

Standard Operating Procedure for Bio-waste and the Systec Autoclave

• Equipment

- Model: Systec DX-150
- Serial #: D1126

• Autoclave Maintenance Team

- Van Redila: 616-4113
- Robin Gibson: 221-6438
- Jialin Shang: 616-4159

• Manuals and Maintenance Logs

MAINTENANCE INFORMATION			
Maintenance schedule:	Yearly	Maintenance contact:	Kelly Hurtt
Company:	Microbiology International	Phone number:	(301)662-6835

- Service and maintenance records and **Certificates of Calibration** are located in CD-040 on the shelf in the Safety Corner.

• Required Supplies

- Red polypropylene Autoclave bag, **VWR Catalog Number 14220*** several sizes of bags are available.
- Stainless steel pans or autoclavable polypropylene trays.
- Distilled or Mineral-free water.
- Chemical Integrator (Thermalog*) Steam Chemical Integrator, **VWR Catalog Number 34010-086.**
- B/T Sure™ Biological Indicator Kit for Steam Sterilization, from Thermo Scientific. **VWR Catalog Number 55710-014.**
- Autoclave Tape, **VWR Catalog Number 58752-781.**

- **Personal Protective Equipment**
 - Nitrile Gloves
 - Heat resistant gloves
 - Eye Protection (safety goggles, glasses)
 - Lab coat

- **Safety and Health**

Physical Hazards

Needle Sticks

Burns

- Caused by contact with outside of autoclave
- Caused by contact when removing autoclaved items
- Caused by contact with pressurized Steam

Warning! Burn Hazards

- Clogged lines, equipment malfunction or a failure in the steam supply may cause the autoclave chamber to fill with scalding water. If water leaks from front of the autoclave, DO NOT open the chamber door. Burns from scalding water may otherwise result.

Health hazards

Potential exposure to infectious agents through either airborne, droplet, or contact transmission.

Special Notes:

When processing liquids, use only flasks and self-venting automatic sealing stoppers recommended by the manufacturer. This will help to prevent sealed bottles from exploding and glass containers from rupturing. Waste that contains bleach may harm an autoclave.

- **Preparation, Monthly Testing, Loading and Unloading**

- **Preparation For Daily Operation:**

1. Immediately after use, clean instruments thoroughly to dispose of any residue.
2. Materials, including materials used for inner wraps, shall be compatible with the item being packed and the sterilizing method selected.
3. Do not place items to be sterilized directly on the chamber's wall. Place the material only on stainless steel trays and racks.
4. All instruments must be sterilized in an open position.
5. With each load, the sterilizer will be evaluated for effectiveness of adequate sterilization conditions by **using the Chemical Integrator (Thermalog*) test strip**. Place the chemical integrator in the center of **each load** to confirm attainment of adequate sterilization conditions.
 - a. ***Please indicate on the autoclave use list attached to autoclave that you have used the Chemical Integrator.***
6. Place instruments with ratchets opened and unlocked or clipped on the first ratchet position.
7. Disassemble or sufficiently loosen multiple-part instruments prior to packaging to permit the sterilizing agent to come into contact with all parts of the instrument.
8. Tilt items prone to entrap air and moisture on their edge.
9. Load items within the boundaries of the tray so that they do not touch the chamber walls, or fall off when the loaded car is inserted into the autoclave.
10. Load trays loosely to capacity. **DO NOT OVERLOAD AUTOCLAVE.**
11. Liquids: Use only heat-proof glass, filled to 2/3 capacity. Ensure that the glass container is covered, but not sealed to prevent pressure build-up.
 - a. **The flexible temperature sensor must be placed in a liquid reference vessel. The reference vessel should have the same size and fill volume as the vessel containing the liquid to be sterilized.**

- **Monthly Testing**

Monthly, **the sterilizer will be evaluated for effectiveness under full loading using the B/T Sure™ Biological Indicator Kit for Steam Sterilization, from Thermo Scientific.** Test by placing in the center of a load processed under correct conditions [(**To be performed by Autoclave Maintenance team**) *Supplies under sink*].

- Use spore ampules or strips with an average population of 10^4 to 10^6 organisms adjusted to the following resistance data: survives steam heat @ 250 F or 121C for five minutes and is killed at 250F in thirteen minutes.
- The most reliable means for determining whether a sterilizing cycle has been successful is by planting biological indicators throughout the load before the load is subjected to the sterilization process.
- Operate the autoclave in accordance with manufacturer's instruction. The cycle (time and temperature) shall be the minimum as required by user's lab procedures.
- Temperature should be recorded on the chart recorder.
- Upon completion of cycle, fast exhaust the chamber and remove test spore strips from the autoclave.
- Incubate the bacillus stearothermophilus test strips according to manufacturer's instructions.
- Record results on the **Quality Control Checklist** in the Autoclave Book located in **CD040 Safety Corner (To be performed by Autoclave Maintenance team)**.
- Following test, dispose of Biological Indicators in **Biohazardous Waste**.

BIOLOGICAL INDICATOR TEST INSTRUCTIONS

Monthly biological indicator monitoring is required for all autoclaves used for sterilization of biohazardous waste at UW. This test validates that the autoclave is sufficiently inactivating biological or infectious materials.

EQUIPMENT	
Biological indicator	Use <i>Bacillus stearothermophilus</i> spore strips or ampoules with an average population of 10 ⁴ to 10 ⁶ organisms.
Supporting laboratory equipment as needed	May include (depending on spore product in use): <ul style="list-style-type: none"> ▪ Incubator or heat block ▪ Refrigerator ▪ Culture media ▪ Glassware
Quality Control Checklist	Use to records results.
PROCEDURE	
1. Place indicator in center of load.	
2. Select cycle used for sterilization of biohazardous waste. The cycle must include a minimum temperature of 121°C or 250°F for 30 minutes or longer , depending on size and compaction of the load. The full cycle time will take 60-90 minutes	
3. Record chamber temperature on the Quality Control Checklist.	
4. Remove indicator when cycle is complete and load is cool enough to handle.	
5. Follow manufacturer's instructions for activating and incubating the indicator. Remember to incubate a non-autoclaved indicator as a positive control.	
6. Record results on Quality Control Checklist.	
7. If growth occurs in the processed indicator, the autoclave has not inactivated the spores. Immediately contact the person responsible for the autoclave. The autoclave must remain out of service until a successful biological indicator test is achieved. The responsible person must inform users not to use the autoclave and post a "Do Not Use" sign while the autoclave is out of service.	
8. Maintain the Quality Control Checklist in the lab/facility for six years.	

- **Lab: Preparation of Biohazardous Waste for Autoclaving**

(FOR RED PLASTIC SHARPS CONTAINERS, SEE "SHARPS" SOP BELOW)

1. Wear a lab coat, eye protection, Nitrile gloves and closed toe shoes.
2. Using a Polypropylene bag(s), place biowaste into bag. **(Do not put sharp or pointed contaminated objects into an autoclave bag. Place them in an appropriate rigid sharp disposal container).**
3. Gather the top of the biowaste bag loosely and secure with a twist tie that comes with autoclave bag, a large rubber band or autoclave tape. **(Leave autoclave bags loosely secured to allow steam to penetrate them).**
4. **Use Caution when handling an infectious waste autoclave bag in case sharp objects were inadvertently placed in the bag.**
5. Transport the biowaste, **using a cart**, to the autoclave room.

Note: Never lift a bag from the bottom to load it into the chamber. **Handle the bag from the top.**

- Do not overfill an autoclave bag.
- Do not overload an autoclave.
- Fill liquid containers only half full.
- Loosen cap or use vented closures.

SHARPS SOP:

1. When Sharps container is no more than 2/3's full, close the lid and put autoclave over the lid and sides, being careful not to block the vent holes.
2. If you have not done so already, **label the container with PI and room number.**
3. Transport to the autoclave room and let VAN OR JIALIN know you have done so.
4. Jialin or Van will autoclave the sharps container (see cycle below) and place it in a rigid secondary container for transport to Laboratory Services in T-276. For transport, the secondary container will be placed on a cart.

- **Cycle, Temperature and Time Selection**

OPERATE				
<ul style="list-style-type: none"> • Close and lock door. Be sure door is secure before starting a cycle. • Select appropriate cycle to achieve minimum of 120°C/250°F for at least 30 minutes. 				
Waste Type (select all that apply)	Cycle Number	Liquid / Dry	Sterilization Time	Sterilization Temperature
<input checked="" type="checkbox"/> Bagged solid biohazardous waste	#5	Dry	30"	134°C
<input checked="" type="checkbox"/> Liquid biohazardous waste	#6	Liquid	30"	134°C
<input checked="" type="checkbox"/> Sharps containers	#2	Dry	90"	134°C
<input checked="" type="checkbox"/> Biohazardous lab glass and plastic	#2	Dry	90"	134°C
<ul style="list-style-type: none"> • Record run on autoclave log sheet. • Do not open the autoclave door during a cycle! If necessary, abort the cycle and wait until chamber depressurizes. • If cycle fails, notify responsible person and follow back-up plan. Post the "Do Not Use Autoclave" poster. The waste still needs to be sterilized even if autoclave tape changed color. Cycle failure includes: <ul style="list-style-type: none"> ▪ Autoclave tape did not change color. ▪ Chemical integrator failed. ▪ Cycle did not maintain sterilization temperature for required time. ▪ Biological indicator test failed (refer to Biological Indicator Instructions). 				

- **CD046: Loading the Autoclave (Solids, Liquids, Waste)**

1. Before starting, ensure the instrument is **OFF** and there is no pressure in the autoclave.
2. Switch on the autoclave, if required; press the **OPEN** key to unlock the door. Open the door as far as it goes
3. Load the autoclave with the item that is to be sterilized. **Bags or sharps containers must be placed in stainless steel bins no plastic.** {Biovaste bags must be double bagged.}
4. Attach the provided **chemical sterilization indicator** on the outside one of the bags using a stapler.
5. Place the flexible temperature sensor, with provided silicon tubing on sensor:

- **For "Solids" cycles:** The temperature sensor should be placed in the holder provided for it in the sterilization chamber, or else freely in the chamber. **DO NOT PLACE THE FLEXIBLE TEMPERATURE SENSOR IN THE ITEM BEING AUTOCLAVED.**
 - As a safety feature, the unit requires the operator to confirm that they are not autoclaving liquids in a non-liquid program. **Programs 1-5 are not for liquids.** When starting programs 1-5, the unit displays "No Solutions!!!" and asks for an access code: **enter code: 1000**
 - **For "Liquids" cycles:** The flexible temperature sensor must be placed in a liquid reference vessel that you provide. The reference vessel should have the same size and fill volume as the vessel containing the liquid to be sterilized.
6. Close the door until the seal comes into contact and hold it in until it locks.
 7. When the following appears in the display: **READY** you must select the cycle.
 - 8. Select the cycle using the arrow keys (appendix 4). Make sure the cycle is the correct one!**
 9. Press the **START** key. The autoclaving procedure runs automatically from this point on.
 10. Complete the **AUTOCLAVE LOG SHEET** (Appendix 2).
 11. When the cycle is complete, an acoustic signal sounds and the display shows **CYCLE ENDED**. Press the **OPEN** key to open the door.

Turn Instrument OFF when not in use.

- **Unloading of autoclave and disposal of Biowaste**

1. **Warning! All items are extremely hot!**

2. Wear a lab coat, eye protection, heat resistant gloves and closed toe shoes.
3. Allow items to stand in the autoclave for at least 10 minutes.
4. Visually inspect bags, boxes and sharps containers for protruding objects.
5. Cautiously remove items and place in a safe area to cool, do not agitate containers.
6. Record run information on the **AUTOCLAVE LOG SHEET** (Appendix 2).

- **Disposal of Biowaste**

DISPOSAL	
<ul style="list-style-type: none"> • Black lines on autoclave tape indicate waste is safe for handling by custodial staff. • Refer to the Biohazardous Waste Flow Chart for your location. 	
Waste Type (select all that apply)	Disposal information
<input checked="" type="checkbox"/> Bagged solid biohazardous waste	<ul style="list-style-type: none"> ▪ Place in regular trash container for custodial pick-up and disposal via municipal waste.
<input checked="" type="checkbox"/> Liquid biohazardous waste	<ul style="list-style-type: none"> ▪ After liquids have cooled, dispose via sanitary sewer.
<input checked="" type="checkbox"/> Sharps containers	<ul style="list-style-type: none"> ▪ Depends on location; refer to the Biohazardous Waste Flow Chart for your location.
<input checked="" type="checkbox"/> Biohazardous lab glass and plastic	<ul style="list-style-type: none"> ▪ Place next to regular trash container for custodial pick-up and disposal via municipal waste.

7. Autoclaved **Bagged Waste Disposal:**

- a. Once the cycle is complete, securely close biowaste bag with twist tie that comes with autoclave bag or tie bag into a knot in order to reduce odors and prevent spills.
- b. After double bagging biowaste, place it in a regular black trash bag and dispose in trash.
- c. LABEL THE BAG WITH PI NAME AND ROOM NUMBER.
- d. Cleaning staff will empty the trash.

e. Transport all items extremely carefully!

8. Autoclaved Sharps container/s

- a. Once they have cooled, place sharps containers in rigid sturdy container such as a covered Rubbermaid or cardboard box and transport to the T-wing using a cart.
- b. Place the containers in the designated custodial pick-up zone in T-276.

9. Close chamber door when you are finished using the autoclave.

Accidents and near misses:

ACCIDENTS AND NEAR MISSES	
<ul style="list-style-type: none"> • Post the Exposure Response Poster near the autoclave. • In the event of an accident, immediately provide first aid and get help. • Report any accidents or near misses via the Online Accident and Reporting System (OARS). 	
BACK-UP PLAN IF AUTOCLAVE IS OUT OF SERVICE (SELECT ONE):	
<input type="checkbox"/>	Use an alternate autoclave. Specify location:
<input type="checkbox"/>	Transport waste to Health Sciences Laboratory Services (T276).
<input type="checkbox"/>	Ship waste off-site via contracted carrier (training and account set-up required).
<input checked="" type="checkbox"/>	Store waste in secure freezer for up to 90 days. Specify location: locked freezer in hallway CD056

• MALFUNCTION, MECHANICAL PROBLEMS AND EMERGENCY PROCEDURES

Notify the Autoclave Maintenance Team with any mechanical problems/failures. The Autoclave Maintenance team should notify the approved vendor for service. If the autoclave is not usable please post the "DO NOT USE AUTOCLAVE" flier located on the wall to the left of autoclave! (Appendix 5.)

Emergency Response:

In the event of an employee injury during regular working hours, go to Employee Health in UWMC Room **NE210 UWMC**. After hours, you will need to go the Emergency Department located at **NE207 UWMC** or call 911 for immediate emergency assistance.

Spills:

If there is a spill inside the autoclave chamber, allow the unit to cool before attempting to clean up the spill. If glass breaks in the autoclave, use tongs, forceps or other mechanical means to recover fragments. Do not use bare or gloved hands to pick up broken glassware.

Accident reporting:

Report any accidents or near misses via the Online Accident and Reporting System (OARS). (<http://ehs.washington.edu/ohsoars/index.shtm>)

Appendix #1

Maintenance on the Systec Autoclave

Before every maintenance or care activity, ensure that the sterilization chamber is pressureless, and disconnect the autoclave from the mains supply

CLEANING THE AUTOCLAVE

Never use steel wool or wire brushes for cleaning, as they scratch the surface and can do long-term damage to the autoclave. As a cleaning agent, use citric acid, of which approx 2530mL should be dissolved in a liter of water. Use only a soft cloth or a sponge.

There is a dirt strainer in the interior of the sterilization chamber. This is located in the middle of the floor and can be taken out without using tools and then cleaned. This should be done weekly, and dirt should be immediately cleaned off.

Appendix #2

Autoclave make/model:	Systec DX-150	Location (building/room number):	CHDD /CD 046
Lab/Facility name:	Rubel/Tempel/Stone Labs Virginia Merrill Bloedel Hearing Research Center	Principal Investigator/ Supervisor name:	Edwin Rubel/Bruce Tempel/Jennifer Stone
Person responsible for autoclave:	Jialin Shang	Phone number:	206 616-4159

Date	Time	Cycle #	Contents/Cycle Type			# of items	Sterilization Time (min)	Pressure (PSI)	Post-run results			Biological Indicator Used? (Y/N)	Operator/tel. ext PLEASE PRINT	Comments
			Contents: Media=M Liquid=L Glass=G Plastic=P	If using liquid media did you remember to use silicon tubing in the reference vessel?	Waste: Dry Bagged=DB Liquid=LW Biowaste=BW Sharps Container=SC				Max Temp Reached	Chemical Integrator Result (pass/fail)	Tape Result (pass/fail)			

Appendix #3

Cycle /Program #	Use	Sterilization Temp [C]	Sterilization Time [min]	Dry Time [min]
#1 Instruments	Glassware flasks/bottles tips/tubes	121°C	20"	10"
#2 Instruments	Sharp collectors Needles/Blades Glass Pasteur	134°C	90"	N/A
#4 Waste (bags)	Dry Bags	121°C	30"	N/A
#5 Waste (bags)	BioWaste Bags	134°C	30"	N/A
#6 Liquid Waste	BioWaste Liquid	134°C	30"	N/A
#8 Liquid	LB Agar Solutions Water	121C	30"	N/A

Appendix # 4

Do Not Use Autoclave.	
_____ was Name	
Notified on _____ Date	
Repairs are expected by _____ Date	
Please contact _____ with any Name	
Questions at _____ Phone / email	
Posted: _____ Date	by _____ Name