Information Sheet

Autoclaves

1.0 Introduction

Autoclaves are common laboratory machines, and the most reliable means of sterilising laboratory equipment. Autoclaves use high pressure steam and high temperatures to destroy microorganisms and are used for the decontamination of waste and the sterilisation of laboratory glassware, media, and reagents.

At Swansea University there are several types of autoclaves used, and all pose similar hazards. This document provides general information and guidance on the most common safety considerations for using an autoclave. This is not exhaustive, and each autoclave and its particular use should be risk assessed to identify and manage the hazards.

Note: Some materials may be unsuitable for autoclaving due to risks of explosion or toxic/corrosive fumes. Therefore, all materials must undergo a chemical risk assessment prior to autoclaving (see Health and Safety webpage for a chemical risk assessment template).

2.0 Hazards

The following are general hazards associated with the use of autoclaves:

- **Explosive displacement of the door**: If not correctly secured during operation under pressure.
- **Residual pressure risks**: Uncontrolled door opening under residual pressure.
- **Scalding and burns**: Resulting from exposure to steam, vapour, or hot liquids.
- **Risk of infection**: From improperly sterilised materials.
- **Explosion of sealed containers**.
- **Manual handling**: Due to overloaded waste container, poor technique when loading/ unloading the autoclave.
- **Cuts**: From damaged/ broken glassware, melted plastics.
- **Toxic/ corrosive fumes**: From certain substances toxic/ corrosive materials such as:
    - Autoclaving Virkon solutions should be avoided as sulphur dioxide can be generated at high temperatures.
    - Chlorine releasing agents (CRA) bleach, PRESEPT, HAZTAB, heat releases toxic Chlorine gas.
    - Solvents, volatile or corrosive chemicals (such as phenol, chloroform, etc.).

3.0 General

See below general guidelines for the safe use of autoclaves.

- Autoclaves produce steam under pressure this means they are a ‘pressure system’ (see university Pressure Systems Policy) and must have a written scheme of examination (WSE)

prior to first use. This is carried out by the University's Health, Safety and Resilience (HS&R) Team.

- Autoclaves should be regularly tested in accordance with manufacturers guidance. Serviced and tested by an authorised engineer (accredited). This should be organised by the faculty technical/ compliance team.
- Records on biological tests, recording thermometers, and service work performed on the unit must be kept for a minimum of 5 years. An external UKAS-accredited contractor typically services and calibrates/validates the autoclaves annually. This is organised by the faculty teams.
    - Calibration
        - Ensures that the autoclave instruments provide accurate readings by comparison with a known standard and that the chamber reaches the required temperature for the required time.
        - This does not guarantee that any load placed in the autoclave will achieve those conditions.
    - Validation
        - Demonstrates the load placed inside the autoclave is achieving the specified temperature and time. This involves 12-point thermocouple testing using user defined mock loads.
        - Autoclaves that undergo 12-point validation testing with a load do not require separate calibration. A thorough risk assessment must be carried out prior to any work involving autoclave.
- Only appropriately trained and competent personnel are authorised to operate autoclaves. These can be technical and research staff (Grade 4 or above) or PhD postgraduate students.
- Safe operating procedures (SOPs) must be written in sufficient detail and include how to operate controls, how to load/unload, load size, cycle settings and types etc. and must be adhered to by trained and authorised personnel.
- SOPs should be stored in a secure location adjacent to the equipment (if hardcopy) or on appropriate shared drive/ Teams site where they can be accessed when required.
- Tape indicators (autoclave tape) with heat sensitive and/or chemical indicators should be used in every autoclave load. Remember - the indicators only verify that the autoclave has reached normal operating temperatures; they do not indicate that the contents were heated for the appropriate length of time or at the correct pressure. Therefore, tape indicators cannot be used to prove organisms are killed during an autoclave run.
- For new loads/ organisms' biological indicators are available from manufacturers to demonstrate the organisms are killed.
- High density wastes or materials that insulate the agents from heat and steam penetration are not suitable for steam sterilisation. Items that are covered with dirt or film require additional retention times. Therefore, it is important to have appropriate procedures to ensure items are properly and thoroughly cleaned before sterilisation.

4.0 Preparation of Material

The most common reason for sterilisation failure is the lack of contact between the steam and microorganisms. To achieve effective sterilisation of materials, it is essential the correct packaging and containment of infectious materials are used.

4.1 Dry Material

- Dry material should be separated from liquid material to achieve correct sterilisation. Ensure that no more than 1 kg of high-insulating materials (e.g., animal bedding, high-sided polypropylene containers) is autoclaved at once. These materials require longer exposure to reach sterilisation temperatures.
- Check the bag specifications are suitable for your needs prior to use. All bags should be resistant to tearing. Bags should still be placed in rigid secondary containers to prevent leakage.
- Autoclave bags should only be filled to maximum 75% of holding capacity.
- Bags should not be tightly sealed, the top should be loosely gathered and secured with autoclave tape to allow steam to penetrate.
- Indicator tape should be used (as mentioned above a colour change only indicates exposure to steam, it does not guaranteed sterility.
- When autoclaving petri dishes containing agar, use a suitable tray to contain any leaks.

4.2 Liquid Material

- To prevent bottles from shattering during pressurisation and to facilitate steam penetration, bottle caps and stoppers must be loosened after placement into the chamber. If left sealed, they may not be properly sterilised and could burst violently if exposed to extreme heat.
- Do not overfill the containers (50-75% of holding capacity) to prevent spill and boil over.
- Bottles/flasks can be placed in an autoclave pan with about 5-10cm of water for even heating, ensure there are no bubbles under the bottle/flask.
- Sterilisation of bulk liquids requires special care to prevent the containers from exploding. Do not autoclave bulk liquids without following the manufacturer's written instructions.

5.0 Guidelines for Operation

Only trained and authorised personnel should operate the autoclave (knowing how to use one autoclave, does not mean you know how to use another). Only use the autoclave you are trained and authorised to use. Before using any autoclave, you should:

- Familiarise yourself with the operating manual.
- Wear appropriate PPE when loading and unloading, this includes:
    - Standard laboratory coat (All personnel should adhere to laboratory rules and ensure legs are covered and closed toe shoes).
    - Eye/ face protection.
    - Gloves (including heat resistant gloves).
- Prepare materials as per section 4.

5.1 Loading

- Avoid rough handling of autoclave bags and containers to minimise the formation of infectious aerosols.
- Use a secondary containment vessel for autoclave bags to collect any leakage.
- Follow the manufacturer's instructions when loading the chamber.
- Ensure autoclave is operating properly before commencing cycle i.e. check the temperature dial and pressure dial and the record of the last run, and cycle is appropriate for the load.
- The autoclave bags should be left open/ loosely secured during autoclaving to ensure steam penetration and sufficient temperatures inside the bag are achieved.
- Materials should be loosely packed in the chamber for easy steam penetration and air removal.
- Ensure the autoclave attains the desired temperature. The temperature is displayed on the outside of the autoclave and/or the printout of the run.
- Select the correct autoclave cycle for the sterilisation of the waste material. **If in doubt, ask!**
- **Always** make sure the autoclave lid is closed and secure before operating.

5.2 Unloading

- Before unloading the chamber, verify that the temperature and duration of exposure were met.
- Do not open the autoclave while the chamber is pressurised, released steam can cause severe burns.
- Wait until the autoclave has cooled prior to opening the door. (Most autoclaves have safety interlocks that prevent the door from opening when the temperature inside is greater than 80°C; however, steam may be ejected if the autoclave is opened immediately after the cycle.)
- Sterilised liquids must be allowed to cool for at least 20 minutes before unloading. Removing hot bottles may cause them to explode.
- Carefully open the door, i.e. avoid standing directly in front of the autoclave door when it is opened after a run.
- Handle waste containers containing liquids with care to avoid being burned by hot liquid splashes or spills.
- Clean up agar spills once the machine has cooled to prevent accumulation and damage.
- Complete the logbook (for faults) as required. All waste runs are logged on printer roll paper.

6.0 Standard Autoclave Cycles

The required autoclave cycle time will vary depending on the load's size and material. For instance, a 2-litre flask with 1 litre of liquid requires more time to sterilise than smaller containers.

It is up to the individual laboratory to prove the autoclave cycle they use is effective in making their waste safe.

7.0 Waste Disposal Procedures

Consult Swansea University's Waste Disposal Procedures for correct waste stream guidelines.

- Autoclaved liquids: May be disposed of via the public sewage system.
- Autoclaved solids: Dispose of as "offensive waste in tiger bags.

8.0 Training and Competence Requirements

The faculty technical teams/ laboratory staff should be trained by the manufacturer or service engineers. Once trained they can train other users.

All autoclave operators must receive adequate training and information on safe use of each autoclave they are required to operate. Training is provided by a competent and experienced member of the faculty technical team/ laboratory staff. Only trained and competent staff/ PG students are authorised to operate the equipment.

All inexperienced staff and postgraduate students must be supervised by trained faculty technical team/ laboratory staff during operation until they are deemed sufficiently competent and have demonstrated the required competency i.e. able to load/ unload the autoclave safely and demonstrate they understand and follow the training independently by qualified laboratory staff or service/ manufacturer engineers.