Standard Operating Procedure

Task/Activity/Equipment: Decontamination and disposal of biohazardous materials, including GMOs and risk group 2 microorganisms.	
Purpose: To outline the requirements and conditions for decontamination and disposal of biohazardous waste including GMO and risk group 2 microbiological waste.	
Location: Flinders University	**Reference Number:** IBC-SOP-06 **Version:** 2.0
Written by: Dr Jess Hall, Biosafety Specialist	**Reviewed by:** Instiutional Biosafety Committee
Approved by: Belinda Cox, Biosafety Officer	
IBC approval date: February 2023	**Revision required date:** February 2028
Replaces the version: IBC-SOP-6 Version 1.1	
Changes to the last approved version: Change of title, reformatting, and minor revision with addition of information for biosecurity waste.	

1. POTENTIAL HAZARDS

Infectious substances	Chemical disinfectants
Risk group 1 or 2 microorganisms	Pressure vessels (autoclaves)
Genetically modified organisms	
Diagnostic specimens	

2. TERMS & ACRONYMS

DAFF	Department of Agriculture, Fisheries and Forestry
DIR / DNIR	Dealing with Intentional Release / Dealing with No Intentional Release
GMO	Genetically Modified Organism
OGTR	Office of the Gene Technology Regulator
NLRD	Notifiable Low Risk Dealing
PC	Physical containment
RG1	Risk group 1
RG2	Risk group 2

3. RELEVANT LEGISLATION, GUIDELINES & STANDARDS

- *Gene Technology Act 2000*
- *Gene Technology Regulations 2001*
- OGTR Guidelines for Certification of a Physical Containment Facility (PC1, PC2)
- *Australian/New Zealand Standard 2243.3 Microbiological Safety and Containment*
- *Environment Protection Act 1993*
- *Environmental Protection Authority (EPA) Guidelines for Medical Waste – Storage, Transport and Disposal*

4. PROHIBITED DECONTAMINATION PROCEDURES

Decontamination of GMOs or risk group 2 microorganisms must not be performed using:

- × decontamination equipment that is defective
- × any heat-based decontamination equipment for which the results of each month's monitoring tests for the previous 12 months and the results of each year's calibration are not available

- chemical decontamination agents that are past their expiry date, and
- any method that has not been validated as effective for decontamination of the GMOs or risk group 2 microorganisms.

5. DECONTAMINATION & DISPOSAL REQUIREMENTS

Except where specific conditions apply below, waste containing GMOs may be disposed directly via the biohazard waste stream for offsite incineration.

Waste containing GMO plant material, lentivirus, or carcasses of animals infected with microorganisms (including GM microorganisms or lentivirus) must be rendered non-viable via autoclaving, disinfection, or irreversible fixation prior to disposal prior to disposal via the biohazard waste stream.

Carcasses and identifiable tissues from PC1 GMO animals must be returned to the carcass freezer in the animal facility. Facility staff shall dispose via the biohazard waste stream at time of collection.

Researchers who hold an OGTR **licenced dealing (DIR or DNIR)** should follow all decontamination and disposal conditions specified under the licence conditions.

Biosecurity waste material is not addressed within this document. Each Biosecurity Approved Arrangement (biosecurity containment facility) will have specific decontamination and waste disposal procedures and locations approved by DAFF, and local SOPs must be consulted in all cases.

6. DECONTAMINATION METHODS & CONDITIONS

Appropriate decontamination methods depend on the type of waste being treated. Please refer to Table 1 at the end of this document for methods appropriate for different types of waste.

Autoclaving conditions

- Autoclaving must be conducted using a combination of temperature and time validated as effective for the decontamination of the GMOs or RG2 microorganisms being treated. Minimum requirements, as specified by the OGTR are noted below:
    - 15 minutes (minimum) at 121°C and 103 kPa; or
    - 3 minutes (minimum) at 134°C and 203 kPa.
    - Note that longer cycles may be required for different sample types or volumes, and that the times specified are the minimum holding times at the specified temperature, not the overall cycle time.
- Any autoclave used must be tested monthly for decontamination efficiency (if you are using an in-built autoclave in CoMPHAF or the Biological Sciences building, routine testing is performed by facility personnel). Results of all monitoring tests must be kept for 12 months and must be made available to the IBC or OGTR upon request. The following monitoring methods must be used:
    - thermocouples or resistance thermometers, to ensure that the required temperature has been reached, and
    - biological indicators such as spore strips.
- Only an autoclave which has been calibrated by a NATA accredited party in the last 12 months can be used. The results of all calibration tests must be kept for a period of 5 years and be made available to the IBC or OGTR upon request.

Chemical disinfection conditions

- Chemical disinfection should be used where autoclaving is not possible (e.g., for large surface areas, or for heat-labile materials or equipment).
- Microorganisms vary in their susceptibility to chemical disinfectants. AS/NZS 2243.3 provides a detailed appendix outlining the types of chemical disinfectants suitable for different applications.
- The effectiveness of disinfectants is affected by a range of chemical and physical factors (e.g., concentration, contact time, pH, temperature, inactivation by organic matter). These factors need to be

considered when choosing optimal methods and disinfectants for use. Refer to Annex 9 in the Biosafety Manual and the disinfectant appendix of AS/NZS 2243.3 for further information about disinfectant selection.

- Disinfectants must be clearly labelled with the date of expiry, disinfectant concentration, and formulation.

Disposal via biohazard waste stream (yellow biohazard or clinical waste mobile bins)

- All solid biohazardous waste must be placed in a double-layered biohazard waste bag or sharps container (for contaminated sharps) that is then sealed and placed in a yellow biohazard or clinical waste bin (see Figure 1).

Figure 1: Typical lab biohazard waste bin (left) for laboratory waste collection, and wheelie bin for disposal for collection by waste contractor (right)

- The biohazard waste bag must be clearly labelled with the biohazard symbol.
- The yellow bin must be labelled with a biohazard symbol and, where GMO waste is disposed, with the acronym 'GMO'. Where no GMO label is supplied, it is the researcher's responsibility to add a zip-tie GMO tag to the bin when they place GMO waste there.
- The lid on the bin must be closable – if the bin is full, please arrange for waste to be collected.
- Within Flinders Medical Centre and Flinders University, all waste disposed of via the biohazard or clinical waste streams (yellow biohazard bins) is sent for off-site incineration by an approved contractor.
- Incineration may be used as both the decontamination and disposal method for most biohazard waste (excluding lentiviral waste, GMO plant waste, animal carcasses containing infectious or GM microorganisms, biosecurity waste, and waste from licenced dealings with an alternate specified waste treatment method).

7. UNINTENTIONAL RELEASE

In the event of an unintentional release, spill, leak, or loss of RG2 microorganisms or GMOs during decontamination or disposal, or misdirection of biohazard waste to a general waste or recycling bin:

- Within Flinders University, refer to the spill or unintentional release flowchart available within each PC facility on campus, and from the Biosafety website.

- Any real or suspected unintentional release of GMOs or RG2 microorganisms outside of a certified PC facility must be reported to the IBC as soon as reasonably practicable, (ph. 8201 5277).

8. APPLICABILITY

These procedures are applicable to all persons involved in the decontamination and disposal of biohazard waste, including GMO and microbiological waste.

9. CONTACTS

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10. DEFINITIONS

Decontamination	Decontamination is the general term used for sterilisation or disinfection processes for the removal of biohazard contamination to a 'safe' level.
Disinfection	Use of a chemical (disinfectant) to reduce the number of microorganisms present in a sample to an acceptable, safe level. Generally, less lethal than sterilisation and does not necessarily kill resistant microorganisms (e.g., spores).

Table 1: Decontamination methods for different types of waste

Waste Type	Example(s)	Decontamination method(s)	Disposal method(s)
General waste	Clean plastic packaging from general consumables.	No decontamination required	Place waste in general waste bin
Recycling	Clean cardboard packaging	No decontamination required	Place waste in designated recycling bin
GMO waste derived from NLRD PC1 and exempt dealings. RG1 microbiological waste.	Tissue derived from a PC1 knock-out animal that has not been infected with PC2 GMO or RG2 microorganisms.	Onsite decontamination is not required for solid waste if waste is disposed of via the biohazard or clinical (yellow biohazard) waste stream. Small volumes (< 25 ml) in sealed containers may be disposed directly to biohazard waste stream. Liquid waste that cannot be disposed of in sealed disposable tubes should be decontaminated by chemical disinfection or autoclaving prior to disposal provided that no other chemical risks are present. Refer to Section 8.5 of the Biosafety Manual for information about handling biological goods where chemicals are present.	Double-bag all waste in yellow biohazard waste bags and place in the yellow biohazard bin for off-site incineration. Biohazard bags containing PC1 animal carcasses or tissue should be placed in the carcass freezer. Animal technicians will transfer the bags to the biohazard mobile bins on collection day. Liquid waste that cannot be disposed of via biohazard waste stream may be disposed via the laboratory sink following chemical decontamination or autoclaving provided that no hazardous chemicals are present. Where chemicals are present, dispose via the appropriate hazardous chemicals waste stream.
Liquid RG2 or GMO microbiological waste (excluding lentiviral waste or licence waste)	Broth culture of RG2 or PC2 GM microorganism.	Small volumes (< 25 ml) in sealed containers may be disposed directly to biohazard waste stream. Liquid waste that cannot be disposed of in sealed disposable tubes should be decontaminated by chemical disinfection or autoclaving prior to disposal provided that no other chemical risks are present. Refer to Section 8.5 of the Biosafety Manual for information about handling biological goods where chemicals are present.	Double-bag all waste in yellow biohazard waste bags and place in the yellow biohazard bin for off-site incineration. Liquid waste that cannot be disposed of via biohazard waste stream may be disposed via the laboratory sink following chemical decontamination or autoclaving provided that no hazardous chemicals are present. Where chemicals are present, dispose via the appropriate hazardous chemicals waste stream.

Solid RG2 or GMO microbiological waste (excluding lentiviral waste or licenced waste)	Plate cultures, small volume cultures contained in sealed Eppendorf tubes	Direct disposal of sealed containers to biohazard waste stream is acceptable. Ensure all sharps are placed into a sharps disposal container. Goods may also be autoclaved prior to disposal via the biohazard waste stream where no chemical hazards are present.	Double-bag all waste in yellow biohazard waste bags, or seal sharps disposal container, then place in the yellow biohazard bin for off-site incineration. Following autoclaving, double-bag all waste in yellow biohazard waste bags and place in the yellow biohazard bin for off-site incineration
GM plant material	Tissue from a GM plant or a non-GM plant infected with a GM microorganism; soil or other growth media in which a GM plant was grown	**Onsite decontamination by autoclaving required before disposal.** Follow autoclave procedures in local area.	Following decontamination, waste can be disposed of via the yellow (biohazard) waste stream. Double-bag all waste in yellow biohazard waste bags and place in the yellow biohazard bin for off-site incineration.
Carcasses from animals infected with or containing RG2 or GMO microorganisms	Includes carcasses from animals infected with either RG2 or GM microorganisms.	**Onsite decontamination by autoclaving required before disposal.** Coordinate autoclaving with animal technicians.	Following decontamination, place carcasses in a biohazard (yellow) waste bag and place the bag in the carcass freezer. Animal technicians will dispose of carcasses via the biohazard (yellow) waste stream for off-site incineration.
Biohazard / clinical waste	Blood samples	Onsite decontamination is not required prior to disposal. Dispose via biohazard / clinical waste stream.	Double-bag all non-sharps waste in yellow biohazard waste bags. Sharps or glass should be disposed of in a yellow sharps container. Dispose of waste into a yellow biohazard bin for off-site incineration.
Reusable lab-ware used with infectious or GM organisms	Flasks, Schott bottles, glass pipettes, secondary containers used during transport of GM or RG2 microorganisms.	Decontaminate glassware by autoclaving. Follow autoclave instructions in your local area. Plastic-ware can be decontaminated using an approved chemical disinfectant.	n/a
Contaminated sharps	Syringes, broken glass, microscope slides	Dispose directly in sharps disposal container.	Contaminated sharps must be disposed of in appropriate sharps disposal containers, which are constructed of rigid plastic resistant to perforation. Full sharps containers should be sealed and disposed of in yellow biohazard bins for off-site incineration.

Lentiviral waste		**All waste must be decontaminated on-site prior to disposal.** Liquid lentiviral waste must be treated with 4% bleach or with Cavicide solution (at manufacturer's specified concentration) or by autoclaving prior to disposal. Solid waste that has been in contact with, or contains, lentivirus must be autoclaved on-site prior to disposal. Place waste in doubled yellow bags in a metal receptacle (e.g., metal bucket) for transport to autoclave.	Following decontamination, liquid waste can be disposed of via the sewer system. Solid waste can be placed in yellow biohazard bins for off-site incineration.