Biological Risk Management and Containment

Use of Autoclaves to Treat Biohazardous Waste

Containment Laboratory Guidelines

Version 5- October 2024

Approved by: Vice-Chancellor
Document Owner: Associate Director, Health, Safety and Wellbeing
Content Manager: Manager, Hazard and Containment

Version: 5
Issue Date: 31 Oct 2024

Amendments to Version 5

Date	Page number	Nature of amendment
28/8/2024	All	University logo updated
28/8/2024	4	More detail on mandatory use of approved autoclaves
11/9/2024	5, 6, 7	Cycle conditions match AS/NZS 2243.3 parameters
11/9/2024	7, 8	Chemical Indicators nomenclature changed from Class to Type, as per ISO 11140
11/9/2024	6,7	Update to latest standard 2243.3:2022
11/9/2024	8	Section 8 rewritten to introduce use of autoclave usage logs
11/9/2024	9	Section 10 rewritten to indicate use of autoclave usage logs
11/9/2024	13	Appendix 1, Section 12.2 edited to indicate different models of Auto-reader
11/9/2024	14	Appendix 2. Autoclave Usage Record format provided

Contents

1. Who is this reference document for?

This document is intended for **principal investigators (PIs)**, **designated persons in charge, designated laboratory persons (DLPs)** and **technical managers**, and to provide information to students and other staff using autoclaves to decontaminate waste from PC1, PC2 and PC3. However, to allow flexibility and customised assessments, PC3 specific procedures are detailed in the local procedure.

2. Autoclave use

Autoclave sterilisation is optimal for cultures of GMOs and for solid and liquid waste Alternatively approved chemical disinfectants can be used depending on settings (see the BRMC guidance *Chemical Decontamination of Liquid Biohazardous Wastes*).

Material decontaminated by autoclavation:

- decontamination of non-liquid GM bacterial cultures from PC1 laboratories
- all culture waste from PC2 microbiology, cell culture and Invertebrate facilities
- plant material and media from PC2 facilities for Plants
- material from PC2 Vertebrate facilities that has been exposed to GM cell lines, GM Bacteria, and viral vectors
- sterilisation of waste generated within quarantine periods
- and all waste from PC3 laboratories.

Autoclaving is strongly recommended for the decontamination of **high organic load liquid waste** from PC1 laboratories.

All autoclaved material is to be disposed of as medical waste as, even if decontaminated on site, it will be subjected to further autoclavation off site. Therefore, it is not to be disposed into the general waste stream.

3. What is an approved autoclave?

Approved autoclaves are generally **pre-vacuum autoclaves** with automatic log records that also have:

1. Annual calibration of temperature probes and pressure transducers.
2. Weekly verification of function using an approved biological indicator while autoclave is in regular use.

Gravity displacement autoclaves may only be used as approved autoclaves for processing waste where written procedures for correct loading and use of the autoclave are available.

Autoclaves are approved in writing by the Hazards and Containment Manager after written evidence is provided that they meet the above requirements.

4. What about safety certification?

All autoclaves (including approved autoclaves) are to be certified annually to show the autoclave is safe to operate under the Health and Safety in Employment (Pressure Equipment, Cranes, and Passenger Ropeways) Regulations 1999. Current certification is to be displayed beside the autoclave. Note that this safety certification does not confirm that the desired sterilisation conditions are actually achieved.

In the case of smaller benchtop or laboratory autoclaves, **designated persons in charge** are to ensure that these autoclaves receive their annual safety certification.

5. How is sterilisation achieved?

AS/NZS 2243.3 requires that all parts of the load (i.e. waste) must maintain *either* a temperature of 121°C for a minimum of 15 minutes *or* 134°C for a minimum of three minutes, in order to achieve Log 6 kill or complete sterilisation. However, to allow for variability in loads and sufficient time for all parts of the load to reach the same temperature, the minimum holding times once the required temperature has been reached will be:

a) 121°C for 20 minutes; *or*
b) 134°C for 10 minutes

These are minimum times only and may not be sufficient to achieve complete sterilisation of all load types and volumes. Note that large volumes of liquid require very long steam holding times.

Any sterilising conditions that are used for decontaminating biohazardous waste are to be validated through the use of biological indicators (as described in Appendix 1).

6. Calibration of approved autoclaves

Approved autoclaves used for treating biohazardous waste are to have temperature probes and pressure transducers calibrated annually.

Calibration is undertaken by a service agent.

Documentation provided as part of annual calibration should include calibration of measuring device to an IANZ-standardised measuring instrument.

For more information, please refer to the BRMC guidance *Calibration, Testing and Certification of Containment Equipment*.

7. Verification monitoring of sterilisation cycles

Verification of sterilisation is primarily achieved by weekly monitoring using Biological indicators.

1. For **pre-vacuum autoclaves** (fitted with automatic controls and logging), verification that steam conditions have been achieved is provided by machine control. This verification method is only valid where the temperature sensors and pressure transducers are calibrated at least annually.

2. In some cases, ISO 11140-1 Type 4, 5 or 6 indicators can be used to verify cycle parameters.

7.1 Verification monitoring using biological indicators[1]

- Approved autoclaves are to be monitored weekly using biological indicators (unless the autoclave is not in use), to ensure that target sterilisation conditions (greater than log 6 kill rates for temperature-resistant bacterial spores) are achieved and are effective.
- In cases where the autoclave does not have a remote load probe, biological indicators are required for each waste load.
- Where an autoclave is not used for periods greater than one month, the first subsequent cycle is to be monitored.
- Approved biological indicators are to have > log 5 spores (i.e. the indicator provides confirmation that sufficient kill rates on heat resistant spores are achieved within the minimum sterilisation times specified in AS/NZS 2243.3:2022).

- Approved biological indicator systems for 121°C or 134°C gravity or vacuum-assisted steam cycles (such as 3M™ Attest™) are to be used. These are self-contained biological indicator ampoules containing *Geobacillus stearothermophilus*. They are suitable for placement within or between items of solid waste sterilised with 121°C or 134°C gravity or vacuum-assisted steam cycles.
- Biological indicators are to be placed within the load in a position that steam is least likely to penetrate. It is recommended that the indicator is placed within a bottle with a cap (with a hole to allow steam penetration) which can be placed within a waste load and easily retrieved. Alternatively, the biological indicator can be placed within a "test load" that is representative of the largest load item that would be processed.
- Any failure of biological indicators is to be investigated immediately as this may indicate that either the sterilisation conditions being used are insufficient to achieve complete sterilisation, or that the autoclave is not functioning properly.
- See Appendix 1 for the correct use of an approved biological indicator system.

[1] "Biological indicators, such as spore strips, should be used at regular intervals (e.g. monthly) to monitor the microbial killing power of the sterilisation process." *(from: AS/NZS2243.3:2022 – Safety in Laboratories Part 3: Microbiological aspects and containment facilities.*

8. Recording of approved autoclave use

- In approved autoclaves where biohazardous material is processed, usage logs are to record every cycle, including non-waste cycles. An Autoclave Usage Record format is provided in Appendix 2. This record includes the cycle type, the verification of cycle parameters achieved, any failure to achieve load completion and the subsequent repeated run.
- Verification of the cycle parameters of sterilisation is to be monitored for every run by *either* the use of ISO 11140-1 Type 4, 5 or 6 indicators *or* operator checks of machine controls, where the autoclave has been calibrated. Note that it is not necessary to keep a record of machine printout or indicator strip.
- Records must also be kept of weekly biological indicator testing results where these are undertaken. Refer to the BRMC guidance *Calibration, Testing and Certification of Containment Equipment* for more detail.
- Any maintenance must be recorded.

- It is recommended that annual calibration certificates are kept alongside the records of biological indicator testing.
- It is recommended that autoclaves used for the purpose of decontaminating biohazardous waste should have the capacity to provide charts or electronic records of the temperature and duration of all sterilising cycles.

9. Documentation of autoclave procedures

In areas where autoclaves are used for the decontamination of biohazardous waste, the designated person in charge is to ensure that the following documentation is provided:

1) A list of approved autoclaves and their room locations, together with a copy of any calibration certificates/reports.
2) Brief protocols outlining how monitoring with biological indicators is carried out, including the indicator system used and the frequency of testing carried out.
3) Evidence that autoclave users receive training in the procedures used.

10. Verifiable outcomes

1) Safety certificates displayed next to all autoclaves.
2) Records of autoclave use and verification of cycle completion when biohazardous waste material is autoclaved.
3) Weekly load sterilisation tests using biological indicators are conducted and logged when biohazardous waste material is autoclaved.
4) For approved autoclaves, there is written evidence of training of operator (s) and annual calibration.

11.Definitions

Downward displacement or gravity sterilizers means those autoclaves that rely on gravity to displace ambient air. These autoclaves typically include the bench-top and small vertical laboratory autoclaves. Steam of the desired temperature is generated separately and is admitted into the top of the autoclave chamber. 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 sterilisation.

High organic load liquid wastes means wastes such as cell cultures (as a guideline $>10^5$ cells per ml), and blood and body fluids that have a high organic load, making chemical sterilisation difficult.

Pre-vacuum sterilisers means autoclaves that employ a pre-vacuum stage to remove any trapped air from the load that might block the penetration of steam. This type of steriliser is recommended for decontaminating porous loads.

Designated laboratory person (DLP) means the trained person in each research group who has been given the authority to receive purchase requests made in SQERM and to make a formal request for a purchase order via PeopleSoft. In containment and transitional facilities DLPs will have additional training to enable them to scrutinise documentation for restricted items and provide support to researchers.

Designated person in charge means a staff member in any of the following roles: sector manager, facility manager, floor manager, technical manager or an appointed delegate.

Principal Investigator (PI): In the context of hazard containment and transitional facilities, a principal investigator is the holder of an independent grant administered by the University and the lead researcher for the grant project, usually in the sciences, such as a laboratory study or a clinical trial. The phrase is also often used as a synonym for "head of the laboratory" or "research group leader." The PI is responsible for assuring compliance with applicable University standards and procedures, and for the oversight of the research study and the informed consent process. Although the PI may delegate tasks, they retain responsibility for the conduct of the study.

12. Appendix 1- Procedure for use of a biological indicator (BI) system (such as 3M™ Attest™)

- Set aside an unprocessed BI as a positive control.
- Place the test BI as deep as possible within the load (i.e. where steam is least likely to penetrate) but in a position from which they can be readily retrieved. If containers such as buckets are being used, place the BIs at the bottom of the bucket.
- Fill in the BI monitoring record with date, load type indicator batch number and cycle conditions.
- After sterilisation, allow the processed BIs to cool for ten minutes.

12.1 Procedure for use 3M™ Attest™ 1262 Spore strips

- Check that the colour strips on the BIs have changed from rose to brown.
- Break the vial of both BIs
- Incubate the two BIs (one processed, one unprocessed) at 56°C.
- For 3M™ Attest™ 1262 spores strips the initial colour change for the positive control (from purple to yellow) is visible after eight hours' incubation.
- Processed BIs require 48-hour incubation for a final result.
- Processed BIs should remain purple (i.e. no growth of spores).
- The positive control must turn yellow for the test to be valid.
- If a processed indicator shows positive growth (i.e. turns yellow) notify the manager in charge IMMEDIATELY.
- Record the final results in the BI monitoring record.

12.2 For Rapid Readout 3M™ Attest™ 1292 Spore strips

- Press the top of the vial down and then break the vial of both processed and unprocessed BI.
- Place both BIs in the recommended auto-reader.
- Positive or negative readout on the instrument panel is given after 3 hours incubation (for Auto-reader model 390) or 24 minutes (for Auto-reader model 490)
- If a processed indicator shows positive growth (i.e. a '+' on reader) notify the Technical Manager IMMEDIATELY.
- Record the final results in the BI monitoring record.

13. Appendix 2 – Autoclave Usage Record

The sections in yellow can be prefilled on the template with information specific for each facility.

- The section below the title is for cycle parameters and the shorthand reference.
- The verification section states which method is used such as Type 4, 5 or 6.
- The cycle type uses the shorthand referred to in the parameters section.

Additional information required by individual facilities should be recorded in extra columns.

AUTOCLAVE USAGE RECORD

Verification:					**Lot#:**		**Expiry:**	
Biological Indicator (BI) - once per week if autoclave is in use. Results must be entered on *Autoclave Validation - BI record*								
Failed runs and repeated runs must be clearly identified in the comments section								
Date	**Cycle Type**	**BI**	**Start Time**	**Cycle number**	**Autoclave**	**Comments & Actions**	**Cycle Pass/Fail**	**Operator Signoff**
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	
		Y/N					P/F	

Approved by: Vice-Chancellor
Document Owner: Associate Director, Health, Safety and Wellbeing Content Manager: Manager, Hazard and Containment

Version: 5
Issue Date: 31 October 2024