24.01.01.W1.41AR WTAMU Autoclave Quality Control Program

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Procedure Summary

Environmental Health and Safety at WTAMU is composed of two distinct but integrated environmental safety departments that report to the Vice President of Research and Compliance. Academic and Research Environmental Health and Safety (AR-EHS) is responsible for research and academic related compliance, which includes laboratory and academic research and the associated compliance committees. Fire and Life Safety (FLS- EHS) is responsible for fire related compliance and conducts fire and life safety inspections of campus buildings and assists with the testing of all fire detection and suppression systems.

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1. Purpose

Materials from microbiology, biomedical, and all laboratories handling materials that may be considered biohazardous, including contaminated equipment and lab ware must be rendered non-infectious prior to washing, storage or disposal, even if they are not classified as biohazardous or medical wastes. An autoclave that uses saturated steam under pressure has over the years become the generally accepted method for inactivation of all microbes.

Operational standards require that the autoclave reach a temperature of not less than 121°C (250°F) for 30 minutes at 15 pounds per square inch pressure; or in accordance with manufacturer's directions. A variety of factors can affect the efficiency of an autoclave; therefore, when treating biohazardous wastes, it is recommended that 115°C (239°F) be reached and maintained for a minimum of 20 minutes within the waste itself. Biohazard waste that has been autoclaved within these standards is considered to be no longer biohazardous and is considered solid waste for disposal purposes.

2. Scope

To ensure health and safety, all biohazardous materials and items potentially contaminated with infectious agents must be decontaminated prior to disposal. In addition, items that could be mistaken for medical or biohazardous (infectious) waste, such as agar plates used to grow non-pathogenic microbes, or items that have come in contact with biological materials should be autoclaved prior to disposal.

3. Responsibilities

It is the responsibility of the principal investigator for each lab that uses an autoclave to develop lab specific procedures for each autoclave/steam sterilizer for which they are responsible. The procedure must address each of the following:

- Time;
- Temperature;
- Pressure;
- Type of waste;
- Type of container(s);
- Closure on container(s);
- Pattern of loading;
- Water content; and
- Maximum load quantity

This standard operating procedure (SOP) outlines the elements that should be considered and included as appropriate in lab specific autoclave procedures. This lab procedure should also include a means to ensure training, recordkeeping, and testing that is conducted for each autoclave used by lab personnel. All personnel using autoclaves must be adequately trained by their PI or lab manager. **Never allow untrained personnel to operate an autoclave.** Each individual working with biohazardous materials is responsible for its proper

disposition.

3.1 Recommended Standard Practices

Review the operator's manual for instructions prior to operating the unit. Different makes and models have unique characteristics. Never exceed the maximum operating temperature and pressure of the autoclave.

Wear the appropriate personal protective equipment (PPE). Such as safety glasses, lab coat, and heat-resistant gloves, when loading and unloading the autoclave. Be especially careful not to stand too close when opening an autoclave. Often a pulse of hot steam escapes when the hatch is opened.

Place autoclavable bags containing waste in a secondary containment vessel to retain any leakage that might occur, never place autoclave bags directly on the autoclave chamber floor. The secondary containment vessel must be constructed of material that will not melt or distort during the autoclave process. **NOTE:** *Polypropylene is a plastic capable of withstanding autoclaving but is resistant to heat transfer. Materials contained in a polypropylene pan will take longer to autoclave than the same material in a stainless steel pan.*

Use heat-sensitive tape or other device to visually check that optimal temperatures have been achieved on each container that is processed. If biohazardous or medical waste is being processed; the biological indicator Geobacillus stearothermophilus (e.g. Sterikon® plus Bioindicator) should be placed at the center of a load processed under standard operating conditions, at least monthly, to confirm the attainment of adequate sterilization conditions.

Position autoclave bags with the neck of the bag taped loosely and leave space between items in the autoclave bag to allow steam penetration. Never place sealed bags or containers in the autoclave. Polypropylene bags are impermeable to steam and should not be twisted and taped shut.

Select the appropriate cycle:

- Liquid cycle (slow exhaust) for fluids to prevent boiling over;
- Dry cycle (fast exhaust) for glassware; or
- Fast and dry cycle for wrapped items.

After the cycle is complete, allow liquid materials inside the autoclave to cool down for 15-20 minutes prior to their removal to prevent boiling over. Ensure that the pressure of the autoclave chamber is at zero before opening the door. Stand behind the autoclave door and slowly open it to allow the steam to gradually escape from the autoclave chamber after cycle completion.

Autoclaving items containing solvents, volatile, or corrosive chemicals are prohibited.

Never leave an autoclave in operation unattended (do not start a cycle prior to leaving for the evening). If operational problems occur they should be reported to the lab PI so requests for repairs can be initiated. Obtaining warranties and preventative maintenance plans are strongly recommended.

3.2 Structural Inspection

Autoclaves are pressure vessels. They must be regularly maintained and repaired by qualified technicians per the manufacturer's recommended schedules. Autoclaves should be inspected regularly for damage, rust,

closure damage, element integrity, etc. For questions or assistance call AR-EHS at 651-2270.

4. Record Retention

Recordkeeping is best maintained by the individual users of the autoclave. Designate a "contact" who must:

- Test and assure proper operation of the unit;
- Notify AR-EHS when an autoclave is removed or installed; and
- Notify users when an autoclave is not functioning properly. Report the contact's name to AR-EHS and post it near the autoclave unit.

A user log should be attached to, or near, the autoclave and must be completed by operators for each sterilizing cycle. Date, time, temperature, pressure, contact time, and the operator must be recorded. This log must be kept for a period of not less than one (1) year. Whenever necessary, operators must maintain records and procedures specified for temperature monitoring, chemical integrator monitoring, and biological indicator monitoring. These records are also kept for a period of not less than one (1) year. Many autoclaves are equipped with a recording device that automatically records this information. In this case, the tape strip should be initialed by the operator. This record must also be kept for at least one (1) year.

No official state records may be destroyed without permission from the Texas State Library as outlined in Texas Government Code, Section 441.187 and 13 Texas Administrative Code, Title 13, Part 1, Chapter 6, Subchapter A, Rule 6.7. The Texas State Library certifies Agency retention schedules as a means of granting permission to destroy official state records.

West Texas A&M University Records Retention Schedule is certified by the Texas State Library and Archives Commission. West Texas A&M University Environmental Health and Safety will follow Texas A&M University Records Retention Schedule as stated in the Standard Operating Procedure 61.99.01.W0.01 Records Management. All official state records (paper, microform, electronic, or any other media) must be retained for the minimum period designated.

5. Training

All users must be trained before operating an autoclave and written operating procedures must be readily accessible. West Texas A&M University Environmental Health and Safety will follow the Texas A&M University System Policy 33.05.02 Required Employee Training. Staff and faculty whose required training is delinquent more than 90 days will have their internet access terminated until all trainings are completed. Only Blackboard and Single Sign-on will be accessible. Internet access will be restored once training has been completed. Student workers whose required training is delinquent more than 30 days will need to be terminated by their manager through Student Employment.

6. Autoclave Quality Assurance Program

An autoclave quality assurance program is required by The Texas Commission on Environmental Quality (TCEQ) for treating biohazardous waste and strongly encouraged. To ensure that biohazardous waste is properly decontaminated during autoclaving, the following procedures must be followed.

- Infectious waste must be treated in an autoclave for a minimum of 30 minutes at 121°C (250°F); however, the total processing time required to decontaminate infectious waste depends on the specific

loading factors, such as container type, water content, quantity, etc. A total processing time of 60 minutes is recommended for gravity displacement autoclaves and 10 minutes for vacuum-type autoclaves (132°C; 270°F).

- Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by superheated steam for an adequate amount of time. Since steam will not penetrate a sealed plastic autoclave bag, bags containing dry loads must not be tightly sealed (rubber band closures will allow bags to "breathe") or adequate amounts of water must be added to the load. Consult the manufacturer's instructions for sterilizing materials inside plastic autoclave bags. Liquid waste and fresh animal carcass waste may be autoclaved inside a tightly sealed bag.

NOTE: *All autoclaved waste must include a steam sterilization indicator (the use of biohazard bags with a "built-in" indicator is recommended) or pressure tape.*

The operating temperature of the autoclave must be verified for each run by maintaining a record of the temperature either as a chart, paper tape recording, or a manual recording in a logbook.

Confirm on a monthly basis (see below) that adequate sterilization conditions are being met through the use of ampoules containing heat resistant spores (Bacillus stearothermophilus) placed in the center of an autoclave load. In conjunction with the B. stearothermophilus testing, measure and record the maximum temperature achieved during the autoclave cycle through the use of a maximum registering (or "holding") thermometer on an Autoclave QC Log. Maintain records of B. stearothermophilus testing and maximum autoclave temperature recordings for a minimum of one (1) year, TCEQ recommends three (3) years.

6.1 Monthly Autoclave Testing Procedure

AR-EHS will conduct monthly certifications on designated autoclaves used in both teaching a research labs at WTAMU:

1. Place ampoule of B. stearothermophilus spores and holding thermometer in the center of an autoclave load
2. Process the load under normal operating procedures
3. The highest temperature indicated on the holding thermometer is entered on the Autoclave QC Log. If this temperature is less than 121°C, the autoclave is not to be used to treat infectious waste
4. Validate Temperature and Pressure gauge readings using PicoVACQ PT and adjust sterilization times or gauges appropriately

Autoclaves not meeting required performance regulations will be removed from service pending troubleshooting or repair.

7. Definitions

Biohazardous Waste: Waste which, because of its characteristics may cause, or significantly contribute to an increase in mortality or an increase in serious irreversible or incapacitating reversible illness; pose a substantial hazard to human health and to the environment when improperly treated, stored, transported, disposed of, or otherwise managed. Pathological, chemotherapy, pharmaceutical wastes and dead or diseased animals subjected to regulations by The Animal and Plant Health Inspection Service is an agency of the United States Department of Agriculture. Biohazardous waste includes each solid waste or waste steam in the following list:

- Human Blood and Human Body Fluids or items that contain or are caked with dried blood
- Cultures and Stocks of Microorganisms and Biologicals likely to contain pathogenic organisms
- Tissues and Other Anatomical Waste from humans
- Contaminated Animal Carcasses, Body Parts, Bedding and Related Waste from animals infected with organisms likely to be pathogenic to humans
- All Sharps
- Trauma Scene Waste
- Isolation Waste
- Any residue or contaminated soil, water or other debris resulting from the cleanup of a spill of any biohazardous waste
- Any solid waste, excluding hazardous or radioactive waste, contaminated by or mixed with biohazardous waste

Infectious: capable of causing infection

Medical Waste: A waste that meets the definition of sharps waste and/or biohazardous waste (as identified above) AND is generated or produced as a result of any of the following actions:

- Diagnosis, treatment, or immunization of human beings or animals
- Research pertaining to the diagnosis, treatment, or immunization of human beings or animals
- The production or testing of medicinal preparations made from living organisms and their products, including, but not limited to, serums, vaccines, antigens, and antitoxins

The medical waste is subjected to TCEQ regulations by the Texas Administrative Code. (30 TAC 330.1207, 330.11(f), 330.1219). Biohazardous waste is also regulated by DOT 49 CFR 173.134.

Sharps: A sharp is any item having corners, edges, or projections capable of cutting or piercing the skin.

- ***Biohazardous Sharps***: any object likely to be contaminated or may become contaminated with a pathogen through handling or during transportation, and is also capable of cutting or penetrating skin or a packaging material. Sharps include, but are not limited to, needles, syringes with needles, scalpels, broken glass, culture slides, culture dishes, broken capillary tubes, broken rigid plastic, Pasteur pipettes, and similar items having a point or sharp edge or that are likely to break during transportation and result in a point or sharp edge.
- ***"Clean" Broken Glass***: Use a broken glass box for clean broken glass disposal. Boxes must be heavy cardboard lined with a heavy plastic liner and marked 'Broken Glass". Once full, boxes are taped shut and put into the dumpster.

- ***Chemically Contaminated Sharps***: Sharps grossly contaminated with hazardous chemicals should be contained in a tight sealing, hard sided container and labeled and handled according to DRI's hazardous waste requirements.
- ***Radiologically Contaminated Sharps***: Contain in a disposable sharps container that is labeled and managed according to UNR's radiological waste requirements.

AR-EHS PERIODIC AUTOCLAVE QUALITY CONTROL PROGRAM

Sterilizer Test Data

Email notification required: ar-ehs@wtamu.edu

Please fill in all information requested for complete and accurate results.

Principle Investigator Name:_____

Office Phone Number:_____

Office Fax Number:_____

Operator Name (in capitals):_____

Operator Signature:_____

Room Number:_____ Stericon Vial ID:_____

Date of Test:_____

Make:_____ Asset Tag Number:_____

Model Number:_____ Serial Number:_____

Temperature:_____degrees C/F Exposure time:_____minutes

Pressure Reading:_____

AR-EHS OFFICE USE ONLY:

Date Received:_____

Test Results: Positive ☐ Negative (No Growth) ☐

Evaluated by:_____ Date:_____

Appendix B

BIOLOGICAL WASTE RECORD

Steam disinfection/autoclave record:

Autoclave Location:_____ Biohazard Waste:_____

PI/Lab Manager/Supervisor:_____

DATE	QUANTITY (by weight – lbs/kgs)	TIME (AM/PM)	PRESSURE(psi)	TEMPERATURE(minutes)	LOAD WASTE DESCRIPTION	OPERATOR SIGNATURE (Print name and sign)

USER MANUAL: STERIKON® PLUS BIOINDICATOR AUTOCLAVING CONTROL

For professional use only:

Application

Using the Merck Sterikon® plus Bioindicator System it is possible to check the efficiency of autoclaving cycle for 15 minutes at 121°C (250°F). Furthermore, it is possible to control the sterilization success of any kind of autoclave-loading after autoclaving.

For example: Pharmaceuticals, especially drugs in ampoule form, canned food, culture media, etc.

In the USP and EP, the use of a bioindicator for the autoclave control of pharmaceutical products is recommended.

Principle

The Sterikon® plus Bioindicator consists of an ampoule that contains a nutrient broth, sugar, a pH indicator, and spores of a non-pathogenic organism, Geobacillus stearothermophilus ATCC 7953 (sporulation optimized). The thermal resistance is such that the spores are completely killed after 15 minutes when heated in compressed steam at a temperature of 121 ± 0.5°C (245kPa). At lower temperatures or lower exposure times a small number of spores can survive and are capable of growing.

The ampoules are placed into the autoclave along with the batch to be autoclaved. After autoclaving, the success of the sterilization process is checked by incubation of the ampoules: No growth of Geobacillus stearothermophilus indicates adequate sterilization, whereas growth shows inadequate sterilization.

Evaluation

If sterilization is adequate, the Geobacillus stearothermophilus spores are killed off. The contents of the ampoule remain a clear red-violet color.

If sterilization is inadequate, some spores of Geobacillus stearothermophilus survive. The contents of the ampules then usually turn yellow-orange within 24 hours due to the formation of acid as a result of sugar fermentation and also become turbid due to microbial growth. In cases in which the spores are partially damaged, the reaction may be delayed. The contents of the control ampoule also turn yellow-orange and become slightly turbid.

Stability

When stored at the prescribed temperature (+2 to +8 °C) in the refrigerator, the bioindicator is stable at least up to the expiry date printed on the pack.

Storage

The ampoules should be stored in the refrigerator at +2 to +8 °C. Storage at room temperature (up to approximately 25°C) is possible for a limited period of about 1-2weeks. Storage at temperatures exceeding +30°C effects the product stability.

Specifications

The specifications of Sterikon® plus Bioindicator are as follows:

n= 5 x 105 - 1 x 107 spores per unit D121= 1.5 to 2.0 minutes

Procedure

An appropriate number of ampoules are included in the batch to be autoclaved. Autoclaves with a capacity of up to 250 liters should be checked with at least 2 ampoules, whilst those with a capacity of more than 250 liters should have at least 6 ampoules. In order to avoid contamination by accidental breakage, it is advisable to place the ampoules in a glass beaker.

The ampoules are placed in the autoclave at sites where the most unfavorable conditions for sterilization are thought to exist, such as at the bottom and in the middle of the autoclave. If a single large volume of material is to be autoclaved, like flasks containing a liquid, a test using the bioindicator is possible only when the ampoule is placed in the center of the vessel in question (e.g. suspended in a flask or immersed in the contents of a tin of food). The Sterikon® plus Bioindicator can also be used to check the functional efficiency, i.e. to test whether the prescribed temperature of 121°C is reached within the entire autoclave and whether the temperature remains constant over the whole of the prescribed period of 15 minutes.

After sterilization, the ampoules are removed and incubated at 60 ± 2°C. **Contact AR-EHS for access to incubation room**. Incubation room is located in Killgore Research Center Room #125. AR-EHS will evaluate the vials after 48hours. A non-sterilized ampoule should also be incubated to serve as a control. Use of the ampoules at temperatures exceeding 125°C sterilization temperature should be avoided to prevent the possibility of damaging the bioindicator.

According to the USP the heat-resistance and the number of spores are optimized, when after a sterilization time of 6 minutes at 121± 0.5°C, all ampoules contain living spores, whereas after 15 minutes autoclaving at 121± 0.5°C all spores are dead. For the period in between there will be some ampoules which contain living spores and some ampoules where all spores are dead.

NOTE: The spores are already in a nutrient broth.