**SOP – Autoclave Operation**

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

**Risks/Hazards**
- Broken glassware if the autoclave door is opened quickly, and sufficient time wasn't provided for glassware to approach room temperature.
- Super-heated liquids if shaken or moved during the cooling process.
- Vapors and gases from 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 if the seal of the autoclave door malfunctions or when autoclave is improperly loaded. Sealed containers may explode during autoclaving.

**Safety**
- Documentation of training includes signature of both the supervisor and individual trained (use Biosafety MOU).
- Personal protective clothing and gear must be worn when using an autoclave.
- Procedural and instructional documents from the autoclave manufacturer must be kept, read, and adhered.
- Autoclaves must be inspected at least annually.
- Inspection services can be performed by the manufacturer (contract). The inspection, service and repair records must be available for request for Health and Safety Officers.

**Personal Protective Equipment**
- Eye/face protection – safety goggles/face shield; if possibility of ocular splash
- Heat resistant gloves.
- Lab coats – long sleeved and knee length.

**Operator Instructions**

**Training**
The fundamentals are covered in **EHS601**. Supervisors must ensure personnel, whether taken **EHS601** or not, have completed a training session on safe operating procedures prior to using the autoclave. All in-house training **must** be documented, dated and signed by the trainee and trainer.
Limitations (Do Not Autoclave):

- Chemicals (includes most disinfectants, e.g. bleach).
- Radioactive materials.
- Some plastics – ensure materials are autoclavable.

Permitted For Autoclaves:

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

Material Preparation

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

Primary Container: is in direct contact with the sample to be autoclaved. The container must allow steam penetration, and not sealed to prevent an explosion. Examples include flasks or vials containing liquids, autoclave bags etc.

Loose seals by:

- Loosening caps.
- Capping containers with aluminum foil.
- Opening polypropylene (PP) bags before loading into the autoclave.
Secondary Container: holds the primary container for preventing spills.
- These containers must be able to withstand repeated autoclaving. Metal or plastic autoclave trays are often used.

Transporting Materials to the Autoclave
- Use a secondary container to collect any spillage in case of an accident during transport.

Autoclave Tape (chemical indicator)
- Apply to each item that will be autoclaved.
- Black lines appear when the tape is exposed to high temperatures.

Autoclave Procedure

Loading an Autoclave
- Verify previous cycle logs (time, temperature, and pressure)
- Wear appropriate PPE.
- Place samples in the autoclave. Do not mix solid and liquid materials.
- Avoid overloading the chamber, or compressing contents that can impede steam penetration.
- Containers holding liquids should not be more than 75% full. This allows liquid expansion and prevents overflow.
- Position items on the side to enhance flow of steam, and ensure containers allow steam penetration (slightly open autoclave bags).
- Close autoclave door firmly.

Operating an Autoclave
- Ensure autoclave door is locked.
- Choose the appropriate cycle. Factors include:
  - 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 = for liquids to prevent contents from boiling over.
- fast exhaust = for glassware.
- fast exhaust and dry = for wrapped items.
- Do not open the door while the autoclave cycle is occurring.
- If there’s a problem with the autoclave, abort the cycle and contact CRAFT staff.

Unloading an Autoclave
- Wear appropriate PPE: heat-insulating gloves, eye protection if necessary, lab coat, etc.
- Ensure the cycle is complete and both the temperature and pressure returned to a safe range. Check chamber pressure gauge is zero.
- Open the autoclave door slightly to allow steam to escape while simultaneously allowing pressure in liquids and containers to stabilize.
• Do not disturb containers of super-heated liquids or remove caps prior to unloading these materials.
• Check autoclave tape for colour change and cycle log for time and temperature attained.
• If disposing biological liquid waste, allow to cool before pouring down the drain.

Maintenance and Repair
• Only qualified professionals are permitted to make repairs to the autoclave.
• Report malfunctions to CRAFT staff so repairs can be scheduled with the supplier.

Incident Response
• All incidents and spills, must be reported to the supervisor and department.
• For injuries, seek first aid and/or medical assistance, and fill out an Accident report within 24 hours.
• If clothing absorbs hot water/steam, remove clothing and apply cool water/ice to the affected body part.

Spill Clean-Up
• Once spills in the secondary containers is cooled, it can be poured down the drain.
• Wait until the autoclave and materials have cooled to room temperature before cleaning up the spill.
• For biological spills before autoclaving (during loading), follow the spill procedures in the biosafety manual/emergency response plan. If the spill occurred after autoclaving, then the biological material should no longer be hazardous.
• Dispose of cracked glassware properly.
• All spills to be reported to principal investigator/supervisor, CRAFT staff.