



# Standard Operating Procedure

## Safe Autoclave Operations

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The purpose of this instructional document is to introduce and familiarize the reader to the standard operating procedures for the safe use of autoclaves.

### Introduction

**Autoclave:** is a specialized piece of equipment designed to deliver heat under pressure to a chamber, with the goal of decontaminating or sterilizing the contents of the chamber. The autoclaving process is typically used to destroy microorganisms and disinfect labware, equipment.

### Risks/Hazards

Since autoclaves use heat, steam and high pressure for sterilization, the potential hazards and safety risks for operators include (risk is highest when unloading the autoclave):

- Broken glassware if the autoclave door is opened too quickly, and sufficient time is not provided for glassware to approach room temperature.
- Super-heated liquids also pose a risk if shaken or moved during the cooling process.
- Vapors and gases from the accidental autoclaving of volatile chemicals.
- Heat burns from autoclave chamber walls and doors and/or hot materials.
- Steam burns from the steam coming out of the autoclave and materials following completion of cycle.
- Scalds from hot fluids due to boiling liquids and/or spillage in autoclave.
- Autoclaving certain chemicals may cause an explosion.
- Explosions can also occur when the seal of the autoclave door malfunctions or when autoclave is improperly loaded. If sealed containers are used they may explode during the autoclave process.

### Safety

In order to insure the health and safety of personnel using the autoclave, it is imperative that each department maintain the autoclave equipment as per manufacturer & safety guidelines and ensure personnel are trained in the proper use of an autoclave.

- Name of the individual responsible for the autoclave is to be posted near the autoclave. This Standard Operating Procedure should be present near the autoclave unit for reference.
- Supervisors are responsible for ensuring that employees are properly trained before operating any autoclave units.



- Documentation of training, which includes the signature of both the supervisor and the individual trained, must be kept by the supervisor (can use Biosafety MOU).
- Personal protective clothing and equipment must be worn when operating an autoclave.
- Procedural and instructional documents provided by the autoclave manufacturer must be kept, read, and adhered to when working with autoclave unit.
- Autoclaves must be inspected at least annually. The individual responsible for the autoclave should perform a basic visual inspection of the autoclave on a monthly basis. Inspection services may be managed by the manufacturer's preventative maintenance contract. The inspection, service and repair records must be available upon request of the Health and Safety Officer.

## Personal Protective Equipment

Autoclaves utilize steam, heat and pressure to complete the sterilization cycle. Therefore, the appropriate **Personal Protective Equipment (PPE)** must be worn.

- Eye/face protection – safety goggles /face shield; worn if there is a possibility of ocular splash
- Gloves for heat
- Lab coats – long sleeved and knee length
- Shoes – with closed toe and heel
- Long Pants or Skirt (covered legs)

## Operator Instructions

### Training

The fundamentals of autoclave safety are discussed in the Laboratory Biosafety course (EHS601). Supervisors must ensure that all personnel, whether they have taken EHS601 or not, have successfully completed a training session on the safe operating procedures prior to using the autoclave. This requirement is applicable to both new and experienced personnel. All in-house training **must** be documented, dated and signed by both the trainee and trainer, and available to view upon request (can use Biosafety MOU). Documentation of this and any other in-house training should be kept by the supervisor for a minimum of 3 years after the person has left the lab.

### Limitations of Autoclaves

Although autoclaving is an economical and environmentally friendly way of sterilizing and decontaminating items, not all materials can be autoclaved. In fact, some materials present specific hazards if autoclaved.

**DO NOT** autoclave the following items:

- Chemicals (includes most disinfectants, e.g. bleach)
- Radioactive material



- Some plastics – ensure materials are autoclavable.

## Uses of Autoclaves

Items that **CAN** be autoclaved include- but are not limited to:

- Cultures and stocks of infectious material
- Culture dishes and related devices
- Discarded live and attenuated vaccines
- Contaminated solid items such as: petri dishes, eppendorf tips, pipettes, gloves, paper towels, lab coats, solid and liquid waste
- Items for sterilization such as: glassware, media, liquid solutions
- Some equipment -ask supervisor for confirmation

## Material Preparation

Ensure that all material is prepared properly prior to autoclaving:

- Check to confirm materials can be autoclaved.
- Inspect glassware for cracks prior to autoclaving.

**Primary Container:** the container that comes into direct contact with the contaminated material or fluid to be autoclaved. The primary container **must** allow steam penetration, while avoiding pressure build-up. Examples of primary containers include flasks or vials containing liquids, autoclave bags etc.

Therefore, the container **CAN NOT** be sealed – sealed containers may **explode** in an autoclave!

**Loose seal** can be achieved by:

- Loosening screw caps or using self-venting caps.
- Capping open containers for sterilization with aluminum foil
- Opening plastic (autoclave) bags slightly prior to loading them into the autoclave
- Using envelope folds for wrapping Kraft paper or muslin

### Autoclave Bags:

- The classic autoclave bag is made of polypropylene (PP) and is strong and puncture resistant.
- They come in a variety of sizes and with or without the international biohazard symbol.
- But polypropylene does not have good steam permeability.
- To ensure that steam reaches the contents – autoclave bags **must be opened** prior to autoclaving.

**Secondary Container:** the container used to hold the primary container, which will prevent any spills from occurring.



- Primary containers must be placed into trays or buckets big enough to contain any materials that may melt or spill during the autoclaving
- These secondary containers must be made of a material that can withstand repeated autoclaving. Typically, metal or plastic autoclave trays are used as a secondary container.

## Transporting Materials to the Autoclave

- Waste should be restricted to laboratory zones and should **never** cross general public areas such as lunch rooms or general elevators.
- Ensure the use of a secondary container to collect any spillage in case of an accident during transport.

## Quality Control

### Indicators:

- **Physical Indicators:** pressure and temperature recording devices. Thermocouples can be placed inside the load to determine the temperature achieved in the bag itself.
- **Chemical Indicators:** change colour after being exposed to specific temperatures e.g. heat sensitive tape.
- **Biological Indicators:** bacterial spores are autoclaved along with the load, and incubated for period stated by the manufacturer, and observed for any sign of growth that would indicate that autoclave is not sterilizing properly. *Bacillus stearothermophilus* spores are used, as they are most resistant to steam autoclaving.

### Autoclave Tape

- Temperature sensitive tape should be affixed to each item to be autoclaved.
- Lines will appear when this tape is exposed to high temperatures.
- **It is not proof that the autoclave cycle was successful at decontaminating or sterilizing the contents** (just means the outside of the container got hot).
- A biological indicator or other means should be used to validate the efficacy of the sterilization procedure.

## Autoclave Procedure

### Prior to Using an Autoclave

Verify that the autoclave has been functioning correctly by reviewing:

- previous cycle log recordings (time, temperature, and pressure)
- the results of efficacy testing with biological indicators if available

## Loading an Autoclave

- Wear the appropriate PPE required to safely handle the material being loaded into the autoclave.
- Place material in autoclave. **Do not** mix solid and liquid materials.
- Avoid overloading the chamber, or compressing the contents: this will impede steam penetration.
- Containers holding liquids **should not** be more than **75%** full. This allows for liquid expansion, thereby preventing overflow.
- Place packages on their edges to enhance flow of steam.
- Place empty flasks or tubes horizontally to prevent trapping air pockets.
- Ensure containers do not touch each other so all surfaces are sterilized.
- Ensure all containers allow steam penetration (slightly open autoclave bags).
- Close and latch autoclave door firmly.

## Operating an Autoclave

- Ensure autoclave door is locked.
- Choose the appropriate cycle for the material.

**Factors** to consider in determining the type of cycle and length of time required:

- whether goal is decontamination or sterilization
- composition of the load (solid or liquid)
- density of the material
- volume and viscosity of liquids (i.e. larger volumes will require more time)

### Cycle Selection:

- **slow exhaust** cycle for liquids to prevent contents from boiling over
  - **fast exhaust** cycle for glassware
  - **fast exhaust and dry** cycle for wrapped items
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- If necessary, consult the autoclave manual for assistance in choosing a cycle. Autoclave Operating Manual should be located adjacent to the autoclave.
  - **Do not** attempt to open the door while the autoclave cycle is occurring.
  - If a problem with the autoclave is perceived, abort the cycle and contact the person in charge of the autoclave immediately.

## Unloading an Autoclave

The greatest risk of personal injury occurs during the process of unloading the autoclave. Refer to the Risks/Hazards section for a list of potential hazards and/or risks operator may encounter; exercise caution to eliminate the possibility of such occurrences when operating the autoclave.

### When unloading autoclave:

- Wear necessary PPE: heat-insulating gloves, eye protection if deemed necessary, lab coat, closed-toe shoes and wear clothing that covers your legs.
- Ensure that the cycle is complete and both the temperature and pressure have returned to a safe range. Check chamber pressure gauge before opening door: should be zero.
- Carefully open the autoclave door a little bit, and take care to avoid the steam. This will allow the steam to escape while simultaneously allowing the pressure within liquids and containers to stabilize.
- Do not disturb containers of super-heated liquids or remove caps prior to unloading these materials. Gently transfer containers to trolley.
- Check autoclave tape for colour change and cycle log recorder for time and temperature attained.
- If disposing of biological liquid waste after autoclaving, first allow to cool then can be poured down the drain.

### Maintenance and Repair

- Autoclave must not be used by any individual until the autoclave has been deemed safe for operation.
- Do not attempt to make repairs- only qualified professionals are permitted to make repairs to the autoclave.
- Report possible malfunctions to the individual responsible for the autoclave, as soon as possible, so that repairs can be scheduled with the autoclave company/supplier.

## Contingency Plans

### Incident Response

- All incidents, including spills, must be reported to the supervisor and department.
- If any injury occurs, seek first aid and/or medical assistance as deemed necessary by the degree of the injury.
- Accident report **must** be filled out in the event of an injury.
- If clothing absorbs hot water/steam, remove clothing and apply cool water/ice to the affected body part.
- A notice must be placed on the autoclave to indicate that the unit is out of service until the cause of the incident is identified, pro-active measures are taken to prevent such incidents in the future, and the autoclave is deemed safe for operation.

### Spill Clean-Up

- Spills may occur due to a boil-over or breakage of containers during the autoclave procedure. Use of secondary containers will make spill cleanup much easier – once cooled can be poured down the drain.



- No operation of the autoclave should be allowed until the spill is cleaned up. Spills not cleaned up will become harder to remove.
- The operator is responsible for the clean-up of the spill. Wait until the autoclave and materials have cooled down to room temperature before attempting to clean-up the spill.
- If spill of biological material occurred before autoclaving (during loading), follow your lab's spill procedures found in your lab's biosafety manual/emergency response plan. If spill is found after autoclaving, then any biological material should no longer be hazardous (autoclaving procedure if done properly will make the biologicals non-viable).
- Dispose of cracked glassware properly.
- All spills to be reported to principal investigator/supervisor – record of spills to be kept.

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