Biological Risk Management and Containment

Use of Autoclaves to Treat Biohazardous Waste

Containment Laboratory Guidelines

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Record of Amendments to Version 4

Date	Page number	Nature of amendment

Contents

1. Who is this reference document for?

This document is intended for **principal investigators (PIs)**, **designated persons in charge, designated laboratory person (DLPs)** and **technical managers**, and to provide information to students and other staff using the facility.

2. What are the mandatory requirements for autoclave use?

Autoclave sterilisation is to be used for cultures of GMOs and for solid and liquid waste from PC2 and PC3 laboratories. The only exception is the treatment of small volumes of liquid waste in PC2 viral vector laboratories with approved high level chemical disinfectants (see the reference document *Chemical Decontamination*).

Approved and calibrated autoclaves (see section 6) are to be used for decontamination of GM bacterial cultures from PC1 laboratories, all culture waste from PC2 laboratories and all waste from PC3 laboratories.

Mandatory autoclaving is not required for solid waste from PC1 laboratories (with the exception of cultures of GMOs or heavily contaminated items) where either chemical decontamination of liquid waste and/or disposal of solid wastes by an approved external contractor can be employed.

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

All autoclaved material is to be disposed of as medical waste and 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

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 reach either a temperature of 121°C for 15 minutes or 132°C for four 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 are:

a) 121°C for 20 minutes; or
b) 132°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 guidelines *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 Class 4, 5 or 6 indicators can be used for cycles with waste material.

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 load is critical (i.e. waste from PC3 labs), records of each cycle are to be retained and biological indicators are required for each load.
- In cases where the autoclave does not have a remote temperature probe, biological indicators are required for each 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 2243.3:2002).
- Approved biological indicator systems for 121°C or 132°C gravity or vacuum-assisted steam cycles (such as Attest™ 3M™) 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 132°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/NZS2243.3:2002 – Safety in Laboratories Part 3: Microbiological aspects and containment facilities.*

8. Recording of autoclave use

- Where biohazardous material is processed, logbooks are to record the nature and amount of load processed, the cycle conditions and the verification of cycle parameters achieved. Note that is it not necessary to keep a record of machine printout.
- In cases where there is a sole autoclave operator, who will check completion of autoclave cycles using machine controls, verification of successful achievement of sterilisation using ISO 1140-1 Class 4, 5 or 6 indicators can be used in place of a logbook. Weekly Bioindicator testing is still required.
- Records must also be kept of weekly biological indicator testing results where these are undertaken. It is recommended that annual calibration certificates are kept alongside the records of biological indicator testing.
- Any maintenance and failure to achieve load completion must also to be recorded.
- 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 wastes, 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) Either records of autoclave use and verification of cycle completion when biohazardous waste material is autoclaved or in the case of sole operators' evidence that ISO 11140-1 Class 4, Class 5 or Class 6 indicators are in use for waste treatment by autoclaving as per section 8. Note that the printout does not need to be retained.
3) Weekly load sterilisation tests using biological indicators are conducted and logged.
4) For approved autoclaves, there is written evidence of training of operator (s) and annual calibration.

NB: Logs for biological indicators (and any helix tests) can be recorded in a number of formats including 3M logbooks.

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 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 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^{O}C.
- For 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 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
- If a processed indicator shows positive growth (i.e. a '+' on reader) notify the manager in charge IMMEDIATELY.
- Record the final results in the BI monitoring record.