UNIVERSITY of NORTH DAKOTA
BIOLOGICAL SAFETY POLICY LIBRARY

AUTOCLAVE USE FOR STERILIZATION OF
MATERIALS AND BIOLOGICAL WASTE

Section 6, Public Safety
Policy 6.31, Autoclave Use for Sterilization of Materials
and Biological Waste
Responsible Executive: VP Finance & Operations
Responsible Office: Office of Safety
Issued: July 29, 2016
Latest Review / Revision: August 24, 2016

POLICY STATEMENT

All University of North Dakota (UND) personnel working with biohazardous agents are responsible for the proper
disposal of agents and the safety of employees, students, the public, the environment, and the public service
interests of the University. All laboratories that generate regulated biological waste (e.g., labware contaminated
with blood, blood products, non-fixed pathological waste, cultures and stocks of infectious agents and associated
biological material, animal carcasses, animal bedding and sharps) must adhere to standard operating procedure
(SOP). These SOPs also apply to the sterilization by autoclaving of different materials and items that need to be
sterile for biological work in the laboratory.

REASON FOR POLICY

This policy was developed as part of the UND Biohazardous Waste Disposal Program, and is in compliance with
Centers for Disease Control and Prevention (CDC) and North Dakota Department of Health regulations.

SCOPE OF POLICY

This policy applies to:

- President
- Vice Presidents
- Deans, Directors & Department Heads
- Area Managers & Supervisors
- Faculty
- Staff
- Students
- Others

WEB SITE REFERENCES

This policy: http://und.edu/finance-operations/_files/docs/6-31-autoclave.pdf
Vice President for Finance & Operations: http://UND.edu/finance-operations/
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RELATED INFORMATION

| Infection Control-Division of Oral Health | http://www.cdc.gov/oralhealth/infectioncontrol/faq/sterilization_monitoring.htm |
| Comprehensive guide to steam sterilization and sterility assurance in health care facilities | http://www.precisionlens.net/pdfs/AAMI_ST_79a_2012.pdf |

CONTACTS

Specific questions should be directed to the following:

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DEFINITIONS

**Autoclave**
An airtight vessel utilized for sterilization of objects by using steam under pressure. During the autoclaving process, each item is exposed to direct steam contact at the required temperature and pressure for a specified time.

**Biohazardous Agent**
All viable infectious, pathogenic, or toxin-producing agents, prions, biologically-derived toxins, or nucleic acid constructs that have the potential to affect the health of humans, animals, plants, or the environment. This includes vectors known to carry and transmit infectious agents, infected or potentially infected animals, infectious material, and recombinant or synthetic nucleic acid molecules capable of producing deleterious effects in humans, animals, plants, or ecosystems either directly through infection or indirectly through damage to the environment.

**Cultures and Stocks**
Includes etiologic agents of disease and associated biologicals, including specimen cultures and dishes and devices used to transfer, inoculate and mix cultures, wastes from production of biologicals, and serums and discarded live and attenuated vaccines.

**Decontamination**
A procedure that eliminates or reduces microbial contamination to a safe level with respect to transmission of infection.

**Disinfection**
A procedure that kills pathogenic microorganisms but not necessarily their spores. Chemical germicides formulated as disinfectants are used on
**Infectious Waste**
Is waste containing, or potentially containing, pathogens of sufficient virulence and quantity so that exposure to the waste by a susceptible host could result in the development of a communicable disease.

**Pathological Waste**
All animal and human non fixed organs, tissues, body parts other than teeth, products of conception, fluids removed by trauma or during surgery or autopsy or other medical procedure and infected animal carcasses.

**Personal Protective Equipment**
Specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts, or blouses) not intended to function as protection against a hazard are not considered to be personal protective equipment.

**Plants, plant associated materials, and plant pests**
Plants, plant associated materials, and plant pests may require autoclaving to render these materials biologically inactive prior to disposal. This is a condition of most Animal and Plant Health Inspection Service (APHIS) permits.

**Regulated Biohazardous/Medical Waste**
Any material such as: sharps; blood and blood products; pathological waste; cultures and stocks of infectious agents and associated biologicals; and animal bedding that contains or has been contaminated by a biohazardous agent. Biohazardous waste can be separated in sharps, liquid waste and solid waste.

**Steam**
The vapor created by heating water to 212°F (100°C).

**Steam Sterilization**
Moist heat in the form of saturated steam under pressure is the most widely used and dependable methods available for sterilization. The exposure of any item to moist heat at 250°F or 121°C under pressure (at least 15 psi) for 15 to 30 minutes allows the destruction of most forms of microbial life on any item.

**Sharps**
Objects that have been used in animal or human patient care or treatment or in medical, research, or industrial laboratories, including:
- Hypodermic needles
- Syringes (If the syringe without the attached needle, contained an infectious agent, blood, or blood products or bodily fluids it must be managed as an infectious waste. All other syringes (i.e., dose syringes, irrigation syringes) may be managed as a solid waste.
- Pasteur pipettes
- Scalpel blades
- Blood vials
- Needles with attached tubing.
- Broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips.

**Universal Precautions**
An infection control method where all human blood and any other potentially infectious materials are treated as if known to be positive for bloodborne pathogens such as Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV).

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**PRINCIPLES**

**OVERVIEW** – UND is committed to providing a safe and healthy work environment for those who work in research and academic laboratory settings. All personnel working with biohazardous agents are responsible for the proper disposal of biohazardous agents and the safety of employees, students, the public, the environment, and the public service interests of the University. The standard operating procedure (SOP) described in this
policy applies to all laboratories that generate regulated biological waste. This waste includes material such as labware contaminated with blood, blood products, non-fixed pathological waste, cultures and stocks of infectious agents and associated biological material, animal carcasses, animal bedding and sharps. This SOP also applies to the sterilization by autoclaving of different materials and items that need to be sterile for biological work in the laboratory.

The purpose of this document is to provide information, requirements, guidelines, and procedures for the handling and disposal of potentially infectious biological waste for all departments and units of the UND. This policy describes the use of autoclave/steam sterilization for general sterilization of labware, liquid and solid materials and biological waste. This policy was developed as part of the UND Biohazardous Waste Disposal Program, and is in compliance with Centers for Disease Control and Prevention (CDC) and North Dakota Department of Health regulations.

PROCEDURES

PACKING, LOADING, AND UNLOADING THE AUTOCLAVE

Steam sterilization can be reliable, if extended exposure periods are used and conditions are optimized for appropriate heat transfer throughout the load.

The time and temperature requirements vary for waste loads depending how loosely and densely these loads are packed, with processing times of more than 30 minutes at 121°C, reflecting variations in penetration and conductivity. The appropriate cycle time to sterilize waste for an autoclave must be based on spore testing (see to Autoclave Validation).

GENERAL INFORMATION

1. Do not sterilize clean material together with biohazardous waste in the same load and run;
2. All infectious waste must be placed in biohazard autoclave bags, and be loosely sealed with autoclave tape to allow steam to penetrate;
3. Sealed autoclave bags must be brought directly to the autoclave room in a secondary container (cart with sides, tote, etc.);
4. Ensure sufficient water in load to allow steam penetration or add 250 mL water to bags containing solids to ensure steam penetration;
5. Biohazard waste bags to be autoclaved later should be located in specific area of the autoclave room and clearly marked with biohazard symbol;
6. Biohazard waste bags cannot be held in the autoclave room for more than 24 hours without being autoclaved;
7. The bags to be autoclaved must be placed in secondary containers or decontamination pans. There should only be one bag per pan or enough space between bags to allow adequate steam circulation;
8. Do not overfill containers (prevent spill and boil over);
9. Do not allow bags to touch or strap sides of autoclave;
10. Complete daily autoclave log (see appendix A).

DO NOT AUTOCLAVE

1. Polyethylene plastics: Low-Density Polyethylene (LDPE) and High-Density Polyethylene (HDPE).
2. Solutions or waste products that contain chemicals characterized as corrosives (this includes bleach), solvents, flammables, volatiles, or radioactive materials.
3. Anything in a sealed container.

PREVENT BURNS OR SPILLS – To avoid burns and spills, observe the following safety procedures during loading and unloading the autoclave:

1. Loosen screw caps on bottles and tubes of liquids before autoclaving;
2. Be sure to wear a face shield when opening the autoclave. Escaping steam may burn face;
3. Check that chamber pressure has returned to zero before opening door;
4. Stand behind door when opening it;
5. Slowly open autoclave door only a crack.
6. Beware rush of steam. Make sure that the door to the autoclave room is closed in order to prevent steam from escaping into corridor;
7. Keep face away from door as it opens. Escaping steam may burn face;
8. Wait five minutes after opening door before removing liquids;
9. Load the bags into the autoclave and operate the autoclave according to the manufacturer’s operating instructions.

LOADING THE AUTOCLAVE
1. Ensure the autoclave is operating properly before commencing;
2. Determine the appropriate exposure time, temperature and pressure for the load to autoclave based on spore testing;
3. Ensure the autoclave attains the desired temperature (normally 121°C) and pressure (minimum 15 psi) for the desired time (minimum 30 min.);
4. Record the information in daily autoclave log (see appendix A);
5. Record results on biological test indicator results log (see appendix B).

UNLOADING THE AUTOCLAVE
1. Wait until the chamber pressure gauge reads zero before opening;
2. Open slightly to allow steam to escape (protect yourself from the steam);
3. Wait 20-30 minutes, more if necessary, for the contents of the autoclave to cool;
4. Carefully remove the secondary container with the waste bag to reduce the risk of spillage;
5. Wait until the autoclaved plastic bag has cooled completely;
6. Verify temperature and duration of exposure has been met;
7. Verify that each autoclave tape or strip has changed color. Proper sterilizing conditions turn the indicator on the autoclave tape or strip black. Similarly, check the electronic dial to ensure that the recommended temperature is achieved for the proper length of time on each load.
8. If any of the autoclave tape or strip has not changed color, then the entire load must be re-autoclaved.

Disposal of Autoclaved Biological Waste

All potentially infectious waste must be collected into a solid walled container marked with the universal biohazard symbol. Non-infectious waste (such as plant material that requires autoclaving) may be collected in bags that are not marked with the biohazard symbol. Do not mix potentially infectious waste with non-infectious waste at any time.

Do not overfill the waste container. Prepare the autoclavable bag by cinching, twisting, and securing the bag closed when it is no more than three-quarters full.

Autoclaved bags of biological waste are noninfectious and can be disposed of in the same manner as noninfectious waste in the regular trash. Regular trash is not regulated by North Dakota Department of Health.
1. After verification that each autoclave tape or strip changed color by turning black, the operator should begin to unload the bags.
2. Transfer the cold autoclaved bag to a regular black plastic trash bag.
3. Close bag tightly and dispose of it in the regular trash.
4. Do not dispose the autoclaved red bag directly into the regular trash.

Recordkeeping
1. Record all data from any run in the daily autoclave log (see appendix A) as date, time-in of treatment; the type of load (clean material or waste); quantity of waste treated; printed name and signature of the person responsible for treatment and any relevant information when applicable;
2. The person in charge of the autoclave will be responsible to maintain all records and logs;
3. Results of biological testing results (growth/no growth), should be kept in the biological test indicator results log (see appendix B) when appropriated;
4. All data collected in the daily autoclave log and biological test indicator results log must be maintained for three years by the department.
5. The autoclave operator (or person in charge) should notify their supervisor and record any incident or problems when working or monitoring the autoclave.
6. Users must also maintain records of any validation testing they perform on the autoclaves.

**Equipment and Supplies**

Supplies as biohazard autoclave bags, biohazard sign labels, biological indicators, and autoclave tape or strips should be purchased by each department. Call the biological safety officer for recommendations.

**Monitoring the Autoclave**

The CDC recommends monitoring the autoclave mechanically and chemically every time that a run is performed. In addition, the biological test should be performed monthly. At UND, the individual labs sharing an autoclave will monitor the autoclave using the *Geobacillus stearothermophilus* once a month, alternating between the participating laboratories. Autoclaves that run more than 10 runs per week (40 loads per month) are recommended to perform a biological test more often. The biological safety officer (BSO) will make recommendations for each particular case.

**AUTOCLAVE VALIDATION PROCEDURE** – Each autoclave must have a functional monitoring or measuring device (electronic or dial) to ensure that the recommended temperature is achieved for the proper length of time on each load.

Each waste bag or container decontaminated by autoclaving should have a heat sensitive indicator such as autoclave tape or strip attached to the outside of the bag. These should be visualized before disposal of each bag and should remain with the bag.

At least once a month, autoclaves that are used to decontaminate waste or to render materials biologically inactive should be tested by using a biological indicator, such as endospores from the bacterium *Geobacillus stearothermophilus*. Testing procedure:

1. Secure a biological indicator test containing endospores such as BT Sure (available from both Fisher Scientific and VWR)
2. Tie a piece of string to the testing vial containing spores to facilitate retrieval of the vial after the autoclave run. Add vial containing spores to the bag of waste, burying it within the waste (Be cautious about minimizing exposure during placement and retrieval of the testing vial. At a minimum use personal protection equipment (PPE) such as gloves, safety glasses, and a lab coat and mechanical methods such as forceps to avoid exposures. If generating high risk waste that presents a risk for potential exposure, the vial may be run inside a bag of waste that has previously been autoclaved). Leave the other end of the string attached to the vial trailing out of the opening of the waste bag.
3. Secure the waste bag and start the autoclave run.
4. Post autoclaving and once the bag has cooled, retrieve the vial.
5. For BT Sure indicators: To activate the media, with gloves on hold the indicator in an upright position, gently squeeze to break the glass ampoule.
6. Follow incubation instructions to complete the test. For the BT Sure vials, this is 55-60° for up to 48 hours.
7. Read the results of the indicator according to manufacturer instructions. For BT Sure vials this is a color indicator and reads as follows:
   a. If after 24 hours the media is yellow this = a failed test (the endospores grew, they were not killed).
   b. If after 24 hours the media is still purple = presumptive pass, but continue to incubate until 48 hours.
   c. If after 48 hours the media is still purple = passed test (all endospores were killed).
   d. If spores survived the autoclave process, growth will lead to fermentation and the production of acid turning the media yellow.
8. Record date, run parameters, autoclave tested, and test results (Appendix B).

**Testing a liquid cycle** – Use the B/T Sure Indicators to validate the autoclave liquid cycle, however, do not submerge the B/T Sure Indicators in liquid during the autoclave cycle. Simply run the B/T Sure Indicator in the
chamber alongside what would be a typical autoclave liquid cycle run (with a normal number of tubes, flasks, etc.). To run indicators submerged in liquid, these types of indicators do exist (see biological indicators, incubator and pouches in Related Information).

**Understand the limits of testing** – Testing with a temperature indicator (tape or strip) only lets indicates if the autoclave reached the approximate desired/operating temperature, but will not indicated how long that temperature was maintained. Using a recording device or computer to track time and temperature will not ensure that the materials inside the center of the waste bag have been sterilized. Validating performance using a biological indicator is the only way to ensure complete inactivation/sterilization.

**Handling an Autoclave that Tests Positive with a Biological Indicator**

1. Remove the autoclave from service. Notify a supervisor.
2. As soon as possible, repeat the biological indicator test in three consecutive cycles. If additional spore tests are positive, the items from the suspect load should be considered nonsterile and be reprocessed. Materials processed since the last acceptable (negative) biological indicator should be recalled/resterilized if possible.
3. Check to ensure that the autoclave was used correctly (for example verify that the correct time and temperature settings were used). If not, repeat using the appropriate settings and recall/reprocess all inadequately processed materials.
4. Check with maintenance support to determine if any irregularities may provide an explanation (electrical for example) or if there were any unexpected changes in the steam supply (from the standard ≥97% steam and <3% moisture). Any abnormalities should be reported to the person who performs sterilizer maintenance.
5. Check to ensure that the correct biological indicator was used, that it was not expired, and that the results were appropriately interpreted. If not, repeat the test using appropriate supplies.
6. If steps 1-5 resolve the problem, and if all three repeat biological indicators from three consecutive autoclave cycles are negative, then put the autoclave back into service.
7. If one or more biological indicators are positive however, do one or more of the following until the problem is resolved:
   a. Request an inspection of the equipment by autoclave maintenance personnel.
   b. Have the steam supply lines inspected.
   c. Discuss the abnormalities with the autoclave manufacturer.
   d. Repeat the biological indicator tests using a different manufacturer’s indicator.
   e. If there still is no resolution to the problem, close the autoclave down until the manufacturer can assure that it is operating properly. Retest at that time with biological indicators in three consecutive autoclave cycles.

**Program Evaluation**

UND expects its faculty, staff, and students to adhere to all aspects of this policy. Without commitment to health and safety in the workplace, employees/students are likely to suffer injury and illness. The Office of Safety will evaluate the effectiveness of this program on a regular basis. It may accomplish these evaluations in a variety of ways including, but not limited to:

1. Safety audits
2. Management and evaluation of employee/student training programs

Individual laboratories are responsible to follow the standard operating procedures provided in this document. Failure to comply with the procedures outlined in this document may include the following consequences:

1. Temporary removal of autoclave privileges
2. Re-training of the lab personnel
RESPONSIBILITIES

| Users                                                        | • Responsible for operating the autoclave in accordance with the parameters outlined in this SOP when the autoclave is being used to decontaminate or inactivate materials.  
|                                                             | • Run monthly tests to ensure proper performance of the autoclave. |
| Department/Facility Supervisors                              | • Responsible for maintaining autoclaves in good working order and having autoclaves tested annually by a qualified technician.  
|                                                             | • Assure that permanent and temporary workers are trained in and follow the requirements of this SOP.  
|                                                             | • Investigate and report accidents and unsafe conditions to Office of Safety.  
|                                                             | • Segregating all biohazardous waste at the point of generation from other laboratory waste. |
| Office of Safety                                             | • Providing information on autoclave testing materials and training and guidance on effective decontamination methods to the campus community.  
|                                                             | • Monitor the implementation of this SOP in various labs.  
|                                                             | • Assure that accidents and other hazardous situations which may unnecessarily expose employees to biological hazards are properly reported, evaluated, and corrected.  
|                                                             | • Act as site-wide liaison with other departments and institutions sharing common facilities and resources pertaining to biological waste management. |

FORMS

There are no forms associated with this policy.

APPENDICES

Appendix A – Daily Autoclave Log
Appendix B - Biological Test Indicator Results Log

REVISION RECORD

| 07/29/2016 – Policy Implementation | Signed by President Mark R. Kennedy |
### APPENDIX A: DAILY AUTOCLAVE LOG FOR

A COMPLETE ENTRY MUST BE MADE IN THIS LOG FOR EACH USE OF THE AUTOCLAVE

<table>
<thead>
<tr>
<th>Date/Time</th>
<th>Generator Name</th>
<th>Location</th>
<th>Time at 121°C</th>
<th>Max. Temp</th>
<th>Max. Pressure</th>
<th>Medical Waste</th>
<th>Biohazardous Waste</th>
<th>Other</th>
<th>Number of Bags/Approximate weight in Lbs.</th>
<th>Comments</th>
<th>Operator</th>
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APPENDIX B: BIOLOGICAL TEST INDICATOR RESULTS LOG

**Test Vial Information**
Test Vial #:
Test Vial Type: For example BT Sure
Test Vial lot #:
Expiration Date:
Each Vial contains: for example for BT Sure vials contain $2 \times 10^5$ Geobacillus stearothermophilus endospores
Incubation conditions: for example 55°C for 48 hours

**Autoclave Information**
Building:
Room:
Manufacturer:
Model:
Serial #:

**Test Conditions**
Time:
Chamber Pressure:
Type of Waste:
Date:
Results:

**Name of Tester:**
**Contact Person:**
**Comments:**