VirginiaTech
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Autoclave Use and Verification Program

Purpose

This program establishes requirements for:

- ensuring all potentially infectious materials and other biohazardous waste generated through research and teaching activities is effectively decontaminated prior to disposal;
- ensuring decontamination of materials is verified using standard and approved procedures; and
- protecting the health and safety of citizens and the environment of the Commonwealth of Virginia.

Scope

This program applies to all autoclaves (floor and tabletop models) used to decontaminate biohazardous waste. This includes:

- **Waste potentially infectious or harmful to humans (i.e., infectious waste)** - Biological waste that is hazardous because of its physical and/or biological nature. All waste that contains infectious material or which, because of its biological nature, may be harmful to humans is included. This type of waste is typically generated in facilities which are designated Biosafety Level (BSL) 1-3, Animal Biosafety Level (ABSL) 1-3, or Arthropod Containment Level (ACL) 1-3.
- **Waste potentially infectious or harmful to animals or plants** - Biological waste that is hazardous because of its physical and/or biological nature and may be pathogenic or harmful to plants or animals. This waste is typically generated in greenhouses or plant growth facilities designated Plant Biosafety Levels 1, 2, or 3 (BSL-P) or in animal research facilities.
- **Waste potentially harmful to the environment** – Biological waste that is hazardous because of its physical and/or biological nature and may be harmful to the environment. This waste may be generated in any type of facility.

Note: Performance verification is highly recommended for autoclaves used to sterilize media, reusable glassware, and medical devices, or decontamination of other biological waste not considered infectious or harmful to humans, animals, or plants. This is considered a best practice and improves the quality and reliability of research data and laboratory operations.

Program Overview

This program emphasizes:

- Appropriate use and management of autoclaves
- Autoclave performance verification
- Training on:
    - Safe autoclave use
    - Cycle choices and use
    - Load configuration
    - Performance verification methods
    - Documenting autoclave use, maintenance, and training

Responsibilities

Environmental, Health and Safety Services (EHSS)

- Provide routine evaluation of autoclaves and their facilities, to include:
    - an assessment of program management and training;
    - a review of all documents related to autoclave use and verification; and
    - confirmation of pressure vessel certification by the Virginia state boiler inspector for autoclaves requiring such certification.
- Consult with departments, responsible technicians, and autoclave users on proper autoclave use and management, verification procedures, and development of training programs.

Colleges/Departments/Research Centers

- Designate a Responsible Technician for every autoclave used to decontaminate infectious and biohazardous waste. If an autoclave is shared by two or more colleges/departments/centers, then these entities shall mutually designate a Responsible Technician. The role of Responsible Technician may be rotated between entities.
- Ensure that all Responsible Technicians and autoclave users are trained and have the necessary support to implement an autoclave use and verification program.
- Manage preventative maintenance contracts and service repairs.

Responsible Technicians

- Provide autoclave-specific training directly and schedule program management training, as needed, through EHSS.
- Develop and maintain standard operating procedures for proper autoclave use and management.
- Ensure that all documentation is current and accurate.
- Monitor autoclave use.
- Serve as the contact person and primary resource for answering questions and resolving problems related to the autoclave.
- Ensure all verification testing is performed and maintain associated testing supplies.
- Maintain supply of autoclave bags.
- Ensure user log sheets, cycle charts, safety warnings, operational notices, and contact information for responsible parties are posted near the autoclave.

Autoclave Users

- Participate in required training
- Follow safe and appropriate autoclave use procedures
- Complete autoclave use log
- Promptly report problems or misuse to the Responsible Technician
- When in doubt, ask questions.

Procedure

1. Cycle Optimization

- Factors which must be considered for determining cycle types and times include:
    - Shape/size/volume of containers and materials
    - Thermoconductive properties of containers and contents
    - Density of solid materials and viscosity of liquids
    - Position of load within the chamber

- Load configuration

- For most biohazardous waste, the autoclave must reach and maintain the following conditions at the most difficult location of the load to heat (e.g., inside of a package, autoclave bag): 121ºC and 15 psi for at least 30 minutes. However, longer lengths of time or greater temperatures or pressures may be needed for some materials.
- Because the **entire** waste load must be exposed to the appropriate conditions, denser loads will require longer run times for effective steam/heat penetration. Cycle parameters for such loads must be developed, primarily by adjusting cycle times, so that successful sterilization is achieved.
- Waste decontamination loads should always be run separately in the autoclave from loads of goods to be sterilized for use.
- For every waste load that is decontaminated, regardless of cycle type, processing time, load composition or load configuration, a Class 5 Chemical Indicator (CI) must be used to determine sterilization effectiveness. This measure is necessary to ensure that waste, after autoclaving, can be handled and disposed of safely, and thus protects the health of research personnel as well as the general public.

2. Load Configuration

- Always configure a waste load in a manner that avoids stacking, crowding, and touching the sides of the autoclave chamber. Load the waste to allow for air flow around waste containers.
- Once a commonly-used load configuration has been proven to decontaminate waste successfully, it is recommended that it be documented and posted for reference by other autoclave users to save time, resources, and utilities.

3. Load Composition and Preparation

- Primary containers must not be filled beyond 75% of holding capacity and must be made of materials that will remain structurally intact during autoclaving.
- All primary containers must allow steam penetration through loosened, vented openings or permeable materials.
- Secondary containers must be used which are deep enough to contain spills (e.g., Nalgene or stainless steel pans).
- SPECIAL CONSIDERATIONS FOR LIQUIDS:
    - Bottles or flasks of liquid waste, as well as liquids to be sterilized for further use, must ALWAYS be run using a Liquids Cycle that includes a slow exhaust to avoid boil-over.
    - Do not decontaminate solid waste and liquid waste (in bottles or flasks) together in the same load.
    - Minute volumes of liquid waste (e.g., in Petri dishes, conical tubes, microtiter plates, or vials), when bagged, can be mixed with solid waste (e.g., disposable gloves, paper towels) and autoclaved successfully using a Solids/Gravity cycle or Prevacuum cycle.
    - Do not autoclave liquid waste containing hazardous chemicals or oxidizers.

4. Performance Verification/Monitoring

New Autoclaves

- Before placing a new autoclave into service, performance verification using a Biological Indicator (BI) test must be conducted and documented for each cycle programmed (i.e., each cycle type, time, and temperature) and used for decontaminating infectious and biohazardous waste.
- Waste must not be decontaminated in a new autoclave until the unit passes this performance verification test.

- To perform this verification, BI vials must be 1) within a commercially available challenge test pack, or 2) placed in a central position within a mock load of waste, to offer sufficient challenge for steam penetration.
- Self-fashioned test packs which simulate commercial products can be tested and used with EHSS approval.
- Results must be recorded in the Biological Indicator Test Results Log.

Each Load

- Each load processed in an autoclave must meet the operating parameters set up for that particular cycle, determined by the use of a Class 5 CI.
- Any load which fails to be exposed to the operating parameters must be processed again.
- Performance verification per load must occur as follows:
    - An externally placed, Class1 CI (i.e., autoclave indicator tape) must be used with each container, bag, or item processed in order to distinguish it from unprocessed materials.
    - For every load, the user must verify and document operating parameters using an internally placed Class 5 CI. Chemical Indicators must be 1) within a commercially available challenge test pack, or 2) placed in a central position within the load. Products are available which aid in the placement and retrieval of CIs from within bags of waste.
- Self-fashioned test packs which simulate commercial products can be tested and used with EHSS approval.

Monthly Performance Testing

- Operating parameters (i.e., time, temperature, and pressure) for each programmed cycle (i.e., cycle type, time, and temperature) used for decontamination must be verified monthly by BI testing.
- To perform this verification, BI vials must be 1) within a commercially available challenge test pack, or 2) placed in a central position within an actual or mock load of waste, to offer sufficient challenge for steam penetration.
- Self-fashioned test packs which simulate commercial products can be tested and used with EHSS approval.
- Results must be recorded in the Biological Indicator Test Results Log.

3. Performance Failures

Sterility Not Achieved

- Any of the following must be taken as an indication that sterility was not achieved:
    - Printout of process parameters shows sterilization conditions not met
    - Autoclave malfunctions, aborted cycles, or alarms during a cycle
    - Failure of monthly Biological Indicator Sterility Test
    - Failure of CIs to show adequate processing occurred

Failure Responses

- **Autoclave malfunction (e.g., printout, alarm, aborted cycle, power failure)**
    - The Responsible Technician must be notified immediately and the autoclave taken out of service for waste processing until the problem is rectified.
    - The Responsible Technician will determine if service personnel are required to restore function and will arrange for repairs, if needed.

- **Failure of a Biological Indicator Test**

- The Responsible Technician must be immediately notified of any BI test failure and the autoclave taken out of service for waste processing until the cause of the failure is found and rectified.
- Positive test results from BI can result from a variety of causes, such as inadequate steam quality, insufficient exposure time or temperature, poor loading practices, or product failure or operator failure.
- All possible causes should be investigated by the Responsible Technician.
- Proper autoclave function must be verified using repeat BI testing by the Responsible Technician.
- Once verified, the autoclave may be returned to service for waste processing.

- **Failure of Chemical Indicators (Class 1 or 5)**
  - The Responsible Technician must be immediately notified of any CI failure and the autoclave taken out of service for waste processing until the cause of the failure is found and rectified.
  - CI failures can result from a variety of causes, such as inadequate steam quality, insufficient exposure time or temperature, poor loading practices, or product failure or operator failure.
  - All possible causes should be investigated by the Responsible Technician.
  - Proper autoclave function must be verified using repeat CI and/or BI testing by the Responsible Technician.
  - Once verified, the autoclave may be returned to service for waste processing.

Autoclave Calibration

- May be performed on an autoclave by a service provider when other causes of performance failure have been eliminated.
- Some preventive maintenance contracts may include an annual instrument calibration check.

Documentation

Users Training Log

- Maintained by the responsible technician

Autoclave Use Log

- Must be maintained separately for each autoclave
- Must be located in close proximity to the autoclave
- All runs must be documented

Service Log

- Must be maintained separately for each autoclave
- Responsible technician must document maintenance, repair, and calibration services in this log

Biological Indicator Test Results Log

- Must be maintained separately for each autoclave
- New autoclave and monthly test results must be recorded in this log

References

- **Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, Centers for Disease Control and Prevention (CDC)**
  - **http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**
- **Bloodborne Pathogens Standard, CFR 1910.1030**
  - **http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051**
- **NIH Guidelines for Research Involving Recombinant DNA Molecules (current edition)**
  - **http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html**
- **ANSI/AAMI ST79:2006 Comprehensive guide to steam sterilization and sterility assurance in health care facilities**
  - **http://marketplace.aami.org/eseries/scriptcontent/docs/Preview%20Files%5CST790607-preview.pdf**
- **A Guide for the Safe Use of Autoclaves**
  - **University of Ottawa, Environmental Health and Safety Service, July 2003**
  - **http://www.uottawa.ca/services/ehss/docs/autoclave.pdf**
- **Laboratory Safety Accidents: Autoclaves**
  - **AIHA Laboratory Safety Committee**
  - **http://www2.umdnj.edu/eohssweb/aiha/accidents/autoclave.htm**

Definitions

ABSL (1-3) **Animal Biosafety Level 1-3** The vertebrate animal vivarium biosafety criteria which includes combinations of practices, safety equipment, and facility design requirements for experiments with animals involved in infectious disease research. These ascending levels, provide increasing protection to personnel and the environment, and are recommended as minimal standards for activities involving infected lab animals.

ACL (1-3) **Arthropod Containment Level 1-3** These ascending levels of arthropod containment add increasingly stringent measures and are similar to biosafety levels. Each level includes a combination of practices, safety equipment, and facility design requirements. The arthropod containment levels address arthropods of public health importance, such as those that transmit pathogens. Arthropods that cause myiasis, infestation, biting, and stinging are not included. Most uses of *Drosophila* spp. are also specifically excluded.

BSL (1-3) **Biosafety Level 1-3** The laboratory biosafety level criteria, designated in ascending order by degree of protection provided to personnel, the environment, and the community. The levels establish combinations of practices, safety equipment, and facility design requirements to address the increasing risk of handling agents requiring increasing levels of containment.

BSL (1-3)- P **Biosafety Level 1-3 Plants** These levels specify the physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms (i.e., viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant diseases), and plant-associated small animals (e.g., arthropods).

Biohazardous Waste Solid or liquid waste potentially contaminated with biological agents which is capable of causing disease in humans, plants and animals or contains materials harmful to the

environment. All decontaminated biohazardous, non-infectious solid waste may be disposed of with household trash provided no "biohazard" wording or symbols are visible on outer containers.

Biological Agent An organic entity, typically microscopic, which may be pathogenic to humans, animals or plants. Examples: bacteria, viruses, prions, protozoa, fungi.

Biological Indicator (BI) A commercially produced, standardized test system containing viable microorganisms (e.g., *Geobacillus stearothermophilus*) which provides a defined resistance to a specified sterilization process.

Chemical Indicators (CIs) Devices used to monitor the attainment of one or more of the parameters required for a satisfactory sterilization process, or are used in specific tests of sterilization equipment.

- **External CI**
  - **Class 1 (process indicator):** CI intended for use with individual units (e.g., packs, containers, bags) to demonstrate that the unit has been exposed to the sterilization process and to distinguish between processed and unprocessed units. This type is commonly referred to as "autoclave or sterilizer indicator tape."
- **Indicators for use in specific tests**
  - **Class 2 (Bowie-Dick test indicator):** CI designed to use in a specific test procedure (e.g., the Bowie-Dick test used to determine if air removal has been adequate in a steam sterilization process). Use of Bowie-Dick indicators is not required for this program.
- **Internal CIs**
  - **Class 3 (single-parameter indicator):** CI designed to react to one of the critical parameters of sterilization and to indicate exposure to a sterilization cycle at a stated value of the chosen parameter.
  - **Class 4 (multi-parameter indicator):** CI designed to react to two or more of the critical parameters of sterilization and to indicate exposure to a sterilization cycle at stated values of the chosen parameters.
  - **Class 5 (integrating indicator):** CI designed to react to all critical parameters over a specified range of sterilization cycles and whose performance has been correlated to the performance of a BI under the labeled conditions of use.

Decontamination Use of physical or chemical means to render an area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission). The primary objective is to reduce the microbial load so that infection transmission is eliminated. Steam sterilization (via autoclave use) is the preferred method when processing biohazardous waste.

Infectious Waste Any solid or liquid waste that is capable of producing an infectious disease in humans. This includes waste potentially contaminated with agents likely to be pathogenic to healthy humans, such as those not routinely or freely available in the community, and which may be present in sufficient quantities and with sufficient virulence to transmit disease. Decontaminated solid, infectious waste must be disposed of as Regulated Medical Waste through EHSS.

Specific categories of infectious waste:

- **Cultures and stocks of microorganisms and biologicals**
  - Discarded cultures, stocks, specimens, vaccines and associated items likely to have been contaminated by them
  - All cell culture materials
  - Discarded etiologic agents

- Wastes from the production of biologicals or antibiotics possibly contaminated by organisms capable of producing human infectious disease

- **Human blood and human body fluids**
  - Includes items contaminated with human blood or human body fluids.
- **Tissues and other anatomical wastes**
  - Human tissues, organs, or body parts
- **Sharps**
  - Items capable of puncture and likely to be contaminated with organisms that are pathogenic to healthy humans
  - Needles, syringes with attached needles, suture needles, scalpels, razors, blades
  - Pipettes, pipette tips
  - Slides, cover glasses
  - *All sharps generated through veterinary practice*
- **Animal carcasses, body parts, bedding and related wastes**
  - Includes animals which are intentionally infected with organisms likely to be pathogenic to healthy humans for the purposes of research, in vivo testing, production of biological materials or any other reason.
- **Any residue from cleanup of an infectious waste spill (soil, water, other debris)**
- **Any solid waste contaminated by or mixed with infectious waste**
  - Specimen containers
  - Disposable gloves, lab coats, masks, etc.

Steam sterilization A validated process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million (10^{-6}). This is referred to as the "sterility assurance level."