contaminated with radioactive materials **MUST NOT** be disposed of with biohazardous waste, except where this is specifically permitted in any relevant guidelines for the hazardous substance/radioactive material involved.

All practical steps should be taken to avoid mixing biohazardous waste with other hazardous wastes (e.g. toxic, radioactive). Where the generation of mixed wastes is unavoidable the Biological Compliance Officer **MUST** be consulted to determine the appropriate disposal method **BEFORE** the waste is generated.

6.1 Sharps

Biohazardous sharps waste includes any of the following;

i) Any potentially biohazardous items that could break or puncture the skin, e.g. needles, scalpel blades, glassware[4].

ii) Any potentially biohazardous items that could break or puncture a biohazard bag e.g. large items of rigid plasticware.

All biohazardous sharps must be decontaminated off-site by the approved external contractor. Sharps waste must be discarded directly into approved sharps containers (labelled with both the biohazard symbol and the word '*Sharps*'). These can be obtained from the approved external contractor and come in a variety of sizes. Sharps containers are also available with 'flip-top' lids (see Figure 2, page 7) or plain lids. Flip-top lids are recommended where bins are used for disposal of small sharps such as needles and scalpels, but plain lids may be more practical for larger items e.g. plastic pipettes, glassware.

Biohazard sharps containers should be filled no higher than 2/3 full or to the indicator line on the outside of the bucket, if one is present (see Figure 3, page 7). The container should then be sealed and labelled with the department/area the waste originates from (for laboratory waste, also label with the lab group/room number). Store containers in a secure area (refer Section 9) until collection by the approved external contractor.

Sharps **MUST NEVER** be placed into biohazard bags as they may puncture the bag, potentially resulting in spillage of biohazardous material and/or injury.

DO NOT attempt to empty the contents of one biohazard sharps bucket into another.

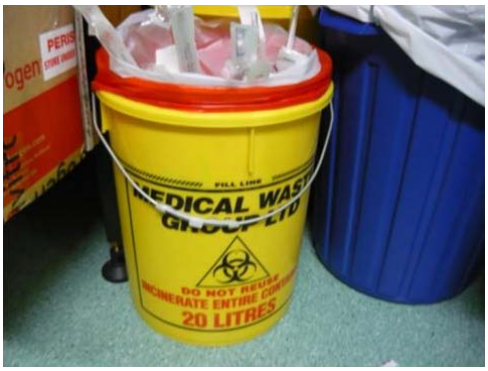
On-site decontamination of sharps waste **IS NOT** permitted.

[4] Including intact glassware, as this can easily break and create a sharps hazard.

Figure 2 – Sharps container with 'flip-top' lid

Figure 3 – Correct and incorrect filling of biohazard sharps containers

Good - Bucket should be filled no higher than this indicator line (red arrow).



Bad – contents are overflowing from the bucket and the lid cannot be closed.

6.2 Solids (non-sharp)

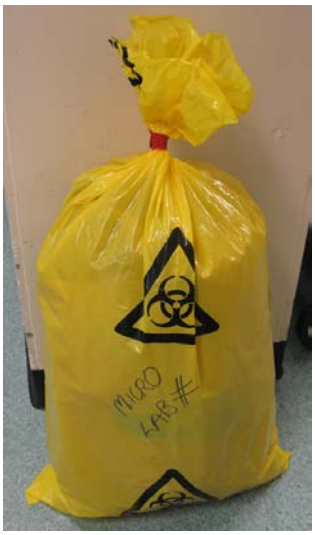
Biohazardous solid waste typically consists of items such as contaminated gloves, paper towels, plasticware and agar. Small volumes of liquid waste may also be included provided that any liquids are sealed in plastic tubes (e.g. microfuge tubes, falcons) of no greater than 50 mL volume and that the total aggregate volume per biohazard bag/bin does not exceed 500 mL.

Biohazardous solid waste may be disposed of either by decontamination off-site by the approved external contractor or on-site by autoclave sterilisation.

6.2.1 Biohazard bags (off-site decontamination)

Where this method of disposal is used, solid waste **MUST** be placed into a yellow biohazard bag that is lined with a paper kleensac (this helps prevent puncturing or tearing of the plastic bag) [5]. When in use (i.e. the bag is open) the biohazard bag and liner should be contained within a solid-sided bin with a lid (these bins may also be used to transport waste through non-laboratory areas). Biohazard bags must be filled no higher than 2/3 full (see Figure 4, below), sealed with a cable tie or tape, then labelled with the department/area the waste originates from (for laboratory waste, also label with the lab group/room number). Store biohazard bags in a secure area (refer Section 9) until collection by the approved external contractor.

Figure 4 – Correct and incorrect filling of biohazard bags



✓ **Good – Bag 2/3 full**

✗ **Bad - Bag is over full**

6.2.2 Biohazard bags (on-site decontamination)

Only specifically approved autoclaves (designated by the Sector Manager or DLM) may be used for decontamination of solid biohazard waste and this option may not be provided in all areas. Where this method of disposal is used, solid waste must be disposed of in either autoclave bags or yellow biohazard bags. Users should take care not to place intact biohazard warning labels into these bags as these will enter the general waste stream intact (unlike commercially treated bags, which are shredded following sterilisation).

Autoclave/Biohazard bags: Follow the guidelines for disposal of waste into Biohazard bags as detailed in section 6.2.1, with the exception that the bags should be sealed with clips and

[5] Solid waste may also be disposed into biohazard sharps containers, in accordance with Section 6.1, although this is a more expensive disposal option.

these should be removed just prior to autoclaving. Autoclave sterilization **MUST** be carried out in an approved autoclave in accordance with the guidelines in Section 7. Following autoclaving, check that no biohazard labels on the exterior of the bag remain legible. If any labels remain these must be defaced prior to disposal (they will usually be destroyed during the autoclave process). The bags may then be disposed of into the general waste stream (e.g. rubbish skip).

6.3 Liquid waste

Liquid waste should be further segregated into either "high organic load" (as a guideline, $>10^5$ cells/mL) , e.g. microorganism/cell` cultures, blood and body fluids, or "low organic load waste" (as a guideline, $<10^5$ cells/mL), e.g. trap wastes, used media, culture supernatants. Note that any liquid waste that may contain spores **MUST** be treated as a high organic load waste.

High organic load liquid wastes **MUST** be decontaminated off-site by an approved external contractor or on-site by autoclave sterilization in an approved autoclave.

Low organic load wastes may be decontaminated in the same manner as high organic load wastes or treated on-site by chemical disinfection.

6.3.1 High organic load waste (off-site decontamination)

Small volumes of high organic load waste that are sealed in plastic tubes (<50 mL per tube and <500 mL aggregate volume) may be placed with solid waste into biohazard bags (refer section 6.2.1) or biohazard sharps containers (refer section 6.1). However, high organic load liquid wastes **MUST NOT** be poured directly into biohazard sharps containers.

Larger volumes (>50 mL per tube or >500 mL total) **MUST** be disposed of on-site by autoclave sterilisation in an approved autoclave (see 6.3.2).

6.3.2 High organic load waste (on-site decontamination)

Only specifically approved autoclaves (designated by the Sector Manager or DLM) may be used for decontamination of liquid biohazard waste. Liquid waste must be placed in an autoclavable container (if it is not already in an appropriate container) and the lid loosened (or vented closure used). Autoclave sterilization **MUST** be carried out in an approved autoclave in accordance with the guidelines in Section 7. The decontaminated waste may then be disposed down a laboratory sink.

6.3.3 Low organic load waste

Low organic load wastes may be decontaminated as for high organic load wastes (i.e. as per Sections 6.3.1 or 6.3.2) OR by chemical disinfection. Guidelines for chemical disinfection using chlorine bleach (sodium hypochlorite) and Virkon are given below (these are examples only, actual protocols may vary depending on the waste and the protocol followed should be

documented). **DO NOT** chemically disinfect materials that will be autoclaved – as this may damage sterilising equipment and expose users to hazardous fumes (especially if sodium hypochlorite is used).

Chlorine bleach disinfection: Chlorine bleach is typically supplied as 5% sodium hypochlorite. To disinfect low organic load wastes, add chlorine bleach to give a final concentration of 0.25% (i.e. add 50 mL of 5% sodium hypochlorite per 1 L of waste). Mix and leave to stand for a minimum of 1 hour. The treated waste may then be disposed of down a laboratory sink.

Virkon disinfection: Virkon is commercially available disinfectant with proven effectiveness across a broad range of fungi, bacteria and viruses and is supplied as powder or tablets (Virkon solutions should be prepared fresh). To disinfect low organic load wastes, dissolve Virkon in waste to a concentration of 1% (e.g. by adding 10 g/L waste), then leave to stand for a minimum of 1 hour. The treated waste may then be disposed of down the laboratory sink.

6.4 Animal (vertebrate) carcasses

Carcasses of vertebrate laboratory animals that are genetically modified, which contain genetically modified cells or tissue (e.g. as a graft or implant), or which are infected with genetically modified microorganisms or pathogenic microorgansims must be treated as biohazardous. Typically, these carcasses will originate from animals that were held in a Vertebrate Laboratory Animal Containment Facility (i.e. PC2 Animal area) when they were alive, but which may have been removed from these areas for experimental purposes.

All biohazardous animal carcasses **MUST** be disposed of as 'Animal waste' through an approved external contractor and are not permitted to be decontaminated on-site[6]. Such carcasses must be sealed in a plastic bag and transferred to either a biohazard bin or bag that is designated specifically for this purpose (i.e. animal carcasses **MUST NOT** be discarded into biohazard bags used for other solid biohazardous laboratory waste). This bin or bag must be kept in a secure area (refer section 9) with appropriate temperature control (e.g. fridge, freezer, cold room) until it can be collected by the approved external contractor. The death and disposal of any animal carcasses from a Vertebrate Laboratory Animal Containment Facility must be recorded in the appropriate register.

PC2 Animal areas must have appropriate disposal procedures in place for the disposal of all biohazardous animal carcasses. However, where animals have been removed from a PC2 Animal area for experimental purposes it is the responsibility of the animal user to ensure appropriate disposal procedures are in place in their area of work, or to dispose of any carcasses through the facility from which the animal originated.

[6] Except where other disposal procedures are specified in a project approval or import health standard – in which case these must be followed.

Note that bedding that originates from cages used to house animals infected with pathogenic microorganisms **MUST** also be treated as biohazardous and may either be decontaminated on-site (by autoclave sterilization) or off-site (by collection and disposal through the approved external contractor) as per the requirements for decontamination of solid waste outlined in Section 6.2. Animal bedding from cages of non-infected animals is not required to be treated as biohazardous and can be disposed of through the general waste stream.

6.5 Non-hazardous laboratory-related waste from PC1 laboratories

PC1 laboratory waste which is obviously laboratory-related in nature, particularly disposable lab-ware such as used gloves, pipette tips, petri dishes and tubes, but which is known to be non-hazardous (i.e. is not contaminated with any known biohazard, hazardous substance or radioactive material), **MUST NOT** be disposed of into general waste bins emptied by University cleaners. Such waste must be disposed of by one of the following two methods;

i. The waste may be treated as biohazardous and disposed of in accordance with sections 6.1 and 6.2.

ii. The waste may be collected in waste bins that are clearly labelled to the effect that they are not to be emptied by cleaners . This waste must be double-bagged and disposed of into a waste skip (or other appropriate receptacle approved for this purpose by the Biological Compliance Officer) by laboratory personnel.

Note that University cleaners are trained not to touch or empty waste bins that contain obviously laboratory-related material. This is to minimise the risk of contaminated waste being accidentally disposed of into the general waste stream and to protect the health and safety of cleaning personnel.

7. Use of autoclaves

On-site decontamination by autoclave sterilization may only be used for those wastes where this is specifically indicated as a disposal option in the preceding sections (6.1-6.4) and in areas where suitable approved autoclaves are available (refer section 7.7).

Autoclave sterilization should be available in all areas which generate high organic load liquid wastes (Section 6.3), but is not required to be provided for solid waste disposal (Section 6.2.2) where procedures are in place for collection and disposal of solid wastes by an approved external contractor (Section 6.2.1).

When autoclaves are to be used for on-site decontamination of biohazardous wastes it is imperative that there are standard operating procedures in place to ensure that these wastes are treated in a manner that renders them safe for disposal into the general waste stream (and in accordance with the requirements of AS/NZS2243.3:2002 and NZS4304:2002).

There are a wide range of different makes, models and ages of autoclave in use across the campus and it is not possible to provide detailed instructions for the correct use of each of these. However, this section provides general guidelines that should be followed[7].

7.1 Sterilization

The correct use of autoclaves to decontaminate biohazardous wastes requires a good understanding of the appropriate autoclave type and sterilizing conditions to use for different types and volumes of waste.

7.1.1 Autoclave type

There are three main autoclave types that are commonly used and each is suited to different waste types;

Pressure cookers: These rely on heating water within the sterilizer to generate steam and pressure. In general, these provide poor control of sterilizing conditions and **MUST NOT** be used for the decontamination of biohazardous wastes without the written approval of the Biological Compliance Officer (they may be approved for certain wastes, e.g. low organic load liquids, with appropriate validation).

Downward displacement (Gravity) Sterilizers: Steam of the desired temperature is generated separately and is admitted into the autoclave chamber, relying on gravity to displace ambient air. Porous materials and large containers (e.g. buckets, biohazard bags) that do not allow the downward escape of air may trap pockets of air and prevent penetration of steam into the load, leading to incomplete sterilization.

[7] Detailed guidelines are also provided in AS/NZS2243.3:2002.

This type of sterilizer **IS** recommended for the decontamination of liquid wastes (**Section 6.3**), but **IS NOT** recommended for treating large containers of solid waste such as biohazard bags.

Pre-vacuum (Porous load) sterilizers: These work in a similar manner to downward displacement sterilizers except that they employ a pre-vacuum stage to remove any trapped air from the load that might block the penetration of steam. This type of sterilizer **IS** recommended for decontaminating 'porous loads', including biohazard bags (**Section 6.2**) or other large containers of solid biohazard waste.

Some modern autoclaves are capable of running as both downward displacement and pre-vacuum (porous load) sterilizers, and for such autoclaves it is important that users understand what type of cycle is being run (and what cycle is appropriate for the waste type being processed). Clear instructions should be present at the autoclave in such cases.

7.1.2 Sterilization cycle conditions

At a minimum, all parts of the load (i.e. waste) must reach either a temperature of 121°C for 15 minutes or 134°C for 4 minutes (in order to achieve complete sterilization)[8]. However, in order to allow time for the load to equilibrate to the same temperature as the autoclave the **MINIMUM** sterilization times that **MUST** be used are[9];

a) 121°C for 20 minutes; or

b) 134°C for 10 minutes

These are minimum sterilization times only and may not be sufficient to achieve complete sterilization of all load types and volumes. Approximate guidelines of the sterilization times required for different volumes of waste at 121°C (using a downward displacement sterilizer) are given in Appendix 2.

Any sterilizing conditions that are used for decontaminating biohazardous waste should be validated through the use of biological indicators (as described in Section 7.2.2).

[8] As specified in AS/NZS2243.3:2002

[9]Sterilization times are taken from the point at which the autoclave reaches the desired temperature and **DO NOT** include the time taken for the autoclave to warm up.

7.2 Monitoring of sterilization cycles

Autoclaves that are used to decontaminate biohazardous wastes **MUST** be regularly monitored to ensure that target sterilization conditions are achieved and are effective[10]. Monitoring shall be carried out by annual calibration and by regular testing with biological indicators.

7.2.1 Calibration

All autoclaves used for the purpose of decontaminating biohazardous waste **MUST** be calibrated annually[11]. An annual calibration service will be organised centrally through the Health and Safety Compliance Office, with the costs met by the departments involved. Individual departments may also organize the calibration of their own autoclaves if they prefer.

7.2.2 Biological indicators

Biological indicators (such as spore strips) **MUST** be used to monitor the sterilisation process at a minimum of once per month or whenever the autoclave is used (if autoclave is used less than once per month)[12]. For high-use autoclaves (those that are typically used at least once per day) monitoring should be carried out weekly.

A number of biological indicators are commercially available and any of these may be used providing the 'kill time' of the indicator is equal to or greater than 15 minutes at 121°C, or 4 minutes at 134°C (i.e. the indicator provides confirmation that the minimum sterilization times specified in AS2243.3:2002 are achieved).

Note that careful consideration should be given to the placement of the biological indicators to ensure these provide meaningful results i.e. biological indicators should be placed within the part of the load that is likely to take the longest time to reach the desired temperature. (or within a 'test load' that is representative of the largest load item that would be processed).

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[10] "Sterilisation and disposal procedures of biological waste must be scientifically validated and shown to be effectively used in the facility to devitalise the waste being treated." *(from: MAF Biosecurity NZ/ERMA NZ Standard Facilities for Microorganisms and Cell Cultures:2007a)*

[11] The annual *certification* that is organized by Property Services only checks that the instrument is safe to operate and **DOES NOT** confirm that the desired sterilization conditions are actually achieved.

[12] "Biological indicators, such as spore strips, should be used at regular intervals (e.g. monthly) to monitor the microbial killing power of the sterilization process." *(from:AS/NZS2243.3:2002 – Safety in Laboratories Part 3: Microbiological aspects and containment facilities)*

Examples of biological indicators that are available are;

Attest™ Biological Indicator System for 121°C gravity or 132°C vacuum-assisted steam cycles (3M™, Cat No. 1262 or 1262P): Suitable for placement within or between items of solid waste sterilized with 121°C gravity or 132°C vacuum assisted steam cycles (distributed by Biolab).

MagnaAMP® Self Contained Biological Indicator Ampoules for Monitoring Steam Sterilization of Liquids. 10^5 or 10^6 *Geobacillus stearothermophilus.* (SGM Biotech Inc., Cat No. MA/6): Suitable for placement into liquid loads sterilized at 121°C (distributed by Austmel Pty Ltd).

An example protocol for the use of the Attest™ (3M™) biological indictor system is provided in Appendix 3. Any failure of biological indicators must be investigated immediately as this may indicate that either the sterilization conditions being used are insufficient to achieve complete sterilization, or that the autoclave is not functioning properly.

7.3 Recording of autoclave use

Logbooks recording the nature and amount of load processed, the cycle conditions used and any biological indicator testing results, should be maintained and available for inspection in close proximity to the relevant autoclave. It is also recommended that autoclaves to be used for the purpose of decontaminating biohazardous waste should have the capacity to provide chart records of the temperature and duration of all sterilizing cycles.

7.4 Approved autoclaves

The Sector Manager or DLM (as appropriate, refer Section 3) is responsible for approving which autoclaves may be used for the decontamination of biohazardous wastes, and for ensuring these are used in accordance with these guidelines.

ONLY THESE 'APPROVED AUTOCLAVES' ARE PERMITTED TO BE USED FOR THE DECONTAMINATION OF BIOHAZARDOUS WASTES.

The approved autoclaves should be clearly signed to indicate what waste types and volumes they are permitted to be used for (e.g. LIQUIDS ONLY <1 L) and the required sterilization conditions to be used. The Biological Compliance Officer must be notified of any autoclaves used for decontaminating biohazardous waste, in order that they can be audited and included on the list of autoclaves to undergo annual calibration.

7.5 Documentation of autoclave procedures

In areas where autoclaves are used for the decontamination of biohazardous wastes the Sector Manager or DLM (as appropriate, refer Section 3) must ensure that the procedures used are documented (and available on audit).

This documentation should include;

i) A list of any autoclaves that have been approved for decontaminating biohazardous waste (including room locations), together with any calibration certificates/reports.

ii) Brief protocols outlining the procedures used for decontaminating biohazardous wastes, including; what waste types are permitted to be treated in which autoclaves, any volume limitations, as well as the sterilizing cycles and conditions to be used.

iii) Brief protocols outlining how monitoring with biological indicators is carried out, including; the indicator system used, the number of replicates per load and the frequency of testing carried out.

iv) Evidence that autoclave users receive training in the procedures used.

8. Transport of biohazardous wastes

The transport of biohazardous wastes through non-laboratory areas shall be carried out in a manner that minimizes the risk of any biohazardous material being released. This shall be accomplished by following the guidelines below for the specific waste types.

Sharps (Section 6.1): These **MUST** be transported in sealed biohazard sharps containers.

Solid waste (Section 6.2): Biohazard bags **MUST** be sealed (e.g. with cable ties, tape or clips) and it is recommended that these be transported through non-laboratory areas within solid-sided, wheeled bins.

Liquid waste (Section 6.3): Containers of liquid waste **MUST** be sealed and transported inside a closed secondary container (e.g. plastic box). Where trolleys are used, these must have a lip to prevent containers sliding off.

Animal carcasses (Section 6.4): These must be sealed in a plastic bag and transported inside a closed secondary container.

9. Secure storage areas

Biohazardous waste must be stored in a secure area at all times. PC1 and PC2 laboratories are considered secure areas. In addition, many departments provide a secure area/bin for storing full biohazard containers until they can be autoclaved or collected by the approved external contractor. Within these areas, waste will be stored in a manner that prevents unauthorised access and contains any spills[13]. The Sector Manager or DLM (as appropriate, refer Section 3) has responsibility for designating and managing the use of these areas.

10. Handling of Biohazardous waste

Biohazardous waste must be handled in accordance with the standard operating requirements for PC1 and PC2 laboratories (refer to the Containment and Quarantine Manual for the University of Otago Containment and Transitional Facility for Microorganisms and Uncleared Biological Products).

11. Emergency Management

In the event of any spill of biohazardous waste or of exposure of personnel to potentially infectious agents follow the spill and exposure procedures detailed in the Containment and Quarantine Manual for the University of Otago Containment and Transitional Facility for Microorganisms and Uncleared Biological Products.

[13] The means by which secure storage areas are provided will depend on the specific area, together with the volumes and types of waste involved. Appropriate secure storage areas and procedures will be designated in consultation with the Biological Compliance Officer. Where any structural alterations are required (e.g. the provision of a locked storage cage), the costs will be met through the Statutory Budget process.

12. Alternative disposal procedures

These guidelines have been prepared with the intention of covering the majority of biohazardous waste types generated at the University of Otago. However, circumstances may arise where a biohazardous waste is not covered by these guidelines, such as;

i) Where the waste is of a type not specified here.

ii) Where, for practical reasons, it is not possible to follow the disposal procedure detailed here (e.g. due to the quantity).

In such circumstances, alternative procedures for disposing of these wastes may be developed in consultation with the Sector Manager or DLM (as appropriate, refer Section 3), as well as the Biological Compliance Officer. However, any alternative procedures must be documented and must not be used without the written approval of the Biological Compliance Officer.

Appendix 1. Standards requiring certain wastes to be treated as biohazardous

A list of standards covering waste types that are required to be treated as biohazardous and which are commonly generated in laboratories at the University of Otago are given below. This list is not exhaustive – other legislation, standards or specific approvals may also require specific wastes to be treated as infectious/biohazardous. It is the responsibility of the waste generator to be aware of any specific requirements that apply to any wastes they generate and to ensure that these wastes are disposed of in accordance with these requirements.

Standard	Wastes to be treated as biohazardous	Disposal
AS/NZS2243.3:2002 Safety in Laboratories, Part 3:Microbiological aspects and containment facilities	• Any Infectious waste • Microbiological waste originating from a PC2 (or higher) laboratory	As per Section 9.2
MAF Standard 154.02.17 Transitional Facilities for Biological Products	• Uncleared biological products and all waste associated with their use.	As per AS/NZS2243.3
MAF Biosecurity New Zealand Standard and ERMA New Zealand Standard - Facilities for Microorganisms and Cell Cultures:2007a	• Microorganisms or animal cell cultures (including genetically modified organisms) that are new organisms/risk species under the HSNO Act, or which are considered 'risk goods' . • Any other material directed to be disposed in accordance with this standard by another standard or approval.	As per AS/NZS2243.3

Appendix 2. Approximate sterilization times

Approximate sterilization times required for different volumes of waste at 121°C using a downward displacement (gravity) sterilizer.

Load Volume[a]/Type	Minimum sterilization time at 121°C (mins)
≤25 mL	20[b]
>25 - 50 mL	25[b]
>50-100 mL	28[b]
>100-200 mL	31[b]
>200-500 mL	35[b]
>500-1000 mL	40[b]
>1000-2000 mL	48[b]
>2000-4000 mL	63[b]
Biohazard bag (approx 4 Kg)	70[c]

[a] Load volume refers to the volume of the largest individual item processed, not to the total aggregate volume of the load.

[b] Burger, D.W. (1988), 'Guidelines for autoclaving liquid media in plant tissue culture.', HortScience Vol. 23, pp.1066-1668.

[c] Ozanne, G., Huot, R. & Montpetit, C. (1993), 'Influence of Packaging and Processing Conditions on the Decontamination of Laboratory Biomedical Waste by Steam Sterilization.', Applied and Environmental Microbiology,Vol. 59, pp. 4335-4337

Appendix 3. Example protocol for use of Attest™ (3M)™ biological indicator system

Attest™ (3M™) Biological Indicator Protocol:

Attest™ (3M™) Biological Indicators for 121°C gravity or 132°C vacuum-assisted steam cycles consist of a *Geobacillus stearothermophilus* spore strip, sealed glass ampoule with growth medium and a bromocresol purple pH indicator system; brown colour-coded cap with holes for sterilant penetration and a hydrophobic filter as a bacterial barrier, and a chemical indicator on the label that changes from rose to brown when processed. After sterilization, the vial is "crushed" to join the growth media with the processed spore strip. The BI is incubated for 48 hours for visual colour change. A colour change to yellow indicates surviving spores and a failed devitalisation.

- Biological indicators (triplicate) are included with biohazardous waste to monitor the sterilisation process. Include with the **first run of the week.**
- Fill in the autoclave log book with name, date, load, indicator batch number and cycle conditions.
- After sterilisation allow the processed indicators to cool for 10min.
- Check the colour strips on the indicators have changed from rose to brown.
- Crush the processed indicators and a control (unprocessed) indicator.
- Incubate the indicators at 56^{o}C.
- Initial colour change of the control (change from purple to yellow) is visible after 8h incubation but 48h incubation is required for a final result with processed indicators. Processed indicators should remain purple (i.e. no growth as spores destroyed by autoclave process).

NB: If a PROCESSED indicator shows POSITIVE growth (i.e. turns yellow) contact the Sector Manager IMMEDIATELY.

- Record the final results in the log book.

A log book shall be maintained recording the details of date, load, biological indicator batch number, autoclave conditions and the results of the processed and control indicators after incubation.