A. INTRODUCTION

An autoclave is a commonly used piece of equipment in research laboratories. Autoclaves pose many hazards including physical hazards (e.g., heat, steam, and pressure) and biological hazards. This guide is intended to provide practical information that can be utilized by all personnel to safely operate the autoclaves at George Mason University. This guide outlines precautions and procedures for the safe use of autoclaves, and includes guidance from the Virginia Department of Environmental Quality (DEQ) Regulated Medical Waste Management Regulations, 9 VAC 20-120.

B. SCOPE

This guide applies to all autoclaves and users of autoclaves used to treat regulated medical waste (RMW) at George Mason University.

C. TRAINING

All personnel must receive proper training before using any autoclave at the university. The Environmental Health and Safety Office (EHS) provides training on autoclave safety as part of Biological Safety for BSL-2 Laboratories training. This training includes the regulations associated with treating RMW. In addition, Principal Investigators and Laboratory Supervisors must provide hands-on training specific to the autoclaves used by their personnel as part of laboratory-specific training.

Different brands of autoclaves have unique control settings for loading, load sizes, cycle types, and settings therefore it is important to review the owner’s manual before using the autoclave for the first time. Owner’s manuals are kept in the autoclave room with the autoclave log.

D. GENERAL AUTOCLAVE SAFETY PRACTICES

Autoclaves operate at high temperature and pressure. When using an autoclave, be sure to follow these safety practices:

- Before using the autoclave, check inside the autoclave for any items left by the previous user that could pose a hazard (e.g., sharps).
- Check the drain and clean the strainer before loading the autoclave.
- Load the autoclave properly as per the manufacturer’s recommendations.
• Place containers and bags within an autoclave-safe tray to provide stability and to capture overflow when autoclaving materials. Never place containers directly on the rack or autoclave floor.
• Ensure the door of the autoclave is fully closed and sealed.
• Never attempt to open the door while the machine is in operation. Always check the jacket pressure gauge to make sure that it is reading 0 PSI before opening the door.
• When the cycle is complete, verify required parameters have been met prior to opening the autoclave door.
• Open the door slowly. Keep your head, face, and hands away from the opening.
• At a minimum, wear heat-resistant gloves and a lab coat when removing items from an autoclave.
• If the machine is not operating properly, notify the appropriate service representative as posted on the autoclave. Do not attempt to make repairs as this should be done only by a trained technician.
• Never autoclave items containing corrosives (including sodium hypochlorite or bleach), solvents or volatiles, or radioactive materials.

E. AUTOCLAVING LIQUIDS

• Loads containing liquids should be treated using the liquid cycle on the autoclave.
• To prevent bottles from shattering during pressurization, do not overfill containers and loosen the caps of containers before loading.
• Use only borosilicate glass (Pyrex™ or Kimax™) which can withstand the high autoclave temperature.
• Use an autoclave-safe tray or catch basin with a solid bottom and walls to contain the contents and catch spills.
• Let liquids stand for a full hour before touching with ungloved hands.

F. AUTOCLAVING DRY LOADS

• Dry loads, such as waste, should be treated using the gravity cycle on the autoclave.
• Check plastic materials to ensure that they are compatible with the autoclave (i.e., polypropylene is autoclavable, polyethylene is not).
• Use an autoclave-safe tray or catch basin with a solid bottom and walls to place materials into the autoclave.
• Allow the dry materials to cool before touching with ungloved hands.

G. MONITORING AUTOCLAVE FUNCTION

The following methods can be used to verify that an autoclave is operating properly:

Heat-Sensitive Tape Monitoring: Autoclave users should use heat-sensitive sterilization indicator tape for each load to indicate that the load has undergone a steam sterilization process. This tape indicates that the proper temperature has been reached; it does not indicate that the autoclave operated at the proper pressure or for the appropriate amount of time.
**Autoclave Readings:** After each cycle is complete, a printout is created indicating the temperature and pressure. The user must verify the desired temperature and pressure were reached for a specific amount of time (RMW is treated at 121°C at 15 pounds per square inch for 2 hours).

**Monthly Bio-indicator Tests:** Autoclaves that are used to treat regulated medical waste are required to be tested for sterilization efficacy using a biological indicator. These tests are conducted monthly for each autoclave, and results are recorded in the Biological Indicator Sheet in the Autoclave Log located in the autoclave room. For more information on bio-indicator tests, contact EHS at 703-993-8448 or safety@gmu.edu.

**H. REGULATED MEDICAL WASTE (RMW)**

Autoclaves that are used to treat RMW are subject to regulations that require specific recordkeeping. Documentation must be maintained of all autoclave usage including the operator’s name, date, amount of waste treated, type of waste treated, and autoclave settings including time, temperature, and pressure. This information is kept in binders within each autoclave room where RMW is sterilized.

- RMW must be treated for 2 hours, at 121°C at 15psi.
- Parameters of the autoclave run must be verified and recorded in the autoclave logbook.
- After autoclaving, place autoclaved RMW in an orange bag.
- Affix a Treatment Certification Label (available in the Autoclave Log in each autoclave room) that includes a certification that the waste is no longer infectious, the date the waste was autoclaved, generator name (George Mason University), full address, and contact number. Discard orange bags in the regular trash dumpster.
- Decontaminate the cart and red waste container after each use.

**I. WASTE MANAGEMENT FACILITY OPERATORS**

George Mason University is required to have Waste Management Facility Operators on staff for each campus where autoclaves are used to treat biohazardous waste. The Fairfax and Prince William campuses and the Biomedical Research Laboratory have a Waste Management Facility Operator whose responsibilities are to:

- Receive Class I and Class III Waste Management Facility Operator training and pass the exam.
- Maintain current Waste Management Facility Operator license for George Mason University (including continuing professional education or training).
- Verify that waste autoclaves are functioning properly by performing bio-indicator tests monthly on each waste autoclave and maintain the Biological Indicator Sheet in the Autoclave Log. The Waste Management Facility Operator may assign this responsibility to a designee.
- Verify that autoclave performance standards are being met as outlined in Virginia DEQ RMW Regulation 9VAC20-120.
- Maintain autoclave user logs according to 9VAC20-120.
- Coordinate maintenance when necessary.
- Maintain service contracts for autoclaves and forward annual certification documentation to EHS.
- See that unautoclaved waste is not left unattended in unrestricted areas and that it is placed on carts provided by EHS instead of the floor.

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<thead>
<tr>
<th>Name</th>
<th>Location of Responsibility</th>
<th>Contact Information</th>
</tr>
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<tbody>
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### J. AUTOCLAVE MALFUNCTION

If an autoclave is not working properly, discontinue use immediately. Post a sign alerting others not to use the autoclave, and return unautoclaved waste to the laboratory. Mechanical failure needs to be attended by a trained technician or contractor. Contact information for a trained technician should be available on or near the autoclave. If contact information is not available, contact the Waste Management Facility Operator listed in Section I for your location.

### K. BURN EMERGENCY

The pressurized steam and heat of the autoclave can cause scalding or burns. If you receive an injury while using an autoclave, seek medical attention as soon as possible. Scald and burn injuries to the face, third-degree burns, or burns over large areas of the body should be treated as emergencies. Minor burns should be treated with first aid.

First aid for scalding and burns include immersing the area immediately in cool water, removing clothing from the area, and keeping the area cool for at least five minutes (preferably longer). Any burns to the face or eyes or any burns that blister should be seen by a physician as soon as possible.

Regardless of the degree of severity, report the injury to your supervisor or Principal Investigator, and complete the *First Report of Accident* form and submit it to the Worker’s Compensation department within Human Resources and Payroll.
Appendix A
Bio-Indicator Procedure

The process outlined below should be followed at least once a month to ensure autoclave effectiveness.

1. Place one Verify indicator (provided in each autoclave room) with the load to be autoclaved.

2. At cycle completion, unload the autoclave and remove the biological indicator.

3. Using the Verify Activator (made of black metal and a blue top), firmly seal the Verify indicator by placing the vial in the holder and pushing down on the blue top. The indicator is properly sealed when the cap is pushed down to the second black bar on the vial label.

4. With the indicator vial in the Verify Activator (pictured on right), push or pull the indicator vial completely through the restricted space on the Activator. The Verify indicator is properly activated when the growth medium is released from the crushed ampoule and is in contact with the spore disc.

5. Take a Verify indicator that has not been autoclaved and label it as the positive control. Activate this indicator as described above.

6. Place both activated Verify indicators in the Verify Incubator and make sure that the incubator is turned on. Leave for 24 hours.

7. Remove indicator vials from the incubator. The unsterilized positive control will indicate growth by changing color to bright yellow and turbidity will be evident. The autoclaved vial should have no turbidity and remain blue (or blue/ brown). If the autoclaved vial turns yellow, notify EHS by emailing labsafe@gmu.edu.

8. Record your results on the Biological Indicator Sheet in the Autoclave Log.

9. Dispose of the biological indicators in laboratory trash (double red bags) for autoclaving. After autoclaving, they may be disposed of as municipal solid waste in the dumpster.