1. **Definitions and Responsibilities:**

   Autoclave: a vessel that uses steam under high pressure for sterilization.

   The Principal Investigator/Laboratory Supervisor is responsible to ensure that their staff and students adhere to the requirements set forth in this document as a minimum. It is the responsibility of each person operating the autoclaves to adhere to these requirements.

2. **Circumstances of Use:**

   Steam sterilization of materials is a dependable procedure for the destruction of microbial life. The pressurized steam that autoclaves use makes them serious burn hazards. Therefore, staff must understand and respect this equipment and the hazards posed by their use. Many autoclaves have unique characteristics, which will impact their operation. Before using an autoclave for the first time, all users should read and understand the owner’s manual.

   Autoclaves are typically used to sterilize glassware or equipment, prepare culture media, or pre-treat biohazardous waste for disposal.

3. **Instructions for Safe Use:**

   **I. Autoclave Cycles**
   
   The autoclave uses different settings of high heat, vacuum, and pressure to sterilize its load. The type of materials to sterilize will determine the “cycle”.
   
   1. Liquids or “slow exhaust”: used for water-based solutions and to prevent sterilized liquids from boiling over
   2. Dry goods without vacuum: used for clean dry goods such as glassware – the autoclave chamber is pressurized with steam for the duration of the cycle and returns to normal
   3. Dry goods with vacuum: Used for porous material, or for large bags or bundles of materials – the vacuum is used to move the steam and heat into the center of the load
   4. Autoclaves usually have an additional “drying cycle” in which hot air is drawn into the chamber to dry materials before they are removed

   **II. Packaging and Loading**
   
   Correct packaging ensures that steam penetrates the load. Containers packed to capacity will not be decontaminated effectively, even if the autoclave parameters are observed.
   
   1. ALWAYS ensure materials are suitable for use in an autoclave prior to beginning cycle.
      a. Suitable materials: glass, polypropylene, polycarbonate
      b. Unsuitable materials: combustibles, polyethylene, high-density polyethylene
   2. Use polypropylene autoclave bags – polyethylene bags will allow for greater steam permeability, but are not appropriate in many situations, as they will melt
   3. Prepare and load materials to ensure steam penetration
   4. Ensure ALL containers including bags are vented – **NEVER PLACE SEALED CONTAINERS IN AN AUTOCLAVE**!
      a. Large bottles with narrow necks (Erlenmeyer flasks) can simulate sealed containers if filled with too much liquid. Do not fill more than halfway full.
      b. The screw caps of containers can seal down during the autoclave process causing an explosion hazard.
5. Ensure there is sufficient water in the load to allow steam penetration – the bag can be opened prior to autoclaving and water add to the contents to generate steam within the bag (NOT ALLOWED FOR BIOHAZARDOUS MATERIALS).

6. Use secondary containers such as polypropylene or stainless steel tubs to prevent damage to the autoclave – if autoclaving liquids, add 1 to 2 inches of water to the secondary containment to prevent breakage of beakers/flasks.

7. Do not mix clean and contaminated materials in the same load.

8. Do not let bags touch the sides of the autoclave chamber; do not let containers touch each other.

9. Never autoclave items containing solvents, volatile or corrosive chemicals (e.g. phenol, ether, chloroform, etc.), or any radioactive materials.

III. Autoclave Operation

Each autoclave has unique characteristics and operating instructions. Consult the manual for specific instructions.

1. Use autoclave tape or biological indicators to validate the process – if the indicator fails, you must contact Facilities at x3-4567 for autoclave maintenance.

2. Maintain a log to record autoclave operation.

3. Processing time starts after the autoclave reaches normal operating conditions of 121°C and 15 psi pressure.

IV. Unloading the Autoclave

Accidents and burns are most likely to occur at this point. Exercise caution when unloading the autoclave and observe the following steps.

1. Once the cycle is complete, allow the chamber pressure to return to zero before trying to open the door – do not attempt to force the door, if it does not open, the internal pressure has not normalized.

2. Open the autoclave door slowly to vent the steam into the canopy exhaust system – keep your head, face, and hands away from the opening!

3. Materials and surfaces inside the autoclave will be extremely hot so allow 10 minutes for cooling before removing materials.

4. Wear personal protective equipment (PPE) when removing materials
   a. Gloves: long-sleeved, heat and fluid resistant – **DO NOT USE GLOVES THAT ARE WET OR HAVE HOLES!**
   b. Face protection: Goggles or a face shield are required when autoclaving liquids.

5. If there is a spill inside the autoclave chamber, allow the unit to cool completely before cleaning up the spill.

6. If glass breaks in the chamber, pick up the pieces using tongs and dispose of in glass waste box or sharps container depending on contamination status of the glass.

IV. Unloading the Autoclave

Accidents and burns are most likely to occur at this point. Exercise caution when unloading the autoclave and observe the following steps.

1. Once the cycle is complete, allow the chamber pressure to return to zero before trying to open the door – do not attempt to force the door, if it does not open, the internal pressure has not normalized.

2. Open the autoclave door slowly to vent the steam into the canopy exhaust system – keep your head, face, and hands away from the opening!

3. Materials and surfaces inside the autoclave will be extremely hot so allow 10 minutes for cooling before removing materials.

4. Wear personal protective equipment (PPE) when removing materials
   a. Gloves: long-sleeved, heat and fluid resistant – **DO NOT USE GLOVES THAT ARE WET OR HAVE HOLES!**
   b. Face protection: Goggles or a face shield are required when autoclaving liquids.

5. If there is a spill inside the autoclave chamber, allow the unit to cool completely before cleaning up the spill.

6. If glass breaks in the chamber, pick up the pieces using tongs and dispose of in glass waste box or sharps container depending on contamination status of the glass.

4. **Site Specific Use:**

   The laboratory must insert their site specific procedure for the use of their autoclave(s) here.

5. **Personal protective equipment (PPE):**

   Gloves: Heat and fluid resistant gloves which cover the wrist and forearm. Gloves should be in good condition with no holes or tears. **DO NOT use gloves if they are wet.**

   Face protection: use goggles or a face shield if autoclaving liquids or if any splash hazards may be present/identified

   Body protection: standard laboratory attire – lab coat, covered legs, full-coverage shoes
6. **In Case of Incident:**

   The most likely incident to occur when using an autoclave is a burn from pressurized steam or from hot glassware or equipment. It is also possible to generate pressure vessels inside the autoclave which can explode upon opening the autoclave. Opening the autoclave door cautiously (only 1-2”) once the pressure normalizes should reduce the potential of containers holding liquids from exploding.

   If a burn or other injury occurs, contact University Health Services at x5-2662 or call Public Safety at x13 in case of emergency.

   Incidents must be reported to the laboratory supervisor or PI. An incident report must be filled out within 24 hours. The University’s Employee Incident Report Form can be found at the Environmental Health and Safety website: [http://www.safety.rochester.edu/SMH115.html](http://www.safety.rochester.edu/SMH115.html)

7. **Training of personnel:**

   The user must demonstrate competency and familiarity regarding the safe handling and use of the autoclave prior to use. Training should include review of:

   - Owner’s Manual
   - University’s SOP for the Effective Use of Autoclaves
   - Lab-specific Procedures

   Training on lab-specific procedures must be documented (topics covered, date, employee names and signatures).

   All personnel shall read and fully adhere to the laboratory-specific SOP, and shall document that they have read it by signing and dating the SOP.

   “I have read and understand this SOP. I agree to fully adhere to its requirements.”

<table>
<thead>
<tr>
<th>Last</th>
<th>First</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>