



3.14 Biological Safety Management

Information Sheet – Autoclave

Purpose

The purpose of this information sheet is to provide information and guidance to workers and supervisors when working with autoclaves. This information sheet should be read in conjunction with the [Biological Safety Management chapter](#) of the HSW Handbook.

Q1 What is an autoclave?

Pressure steam sterilisers (autoclaves) are used in laboratories both for sterilisation of media and equipment required for the culture of microorganisms, and for sterilisation of discarded cultures and waste materials. Pressure steam sterilisers operate at high pressures and temperatures, and appropriate measures must be taken for to ensure the safety of workers.

Autoclaves utilise moist heat, in the form of saturated steam under pressure, to destroy microbial life. Steam sterilisation is the most reliable sterilisation method for the majority of situations.

The autoclave is used to sterilise:

- reusable equipment after it has been disinfected and washed;
- biological waste before disposal.

Q2 What contributes to the effectiveness of an autoclave?

The following elements all contribute to autoclave effectiveness.

Temperature: The temperature inside the autoclave must be at least 121°C.

Time: The time required for sterilisation varies, but it will never be less than 30 minutes. The time is measured from the point at which the temperature of the material to be sterilised reaches the required autoclaving temperature - the tighter the autoclave is packed, the longer it will take for the material in the centre of the load to reach the temperature required.

Contact: Saturated steam must contact all areas of the load. Sterilisation will fail if:

- air pockets or inadequate steam supply prevent steam saturation; or
- the load exceeds the capacity of the autoclave so that steam cannot contact all areas of the load.

Autoclaves must be used properly to effect successful steam sterilisation.

Q3 What are the general guidelines for ensuring autoclave effectiveness?

There are a number of general guidelines that should be followed when using autoclaves to ensure effective sterilisation.

Indicators: Indicators, such as autoclave tape, indicate with a visible colour change that they have been through the autoclave process. At the end of the autoclave cycle, indicators should be checked to ensure they have changed colour.

Biological indicators: There are commercially available test kits that use bacterial spores to test the autoclave efficiency. Autoclaves should be tested using biological indicators at regular intervals in accordance with the manufacturer's instructions, and results recorded.

Overfilling

- Steam and heat cannot easily penetrate a densely packed autoclave bag. If an autoclave is overfilled the outer contents of the bag will be sterilised, but the innermost part will be unaffected.
- An overpacked autoclave chamber does not allow efficient steam distribution, and so sterilisation efficiencies will be reduced.

Continued

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Q3 What are the general guidelines for ensuring autoclave effectiveness? Continued

Monitoring: Autoclave temperature, pressure and cycle duration time should be monitored during each cycle. (Some autoclaves have charts that trend the temperatures and pressures inside the autoclave chamber throughout each cycle).

Maintenance: Autoclaves must be covered by a regular preventative maintenance program that is performed by a technician certified by the manufacturer.

Q4 What do I need to know about autoclave bags?

Most items to be autoclaved are packed into autoclave bags, which come in a variety of types and sizes.

Paper autoclave bags

- Paper autoclave bags are commonly used when autoclaving reusable equipment after it has been disinfected and washed.
- Paper autoclave bags should be closed by folding down and taping the open end (as they are permeable to steam).

Plastic autoclave bags

- Plastic biohazard bags are commonly used when autoclaving biological waste before disposal.
- Autoclave bags should be left partially open, to allow steam to penetrate the bag.
- Where possible, water should be added to autoclave bags to facilitate saturated steam contact. However, water should not be added if by doing so there is a chance that biohazardous materials may splash out of the bag
- Bags should be loosely packed and no more than $\frac{3}{4}$ full.

Q5 What do I need to know when autoclaving without an autoclave bag?

Some reusable equipment is autoclaved without autoclave bags, and in this instance the following guidelines should be followed.

- Tubular equipment such as pipettes and equipment such as conical flasks should be plugged with a wad of cotton wool, so that the interior of the equipment remains sterile after autoclaving.
- Small bottles and tubes should be loaded in open mesh baskets.
- Lids or caps should be loosened.
- Instruments such as forceps can be wrapped in aluminium foil.

Q6 What safety considerations should I implement when using an autoclave?

Autoclaves must be used in accordance with the manufacturer's instructions. Safety precautions that should be followed when using autoclaves include the following.

- Wear personal protective equipment, including heat-resistant gloves, safety glasses and a laboratory coat when operating an autoclave.
- If using a large autoclave consider using a trolley for loading & unloading items into the autoclave.
- Be careful when opening the autoclave door. Opening the autoclave door too quickly can result in glassware breakage and steam burns. Check that the pressure is close to zero before opening the door.
- Allow steam to escape from the autoclave before attempting to remove the contents of the autoclave.
- Be careful of hot temperatures when handling autoclaved equipment and any liquids.
- Never autoclave sealed containers - this could cause an explosion inside the autoclave. Large bottles with narrow necks can simulate sealed containers if filled with too much liquid.
- Never autoclave solvents, volatile or corrosive chemicals, or any radioactive materials.
- If there is a spill inside an autoclave, allow the autoclave to cool before attempting to clean up the spill.
- If glass breaks in the autoclave use tongs, forceps or other suitable tools to clear it away. Do not use bare or gloved hands to pick up broken glassware.
- Do not put sharp or pointed contaminated objects into an autoclave bag, as this could cause a needle stick injury. Instead, place sharp or pointed objects in an appropriate rigid sharps disposal container.
- Be careful when handling an autoclave bag full of infectious waste, in case sharp objects have been inadvertently placed in the bag.
- Never lift an autoclave bag from the bottom: instead handle it from the top.
- Ensure any equipment containing liquids is no more than $\frac{3}{4}$ full when placing into the autoclave. This will allow for heat expansion and decrease the likelihood of a pressure-induced breakage.

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Q7 What are the common pressures and times for autoclave?

The time and pressure depend on the contents of the run so it is important not to mix items/media with greatly different temperature ranges in the same run.

Temp.(°C)	Pressure(psi)	Time (minutes)	Exhaust	Applications
121	15	15	Slow	Liquids/agar
121	15	20	Slow	Large volumes of liquid/agar
121	15	20	Fast	Solid waste (no liquids)
109	5	45	Slow	Heat sensitive media
115	10	15	Slow	Heat sensitive media
118	12	10	Slow	Heat sensitive media
134	30	4	Fast	Glass/equipment

Further Information

If you require further information, please contact a member of the [local HSW Team](#)