

Autoclave Operations and Verification Program

rev. 2017.0601

Objective

To describe policies and procedures related to safe autoclave operation, installation of new autoclaves, routine preventive maintenance and repairs, appropriate signage, training and verification of autoclaves. These policies and procedures are best practice recommendations; however, specifics of daily use and routine maintenance shall be performed according to manufacturers' recommendations. These policies and procedures apply to all laboratories at the University of Kentucky which generate biohazardous waste with the exception of laboratories operating at Biosafety Level 3.

Implementation of this program by all research departments within the University of Kentucky will ensure that all potentially infectious waste or biohazardous waste as defined in the University of Kentucky Biosafety Manual will be decontaminated prior to disposal as solid waste in a public landfill pursuant to guidelines set forth in Guidance for Evaluating Medical Waste Treatment Technologies , US. EPA, 1993.

Definitions and Acronyms

Animal Carcasses and Tissue Waste: Animal carcasses and gross tissue specimens are to be returned to the Division of Laboratory Animal Resources (DLAR) for disposal via incineration. Follow DLAR policy for disposal of this material.

Autoclave: A self-locking apparatus for the sterilization of materials by steam under pressure. The autoclave allows steam to flow around each article placed in the chamber. Autoclaving is one of the most effective methods for decontamination of potentially biohazardous waste. The amount of time and degree of temperature necessary for sterilization depends on the materials to be sterilized and how they are packaged and loaded into the autoclave.

Biohazardous Waste: Solid and/or liquid waste that contains or has been in contact with infectious agents, potentially infectious materials or recombinant or synthetic nucleic acids. This may include waste from tissue culture, pipets, flasks, gloves, tubes, tips, etc. Any disposable laboratory items that have been in contact with or contain material which could be potentially infectious or hazardous to humans, animals, plants or the environment.

Mixed Waste: Waste material that is contaminated with a combination of chemical, biological or radiological hazards. Special consideration must be given to the disposal of this type of waste and many times autoclaving is inappropriate.

Regulated Medical Waste: Pathological waste. Gross specimens of human tissues or organs and large amounts of human blood. Special consideration must be given to disposal of this type of waste and incineration is typically required.

Responsible Individual: The individual, designated by the Department or Facility owner of the autoclave facility, tasked with ensuring verification, recordkeeping, preventative maintenance and training for autoclave facility equipment.

Verification: Process involving a biological challenge to ensure autoclaves are performing to an appropriate standard to effectively inactivate biohazardous waste.

Procedure

Safe Autoclave Operation

- Loading
 - Prepare autoclave load to allow steam penetration.
 - Check that all containers including bags are vented. Vent lids on bottles containing solutions.
 - Loosely close autoclave bags.
 - Place packaged material in a secondary container.
 - Stainless steel tray
 - Autoclavable polypropylene bin
 - Load material to allow efficient steam penetration.
 - Include appropriate verification materials with load as needed.
 - Autoclave clean items and waste separately.
 - Do not allow material to be autoclaved to touch the sides, top or bottom of the chamber.
 - Visually inspect the autoclave to ensure it is functioning properly before use.
 - Record information in user log for the load you are processing.
 - Close the door properly and securely.
 - Choose the appropriate cycle for your materials and initiate the cycle.
 - Biohazardous waste is typically processed at a temperature of 121°C and 15 lb/in² steam pressure with an exposure time of at least 20 minutes on a slow exhaust/liquid cycle.
 - Responsible Individuals should provide training to users on which cycle is appropriate for waste as cycles can vary greatly depending on the model of autoclave.
- Unloading
 - Allow the autoclave to completely finish cycle before attempting to open the door or unload. Pressure gauge must read zero.
 - Verify cycle conditions were met on autoclave readout or by visualization of the chemical integrator.
 - Wear appropriate personal protective equipment including lab coat, eye protection, closed-toe shoes and heat-resistant gloves.
 - Record results of processing on user log.
- Malfunctions
 - If the autoclave malfunctions or the Chemical Integrator and/or Biological Indicator tests fail, personnel shall notify the Responsible Individual so that the autoclave may be repaired in a timely manner.
 - Waste may be removed to an alternate autoclave which has been verified for processing until repairs are completed and the equipment is re-verified.

New Autoclaves

Before newly installed autoclaves can be used for decontamination of biohazardous waste at the University of Kentucky the following conditions must be fulfilled:

- Inspection and Certification as a Pressure Vessel
 - Performed by Boiler Inspection Section of the Kentucky State Fire Marshal's Office as required by KRS 236.110.
 - Each boiler or pressure vessel used or proposed to be used within this state, except boilers or pressure vessels exempt under KRS 236.060, shall be thoroughly inspected as to their construction, installation, and condition.
- Initial Autoclave Verification
 - Calibration services should be completed by the manufacturer on all new autoclave units. Documentation of this calibration shall be maintained with the maintenance records for the unit.
 - To verify calibration, the typical monthly biological indicator verification shall be completed prior to processing biohazardous waste in the unit.

Maintenance

- Autoclave operation and maintenance manuals shall be maintained by the Responsible Individual and provided to service technicians as needed during preventive maintenance and repair activities.
- Preventive maintenance shall be performed according to manufacturers' suggested procedures.
- Following significant maintenance activities or repairs, the monthly biological indicator verification should be completed prior to processing biohazardous waste in the unit.
- A log of all maintenance activities shall be maintained by the Responsible Individual for each autoclave.

Training

- Autoclave Training for the Responsible Individual
 - For new autoclaves, model specific autoclave training from manufacturer (or manufacturer approved contractor) completed by personnel responsible for autoclave maintenance. This training should also include a representative from the Department of Biological Safety.
 - For existing autoclaves, training will be provided by the previous Responsible Individual or an autoclave repair technician will be contracted to provide this training.
 - UK Autoclave Verification Program training provided by the Department of Biological Safety to responsible personnel is available online at the departmental website.
- Autoclave Training for Users
 - Training is provided by Responsible Individual or designee within the facility or department to users on standard operating procedures for usage of specific facility autoclave(s).

Verification Program

Each set of conditions (time, temperature, and pressure) used by each autoclave must be verified monthly using a biological indicator. Biological Indicator Test Packs are utilized and processed according to manufacturer instructions. Results shall be recorded in the Biological Indicator Test Result Log, listing the conditions, date, and initials of person performing the test

Autoclave Facility Supply Recommendations

- **Biological Indicators**
 - Self-contained vials containing spores from thermophilic bacterial species and appropriate growth media.
 - These spores represent a microbiological challenge of the autoclave operating parameters as they are typically more difficult to inactivate than the organisms present in a load of biohazardous waste.
 - 3M Attest Biological Indicator Incubators – Specially designed to provide required set temperature and to activate growth medium and spore contact. Please contact the Department of Biological Safety to receive an incubator.
 - 3M Attest Biological Indicator Test Packs and Control Vials – Test vials packaged to provide a challenge similar to the inside of a filled autoclave bag. Available through Fisher Scientific, catalog number NC9991962.
- **Chemical Indicators**
 - The Department of Biological Safety recommends the use of a Class 5 Chemical Integrator in every biohazardous waste load for autoclaves that do not generate a print-out of operating parameters reached.
 - Class 5 Chemical Integrators respond to three separate cycle parameters, time, temperature and the presence of steam in contrast to autoclave tape which only indicates that the temperature reached 121°C at some point during the process cycle.
 - 3M Comply Steam Chemical Integrators are available from Fisher Scientific, catalog number NC9813091.
- **General Equipment Recommendations**
 - Polypropylene or stainless steel sterilizing trays of an adequate size to contain autoclave bags when 2/3 full without allowing the bag to touch the sides, top or bottom of the autoclave chamber.
 - Autoclavable orange or clear bags. **DO NOT USE RED BAGS FOR AUTOCLAVED WASTE.** Red bags are used to denote regulated medical waste and they will not be accepted as regular trash by custodial staff or placed in dumpsters for subsequent landfill disposal. If your lab generates waste that you consider regulated medical waste, please contact the Department of Biological Safety to determine the proper waste procedure.
 - Heat resistant, extended cuff autoclave gloves.

Signage and Logs

- Autoclave Signage – Verified for Biohazards
- Autoclave Signage – Not for Biohazards
- Autoclave User Log
- Maintenance Log
- Biological Indicator Test Result Log

References and Regulations

- Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, <https://www.cdc.gov/biosafety/publications/bmbl5/>
- NIH Guidelines for Research Involving Recombinant DNA Molecules, <https://osp.od.nih.gov/biotechnology/nih-guidelines/>
- DOT/IATA Regulations for Shipping and Receiving Hazardous Materials, <https://www.gpo.gov/fdsys/pkg/CFR-2003-title49-vol1/content-detail.html>
<http://www.iata.org/publications/dgr/Pages/index.aspx>