LTIBC Guideline		
Title: Waste Management Guidelines	**Approval Date**	June 2019
	Revision Date	October 2019
	Version	1
Purpose: To provide guidance of appropriate waste management procedures in relation to the disposal of biological waste.		

1. What waste this guideline refers to:

Examples including but not limited to; infectious, biological, clinical, GMO, human blood or body fluids, infectious animal carcasses or material, GM animal carcasses or material, quarantine waste and contaminated PPE.

Please note other hazardous waste such as cytotoxic waste, pharmaceutical products and radioactive substances as well as Schedule 4, 8 and 9 Drugs are **NOT** covered in this guideline and should not be handled under biological waste procedures.

A copy of the laboratory's protocol of waste management and spills' handling should be posted, read and understood by everyone working in the teaching or research laboratory.

2. Levels of decontamination:

- **Sterilization**

  Sterilization uses heat, radiation, chemical or filtration to abolish all biological agents (e.g. virus, fungi, bacteria, spore, prion, etc.).

- **Disinfection**

  Disinfection uses a liquid chemical to eliminate microorganisms (apart from bacterial spores) on work surfaces and equipment. Disinfection's effectiveness is influenced by number of factors such as types and numbers of organisms, the amount of organic matter, the object to be disinfected, and chemical exposure time, temperature, and concentration.

- **Decontamination**

  Decontamination is the process of reducing the quantity of contaminants (e.g. microorganisms or hazardous materials) on an object or substance. The purpose of decontamination is to prevent the spread of microorganisms and other noxious contaminants that may threaten the health of people, animals and/or damage the environment.

- **Antisepsis**

  Antiseptics are antimicrobial substances that can be applied to skin or living tissue to inhibit or destroy microorganisms. It includes swabbing an injection site on a person or animal and hand washing with germicidal solutions. Manufacturer recommendations for appropriate use of germicides should always be followed.

- **Cleaning**

  Uses water, detergent, and some mechanical action such as scrubbing with a gloved hand or brush. Cleaning is often a required step before sterilization or disinfection of an equipment or a surface because it removes all material

such as soil or organic material and reduces the number of microorganisms on an object.

3. How to apply good practice for effective waste management in laboratories:

Biological waste can be divided into two categories; liquid waste and solid waste. The diagram below outlines the biological waste streams at La Trobe University.

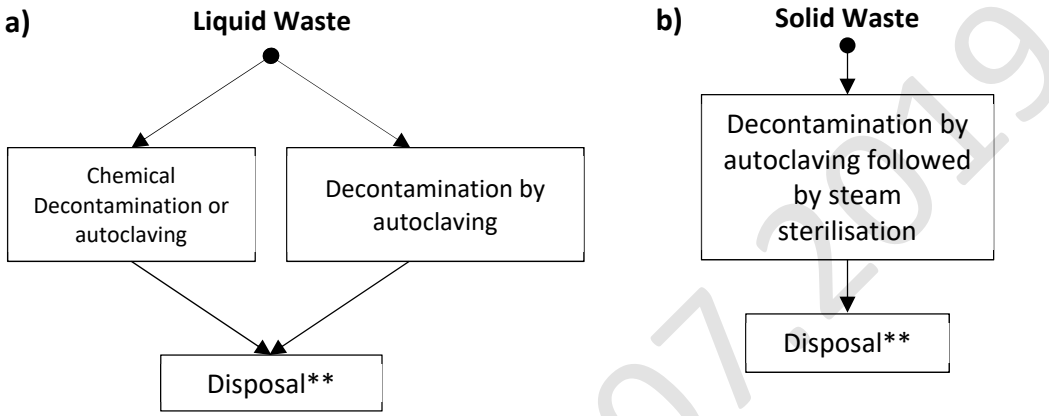

Figure 1. Schematic figure for biological waste streams at La Trobe University.
**Disposal of laboratory waste is subject to various regional, national and international regulations and adequate handling, transportation and disposal of laboratory waste should be adapted. In general, decontaminated biological waste can be disposed to the landfill following incineration.

3.1. Biological liquid waste

All liquid waste that contain (or potentially contain) biological agents should be treated to make them non-viable and minimize the risk of release by using the appropriate chemical decontamination procedure (*section 5*) or autoclaving (*section 3.2.1*). Please refer to required guidelines stated by *AS/NZS 2243.3:2010 Appendix F* and/or OGTR's *Guideline for the Transport, Storage and Disposal of GMOs*.

Biological agents differ in their susceptibility to chemical disinfectants. Therefore, the effectiveness of any disinfection procedures should be validated with the biological agents used. Consideration should be made of potential health hazards that each disinfectant imposes (e.g. corrosiveness, flammability, bleaching, toxicity and carcinogenicity). Please ensure that you read and understand chemical disinfectants' safety data sheet (SDS) and warrant use of adequate PPE.

3.2. Biological solid waste

At La Trobe University we adapted different biological waste streams for processing and disposing solid biological waste. Main waste management streams of different LTU schools are summarized below (*Please consult with the department manager to seek advice on potential procedural modifications*);

i. **LIMS (La Trobe Institute for Molecular Science)**: Decontamination by autoclaving followed by steam sterilisation through authorised contractors.
ii. **SLS (School of Life Sciences)**: Incineration through authorised contractors without preceding decontamination.

iii. **LARTF (La Trobe Animal Research and Teaching Facility)**: Decontamination by autoclaving followed by high temperature incineration through authorised contractors.

NOTE: above mentioned biological waste streams comply with the *AS/NZS 2243.3:2010* and the OGTR *Guidelines for the Transport, Storage and Disposal of GMOs*.

As mentioned earlier, all biological waste should be treated to make them non-viable and minimize the risk of release by using appropriate decontamination procedures as addressed in *AS/NZS 2243.3:2010 Appendix F* and the *OGTR Guidelines for the Transport, Storage and Disposal of GMOs*.

3.2.1. Autoclaving *(AS/NZS 2243.3:2010 Clause 10.6 and 12)*

Autoclaving is a process where high pressure and high temperature is used to produce pressurised steam which sterilises the load. Enough penetration time should be allowed for all parts of the load to reach the desired temperature. Minimum holding times after attainment of temperature shall be:

a. 15 minutes at 121°C and 103 kPa; or
b. 3 minutes at 134°C and 203 kPa.

Visual indicators (e.g. sensitive papers or autoclave tapes), demonstrate that the load has reached a specified temperature, yet they do not indicate how long the load has been exposed to that temperature. Bacterial enzyme indicators can be used to monitor sterilization cycles. These indicators are designed so that the loss of enzyme activity parallels the loss of spore viability. Advantage of bacterial enzyme indicators is that enzyme inactivation can be easily and rapidly determined, e.g. within minutes or hours, by the addition of a substrate and observation for absence of a coloured or fluorescent end-point.

NOTE:

a. Biological waste must be double bagged.
b. Autoclaves operate at high pressures and temperatures, therefore appropriate safety measures must be used and operation should be limited to trained personnel only. Follow manufacturer's guidelines for use.
c. Areas for the temporary holding of material awaiting sterilization shall provide appropriate storage conditions and adequate protection from unauthorized access and vermin.
d. Appropriate chemical disinfectants must be provided for spills and leaks. Easy access to hand washing facilities, safety showers and eyewash facilities must also be provided.
e. Once autoclaved, the treated waste is placed into a yellow biowaste bin for collection by the Bio-waste authorised contractors for incineration.

The efficiency of the autoclave machine must be validated on a monthly bases and the results of each month's testing must be kept in the autoclave logbook. This record must be made available to LA Trobe University's OH&S, LTIBC and OGTR (Office of the Gene Technology Regulator) on request and may be requested. Monthly validation Methods include:

a. Chemical indicators which use a combination of moisture, heat and time and which progressively change colour with the time exposed at the specified temperature; or
b. biological indicators such as spore strips; or
c. enzyme indicators.

3.2.2. Incineration *(AS/NZS 2243.3:2010 Clause 12.2.3.2)*

Incineration of waste that contain (or potentially contain) biological agents (e.g. sharps containers, laboratory waste), shall be done using a high-temperature, high efficiency EPA (Environment protection authority)-approved incineration facility. Waste must be packaged in lockable wheelie bins labelled with the biological hazard and GMO symbols (*AS/NZS 2243.3:2010 Clause 13.4.2 (c)* and the *OGTR Guidelines for the Transport, Storage and Disposal of GMOs*).

4. Spill decontamination

This section outlines required procedures for dealing with the biological spills in research laboratories. Please note that;

a. all biological spills must be attended to immediately;
b. spills must be evaluated for their level of infectious risk, concentration and location; and
c. all staff and students must be adequately trained and competent in spill clean-up procedures.

NOTE: for more information on disinfectants and antiseptics refer to: *AS/NZS 2243.3: 2010*.

4.1. Spills of up to 10mL that occur INSIDE a biological safety cabinet (BSC):

You must keep the cabinet **ON** during the following procedures:

1. For spills up to 1mL, wipe the area with paper towel and 70%-80% ethanol. Dispose of the contaminated paper towel and gloves in a biohazard waste bin together with your used gloves. Switch on the UV light of the BSC for 15 minutes to ensure full decontamination.
2. For spills greater than 1mL (and up to 10ml);
   a. Remove your contaminated gloves and leave them inside the BSC and wear new pair of gloves;
   b. Remove contaminated protective gown/laboratory coat and place them in an autoclavable waste bag;
   c. Wash hands and arms with an anti-microbial soap, then put on a clean pair of gloves, fresh gown/laboratory coat and required PPE (wear safety glasses and masks where there is a risk of splashing);
   d. Isolate and inspect the area and asses risks specially electrocution hazards. If there is risk of electrocution, immediately isolate the area and contact OH&S (03 9479 **2462**) and facility laboratory management team.
   e. If there is no risk of electrical hazard, cover the spill with paper towel containing 70%-80% ethanol or disinfectant granules and leave it for 10 minutes.
   Note: *the risk of fire when using flammable material and remove any potential ignition sources first. Also, to minimize risk of electrical hazard avoid flooding the spill area with ethanol.*
   f. Remove paper towels or absorbent granules used for disinfection and discard them into a biohazard bin. All contaminated disposables must also be disposed into a biohazard bin (**NOTE:** put all contaminated sharps into a sharps bin).
   g. Decontaminate all equipment, walls and work surfaces by wiping them with 70%-80% ethanol followed by use of the UV light for 15 minutes. Note that sodium hypochlorite solutions should not be used on metal surfaces. However, if hypochlorite is used for any reason (e.g. risk of ignition of highly flammable 70%-

80% ethanol) the metal surfaces must subsequently be cleaned with detergent and water and rinsed to remove any residual hypochlorite. Also bare in mind that waste containing hypochlorite solution should **NOT** be autoclaved due to production of toxic gas.

h. Reassemble the BSC, shut it down and irradiate with UV light for at least 30 minutes.

4.2. Spills of 10mL that occur OUTSIDE of the biological safety cabinet (BSC):

a. Assess the RISK to personnel/environment and act accordingly. Bare in mind that the first aid officer should be called, and medical aid should be sought in all cases of injury or doubt.
b. A biological spills kit (with procedure) should be readily available for spills management and should be stored in an area known to all individuals working in the laboratory.
c. Where particles/aerosols have been generated, the area should be evacuated, closed and signs should be put up to prevent people entering the affected area.
d. La Trobe University's OH&S (03 9479 **2462** or ohs@latrobe.edu.au) and LTIBC (03 9479 **1443** or biosafety@latrobe.edu.au) should be notified of spills of pathogens or GMOs as advice from the OH&S and IBC may be required.
e. Allow time for particles/aerosols to settle before disinfecting contaminated surfaces. Remove contaminated PPE and wash hands and arms with anti-microbial soap. Apply clean PPE (lab coat, gloves, safety glasses, mask) and proceed with decontamination.
f. Disinfection procedure must be rapidly effective and aim at containing the spill in the affected area. Disinfectant solution (e.g. 70-80% ethanol or 1% Virkon™ solution) can be poured carefully **around** the spill, allowing it to mix gradually with the contaminated material.
   NOTE:
   - avoid pouring the disinfectant solution directly onto the spill, as this can produce more aerosols;
   - where a spillage of a known or suspected viral materials occurs, a fresh sodium hypochlorite solution (5000 p.p.m.) should be used. Bear in mind that waste materials containing hypochlorite must not be autoclaved due to the subsequent production of toxic gas.
g. Use paper towels soaked with disinfectant solution to cover the area (alternatively disinfectant granules can be used to soak up and disinfect spills containing pathogens or GMOs).
h. Allow at least 30 minutes for the disinfectant to act.
i. Using disinfectant solution, wipe down surroundings of the spill area which are likely to have been contaminated with particles/aerosols.
j. Allow at least another 30 minutes for the disinfectant to act.
k. Carefully clean the decontaminated spillage. If disinfectant granules were used, disposable pan and scoop can be used to carefully collect granules.
l. A full report of any accident and measures taken, along with injury report and hazard notification (where applicable) must be made as soon as possible to La Trobe's "Incident and hazard reporting" (https://www.latrobe.edu.au/incident-reporting/login.php or via direct contact to LTU Health and Safety at ohs@latrobe.edu.au or 9479 2462).

4.3. Spills follow up:

4.3.1.1. All incidents must be reported to the project's chief investigator; School/department/facility's Management; and incident report lodgement by the project's chief investigator.

4.3.1.2. Contents of the spill kit must be replaced after use.

5. Properties of commonly used disinfectants

5.1.1.1. Chlorine

Chlorine is the active agent in chlorine-releasing chemical compounds (e.g. sodium hypochlorite NaOCI) against vegetative forms of bacteria and viruses but are inactive against spores. Chlorine combines rapidly with proteins, so in the presence of organic materials, the concentration of chlorine needs to be increased to overcome this organic demand. For example, an equal volume of 5000-10000 p.p.m. (0.5-1%) available chlorine is required for the inactivation of viruses (e.g. Herpes simplex Virus*)* in blood/serum or tissue culture and microbial liquid waste (minimum of 15 minutes decontamination is required). Commercially available chlorine solutions vary in their concentration of available chlorine. Therefore, care should be taken when diluting these solutions to ensure the correct final working concentration is achieved.

NOTE:

- The effective strength of chlorine solutions decreases on storage. Therefore, working solutions should be freshly prepared. Stabilized solutions of sodium hypochlorite with added sodium chloride are preferred as these solutions maintain a greater effective chlorine concentration.
- For effective biocidal action, a pH range of 6-8 is optimum.
- Chlorine gas is highly corro when it contacts moist tissues such as the eyes, skin, and upper respiratory tract. Therefore, when diluting chlorine solutions make sure to use a fume hood and wear personal protective equipment such as safety goggles, gloves, lab coat and enclosed shoes.
- High concentrations of hypochlorite solutions are corrosive to stainless steel and other metal surfaces.

5.1.1.2. Alcohols

A 70% *v/w* (approximately 80% *v/v)* solution of ethanol provides a useful disinfectant for clean surfaces and skin. Alcohols are active mainly against vegetative bacteria and lipid-containing viruses but are inactive against spores. The 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 microorganism's present. Moreover, ethanol is ineffective against Mycobacterium spp. and HIV dried on surfaces in the presence of sputum or serum.

NOTE:

- Due to ethanol's flammability, it should be used sparingly in biological safety cabinets and not with equipment that is likely to produce sparks. In biological safety cabinets, alcohol disinfectants may be used from a dispensing bottle but should not be sprayed.

5.1.1.3. Acids and alkalis

All acids are corrosive, and care should to be taken with their use. Acids are effective against a wide range of microorganisms, for example 2% hydrochloric acid solution can be used for areas contaminated with urine, blood and faeces.

Alkalis have activity against a wide range of microorganisms even in the presence of heavy organic loads, for example contaminated drains. In general, alkalis are disinfectants of choice for many animal holding areas or animal facilities. 1 M sodium hydroxide is a very effective and readily available decontaminant. It retains a high level of activity in the presence of organic matter and is recommended in many situations, such as decontamination of drains and animal houses. Moreover, 4% sodium carbonate solution can be used as a wash for animal cages and animal transport vehicles.

5.1.1.4. Virkon™

Virkon™ is broad spectrum disinfectant that contains a balanced composition of peroxygen compounds, surfactant, organic acids and an inorganic buffer system which is mainly used for disinfection of non-metallic surfaces, medical devices, textiles, laboratory benches and equipment. Virkon™ is effective against a wide variety of viruses, bacteria and fungi, but it is NOT effective for the decontamination of prions.
As a surface disinfectant, 1% Virkon™ solution needs a pH of approximately 2-3 with minimum of 10 minutes contact time. Also the powdered form of Virkon™ can be directly used onto biological liquid spills, then scooped up for disposal as chemical waste.

NOTE:

- The use of Virkon™ as a liquid tissue-culture decontamination aid is not supported as there is no evidence as yet for the effectiveness of Virkon™ to decontaminate liquid tissue-culture waste.
- To ensure good infection control, it is recommended that 1% Virkon™ solutions are prepared fresh.

Below Table is adapted from the *AS/NZS 2243.3:2010* and summarises some recommended applications for chemical disinfectants.
NOTE: This summary is provided only for assistance. Before using any disinfectant agent, refer to products safety data sheet and ensure that the correct personal protective equipment is worn.

Site or equipment	**Routine/preferred disinfection method**
Benches/ surfaces (with no obvious sign of contamination)	Alcohols e.g. 70% w/w (equivalent to 80% v/v) ethanol.
Biological safety cabinet (BSC) work surfaces	• Alcohols e.g. 70% w/w (equivalent to 80% v/v) ethanol. • Chlorine e.g. 5000- 10000 p.p.m (0.5-1%)
Centrifuge rotor or sealable bucket after leakage or breakage	Autoclaving (at 121°C for 15 mins), chemical disinfection is not recommended. **Note:** spills and aerosols must be contained and decontaminated appropriately.
Centrifuge bowl after leakage or breakage	Glutaradehyde for 10 mins (swab twice within the 10 min period then wipe with water). **Note**: Dilute according to manufacturer's guidelines.
Discard container (e.g. pipette jars)	Chlorine e.g. 2000- 2500 p.p.m (0.2-0.25%)
Hand disinfection	Chlorhexidine (0.5-4% w/v) in alcoholic formulation for 2 mins.
Hygienic handwash	Chlorhexidine (4% w/v) in detergent formulation for 15 secs.
Spills of blood/serum (or viral culture)	Chlorine e.g. 5000- 10000 p.p.m (0.5-1%) for 10 mins.
Spills of bacterial cultures	Chlorine e.g. 5000- 10000 p.p.m (0.5-1%) for 10 mins.
Animal cages	Chlorine e.g. 5000- 10000 p.p.m (0.5-1%) for 10 mins

Table 1: summary of some chemical disinfectants and their relevant recommended applications.

6. Chemical waste disposal

Chemical wastes can be categorised into liquid and solid. Under no circumstances, hazardous chemicals should be allowed to enter storm water drains or being discarded through municipal sewage system. The safety data sheet (SDS) for each chemical must be checked prior to disposal and mixing of chemicals should be avoided to prevent unexpected reactions from occurring.

NOTE:

- Appropriate and unbreakable container which is compatible with the residue chemical must be used for storage.
- Chemical waste sign should be placed on the container.
- Labelled chemical waste containers should be stored appropriately to ensure any potential leakages do not enter the drainage system.
- Each laboratory should keep record of generated, stored and disposed chemical waste should be tracked using a chemical logbook.
- On completion of research projects, and before leaving the University, staff and students must either dispose of their chemicals and samples or pass them on to the chief investigator of the project.
- If a chemical reagent bottle/container has lost its labelling and the identity of the substance is unknown, container must be labelled with "**Caution unknown substance - Do not use**". These bottles/ containers should be taken directly to waste store in your department/facility.
- A bulging waste container must be dealt with immediately by contacting OH&S at 9479 2462 and facility management of the school/department.

6.1. Solid chemical waste

There are various forms of solid chemical waste therefore adequate storage containers must be utilized according to the type of the solid chemical waste.

6.1.1. Powder waste:

To avoid risk of inhalation, container must be opened inside laminar flow cabinet and mask should be wore. It is preferred to use the original container if no longer needed, or an appropriate lidded container to store powered chemical waste. These containers can be disposed of through chemical-waste stream appropriate to the school/facility.

6.1.2. Chemically fixed biological samples

Biological tissues that have been chemically fixed or treated and are therefore no longer considered a biological hazard, should be placed into appropriate lidded containers. Containers should be leakproof and tissues should not be visible or recognizable though the container. These samples can be disposed of through chemical-waste stream appropriate for the school/facility.

6.1.3. Chemically contaminated glasses and sharp

Glasses and sharp that are or may be contaminated with chemical waste, can be stored in lidded and appropriate containers and be disposed of through chemical-waste stream appropriate for the school/facility.

6.1.4. Chemically contaminated laboratory items (disposable)

General laboratory waste, such as gloves, paper towels, rags etc, that are or may be contaminated with chemical waste, can be disposed of in chemical-waste plastic bags. If there is risk of items puncturing the bag, more robust lidded containers can be appropriate (e.g. 20 L lidded buckets).

6.2. Liquid chemical waste

Liquid chemical waste must be stored in unbreakable and leakproof containers with proper labelling. These containers must always be kept sealed except when liquid chemical waste is added. If the liquid chemical waste is likely to generate gases during storage, vented caps must be applied and containers must be stored in fumes hoods. Liquid chemical waste can be then disposed of through chemical-waste stream appropriate for the school/facility.