***STANDARD OPERATING PROCEDURE – E011***

**Autoclaving**

1. **Objectives**

The objective of this document is to establish standard operating procedures for autoclaving, ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials, and injuries. Additionally, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure safety during autoclaving, appropriate personal protective equipment (PPE) must be worn. This includes:

* Long pants and closed-toe shoes to protect against spills.
* A long-sleeved, buttoned lab coat to minimize skin exposure.
* Disposable nitrile gloves to prevent contact with biological materials.
* Safety glasses/goggles to prevent splashes, aerosols or steam during autoclaving.
* A face mask to reduce inhalation risks
* Heat-insulated gloves to protect hands when handling hot items or surfaces after autoclaving
* Long hair should be tied back during the operation of the autoclave.

1. **Potential Hazards**

Autoclaving presents various hazards that must be managed to maintain a safe working environment. These include:

* **Thermal Hazards:** Risk of severe burns from high-temperature steam or contact with hot surfaces immediately after autoclaving. Superheated liquids can cause scalding if containers are opened prematurely.
* **Physical Hazards:** Injuries from improper handling or lifting of heavy bins filled with autoclave bags. Pressure-related hazards due to the high operational pressure inside the autoclave chamber.
* **Biological Hazards:** Contamination risk from mishandling biological materials or improper sterilization.
* **Chemical Hazards:** Release of chemicals from autoclaving inappropriate materials, such as: Corrosive substances (e.g., acids, bases), flammable or volatile chemicals (e.g., solvents, alcohols), bleach (sodium hypochlorite), which can release toxic chlorine gas when heated, radioactive materials, which require specialized disposal methods. Release of toxic gas/vapor if heat-sensitive chemicals (e.g., formalin, paraformaldehyde) are autoclaved.
* **Mechanical Hazards:** Explosion risk from malfunctioning autoclave doors, improper loading, poor maintenance, or the use of sealed containers.

1. **Procedures**
2. Material Preparation and Packaging
3. Preparatory Steps:

* Ensure that all personnel have received training and related safety protocols.
  + MC05 Pressure Safety
  + MC06 Biological Safety
  + MC03 Chemical Safety II / Hazardous Waste Management
  + MC07 Chemical Safety I / Chemical Safety for Laboratory Users
* Verify that the materials are suitable for autoclaving.
  + **Note:** Avoid autoclavingoils, waxes, certain plastics (especially thermoplastic), chemicals, radioactive materials, and samples containing solvents as they may emit toxic gas/vapor during the process.
* Ensure that glassware and plastic items are heat-resistant and inspect for any cracks prior to autoclaving.
* Use only heat-resistant plastics, i.e. polycarbonate (PC), polytetrafluoroethylene (PTFE) Teflon-based material, and most polypropylene (PP) items.
* Select the appropriate autoclave bags for putting laboratory waste; do not use standard black rubbish bags. Autoclave bags should be labeled with biohazard symbols.

1. Packaging Procedures:

* Wrap or bag any loose, dry materials in steam-permeable paper or cover them loosely with aluminum foil.
* Loosen all lids of containers to prevent pressure buildup; containers should be covered with a loosely fitting lid or a steam-permeable stopper.
* Fill liquid containers to no more than two-thirds of their capacity.
* Do not overfill autoclave bags.
  + **Note:** Large quantities of pipette tip waste or a large number of stacked cell culture plates may puncture the autoclave bag; it is recommended to “double-bag” if the bag contains most of such waste.
* Adding some deionized (DI) water to the secondary containment pan will help heat items more evenly. Deionized (DI) water is recommended to reduce the chance of rusting inside the chamber.
* Position a strip of indicator tape on the outside of the autoclave bag.

1. Item Placement in Autoclave

* Position items in a stainless steel pan or another appropriate autoclave container to ensure stability and facilitate handling.
* Place liquid containers, bags containing agar plates, or any materials that may overflow or leak inside a secondary pan within the autoclave.
  + Note: It is recommended biohazard wastes are “double-bagged” in preparation for autoclaving.
* Verify that the pan is sufficiently large to accommodate any possible spills.
* Ensure that bags are not sealed or tied tightly to allow for steam penetration of the waste.

1. Loading the Autoclave
2. Preparatory Steps:

* Wear the appropriate PPE necessary for safely handling the materials being loaded into the autoclave.
* Utilize a cart to transport items to be autoclaved, particularly if they are fragile or breakable (e.g., glass flasks and beakers).

1. Loading Procedures:

* Load materials into the autoclave, ensuring that solid and liquid items are not mixed.
* Avoid overloading the chamber or compressing the contents, as this can hinder steam penetration.
* Position the secondary containment pan containing the items to be sterilised on the shelf or rack that is inside the autoclave.
* Arrange packages on their edges to enhance steam circulation.
* Place empty flasks or tubes horizontally to prevent the formation of air pockets.
* Ensure that containers do not touch each other to allow for complete sterilization of all surfaces.
* Make sure all waste can be penetrated by steam by leaving the autoclave bags slightly open.

1. Operating the Autoclave
2. Preparatory Steps:

* Confirm autoclave is validated by conducting a spore test using the appropriate biological or chemical indicator prior to autoclaving waste.
  + **Note**: This is recommended to be conducted monthly.
* Ensure the autoclave is functioning properly by reviewing logs from previous cycles, including time, temperature, pressure, and any additional notes.
* Confirm that the door can be securely latched during autoclaving cycles.
* Ensure all operators of the autoclave have completed the Boiler and Receiver Safety Course organized by the Occupational Safety and Health Council and possess valid documented in-person training.
* Ensure the certificate of fitness (if any) of the autoclave is valid.
* Place the autoclave operating manual at an accessible place near the autoclave.

1. Operating Steps:

* Select the appropriate cycle (e.g., gravity, liquid, or dry cycle) based on the type of material being autoclaved. Consult your supervisor, Principal Investigator, or the individual responsible for the autoclave operation for assistance.
* Check the pressure and temperature gauges of the autoclave before starting the cycle.
* Start the cycle and record your contact information in the autoclave user log. Typical cycle durations range from 0.5 to 1.5 hours, depending on the selected cycle.
* Do not attempt to open the door while the autoclave is in operation.
* If any issues arise with the autoclave, abort the cycle immediately and notify the person in charge.

1. Unloading the Autoclave
2. Preparatory Steps:

* Confirm that the autoclave cycle is complete and that both the temperature and pressure have returned to a safe range. Typically, this indicates that a temperature below 100°C and pressure back to 0 psi.
* Ensure you are wearing PPE and standing to the side of the autoclave (do not stand directly in front of the door).

1. Unloading Steps:

* Slowly unlatch the door to allow excess steam to escape.
* Once the door is fully unlatched, open it carefully to a maximum of 1-2 inches. This will release any remaining steam upwards and help normalize the pressure within liquids and containers.
* Allow autoclaved load to sit in the chamber for a few minutes prior to unloading. This will help steam dissipate and enable trapped air to escape from hot liquids, reducing the risk to the operator.
* Do not disturb containers with superheated liquids or remove their caps before unloading.
* Avoid agitating or removing caps until the liquids have cooled to a safe temperature.
* Wearing heat-insulated gloves, carefully take items out of the autoclave and place them on a stable surface to cool.
* Check the autoclave tape on the autoclaved waste bags for any color changes and review the cycle log for recorded time and temperature.
* If you need to dispose of biological liquid waste after autoclaving, let it cool first before pouring it down the drain.
* Close the autoclave door and partially latch it if necessary to keep it securely closed.

1. Disposing of Autoclaved Waste

* After the waste has been autoclaved and cooled, place the autoclaved bag into an opaque (black) trash bag prior to discarding. Dispose of the autoclaved waste in a black trash bag at an approved location.

1. **Incident Reporting**

* Report any accidents resulting in injuries to the Principal Investigator and/or the departmental safety officer (DSO) immediately.
* For serious incidents, notify the security unit immediately by calling the 24-hour hotline on **23588999**.

1. **References**

* Monaghan, B. (2016). *SOP\_SMB003.2: Autoclaving.* Risk Assessment. The University of Sydney.
* Coleman, N. & Monaghan, B. (2014). *SOP SMB003.1 (BM NC 0214): Autoclaving.* The University of Sydney*.*
* Dartmouth College and the National Institutes of Health (n.d.). *Autoclave Safety Animation in English*. Retrieved on June 30 2025 from <https://hseo.hkust.edu.hk/node/3641>
* Health, Safety and Environment Office, The Hong Kong University of Science and Technology (2023). *Chapter 9: Biological Safety.* Retrieved on June 30, 2025, from <https://hseo.hkust.edu.hk/sm_09>
* Health, Safety and Environment Office, The Hong Kong University of Science and Technology (2025). *Steriliztion Assurance: Spore Testing for Reliable Autoclave Operation.* From: <https://hseo.hkust.edu.hk/sites/default/files/autoclave%20quality%20control%20%282%29.pdf>