



Applied Microbiology  
&  
Biotechnology Laboratory

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## Standard Operating Procedure

### AMBL-010-B

<b>Prepared:</b>	5/12/2021
<b>Prepared by:</b>	Terry E Baxter
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<b>Revised by:</b>	Terry E Baxter

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## Portable Sterilizer Operation and Performance Verification

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### SOP SUMMARY

This SOP describes the procedure for safe operation of the portable steam sterilizer located in room 241 of the environmental engineering laboratory, as well as the basic procedure for verifying its ability to perform sterilization.

This procedure is based on *Autoclave Operations and Verification Standard Operating procedure (SOP) 2017*, NAU Environmental Health and Safety, and on the equipment manufacturer's recommendations.

### EQUIPMENT IDENTIFICATION

**Sterilizer make/Model:** Zhejiang Xingfeng Medical Apparatus Co.  
Model XFS (K) - 280 CB

**Location (Building-Room):** Building 69 (Engineering), Room 241

**Responsible Department:** CECMEE

**Responsible Individual & Phone:** Terry Baxter, 928-523-2008

**Responsible Individual & Phone:** Adam Bringhurst, 928-523-1164

## ENVIRONMENTAL HEALTH AND SAFETY

**Hazards Assessment:** This procedure involves the use of a portable steam-generating sterilizer that is operated under conditions of high temperature (121°C) and pressure (15 psi), and presents a severe burn risk. More specific hazards associated with using the sterilizer include:

- Burns from high-temperature steam exiting the lid when opened or from containers of sterilized liquids after cycle completion
- Burns from high-temperature steam emitted from the steam release valve or security valve
- Burns from touching sterilized materials or the sterilizer walls, lid, or clamps.
- Burns from the hot water in the bottom of the sterilizer if drained from the sterilizer or from hot sterilized liquids
- Injury to fingers or hands when securing the sterilizer lid
- Bodily injury, dismemberment or death should an explosion occur

**Safety Equipment and Engineering Controls:** This procedure requires that the sterilizer operated when other work in the immediate proximity to the sterilizer can be minimized. **The sterilizer cannot be left unattended during operation.**

**Personal Protective Equipment (PPE):** This procedure requires adhering to the environmental engineering laboratory's safety standard (SOP 002A) and the use of the following PPE.

- Closed-toe footwear
- Safety goggles or glasses,
- Laboratory coat, and
- Heat resistant gloves when unloading the sterilizer within 2 hours from the end of a cycle, or
- Gloves (nitrile, PVC or neoprene) when unloading the sterilizer after 3 hours from the end of the cycle or when materials sterilized have cooled to room temperature.

### **Analysis-derived Wastes and Disposal:**

Waste Generated	Hazardous ( Y / N )	Disposal
This procedure is does not generate wastes unless spillage or boil-over of growth media or liquid agar occurs inside the sterilizer.	N <sup>1</sup>	Disposal of liquid waste from spillage or boil-over of growth media must follow the disposal requirements given in the particular growth media's preparation and use.

<sup>1</sup> Significant health or environmental effects are not anticipated from the routine use of growth media when good laboratory practices are followed.

## PROCEDURE DESCRIPTION

### 1.0 Introduction and Applicability

The Environmental Engineering Laboratory uses a portable steam-generating sterilizer for biological growth media, glassware, instruments and water used to conduct tests for detecting indicator coliform and fecal coliform bacteria, as well as tests involving the growth and physiochemical influence of phytoplankton on water chemistry. Some of these tests also produce liquid wastes that require sterilization before their disposal. Because of the severe hazard associated with high temperature steam and a high-pressure condition, users of this sterilizer must be approved according to the procedure described in this SOP.

The procedure and policies described herein are considered applicable to all individuals operating the sterilizer.

### 2.0 Definitions for Terms and Abbreviations Used

Previously defined terms and abbreviations used may be found in SOPs that precede this SOP and may not be repeated here.

- a. **Authorized Operator.** See SOP 010A.
- b. **Biohazardous Waste Materials.** See SOP 010A.
- c. **Biological Waste Materials.** See SOP 010A.
- d. **Mixed Waste.** See SOP 010A.
- e. **Performance Verification.** See SOP 010A.
- f. **Responsible Individual(s).** See SOP 010A.
- g. **Sterilizer.** A steam sterilizer maintains steam under conditions of high temperature and pressure for an appropriate amount of time in order to sterilize materials.
- h. **Sterilized Materials.** Materials that are sterilized include, but may not be limited to, any waste, media, glassware or instruments that have completed at least one sterilization cycle. Sterilized materials that are not considered wastes are labeled as "sterilized."

### 3.0 Training Requirements and Approved Users

Any individual operating the sterilizer in the environmental engineering laboratory regardless of prior experience must have successfully completed a sterilizer training session conducted by the Laboratory

Manager or Laboratory Director. This training requires the individual to complete the following.

- Read this SOP (AMBL 010B *Portable Sterilizer Operation and Performance Verification*),
- Read NAU Office of Regulatory Compliance's, *Autoclave Operations and Verification Standard Operating Procedure (SOP) 2017*,
- Complete a hands-on instruction session on operating the sterilizer, and
- Conduct a final demonstration of proper sterilizer operation under the supervision of the laboratory Director or Laboratory Manager.

Authorized operators who have completed the sterilizer training and demonstrated their competency to operate the sterilizer are added to the Authorized Sterilizer Operators list that is to be kept current by the responsible individual and visibly located near the sterilizer. A copy of this SOP is also required to be kept readily available near the sterilizer.

#### **4.0 Restricted Materials**

Certain materials are restricted from being sterilized in the environmental engineering laboratory. In addition to mixed wastes, which are strictly forbidden, animal or human tissues, cells, carcasses or specimens, and bases, acids, or oxidizing agents (such as chlorine bleach), flammable or explosive agents, or water reactive chemicals are not to be sterilized.

Do not sterilize individual liquids greater than 2.0 liters in volume.

Do not sterilize cracked glassware or glassware unable to be sterilized, and plastics that are not heat resistant, with the exception of disposable plastic petri dishes contained in a biohazard bag. When in doubt whether an item is able to be sterilized, check the manufacturer's specifications for that item's material.

Any materials that do not need to be sterilized, are not to be sterilized.

#### **5.0 Sterilizer Operating Requirements and Recommendations**

- a. Operation of this portable sterilizer requires that the trained and authorized sterilizer operator remain in the laboratory and is actively monitoring the sterilizer while the sterilizer is plugged in and operating. Make certain that you have scheduled your time appropriately to satisfy this requirement.
- b. It is recommended that all materials sterilized are prepared before your set the sterilizer up for operation.

- c. It is recommended that a minimum of 90 minutes be scheduled for setting up the sterilizer for operation and continued monitoring until it is unplugged.
- d. Plan well in advance of when you need to use the sterilizer and coordinate with others who you know are currently and routinely using the sterilizer or will be working in the vicinity of the sterilizer during its operation.
- e. Do not unload the sterilizer until after 2 hours have elapsed since the time when the sterilizer has been unplugged at the end of a cycle, unless agar-based media is sterilized prior to preparing pour plates or slant tubes.
- f. When sterilizing agar-based media and releasing pressure after a sterilization cycle has completed, slowly release the pressure over a period of 15 minutes to prevent boil-over.
- g. Under no circumstance is the sterilizer lid to be removed when the gauge pressure is greater than zero.

## **6.0 Preparing Materials for Sterilization**

Materials that are otherwise not restricted may be sterilized once they have been properly prepared for sterilization. When preparing materials for sterilizing, wear personal protective equipment that is appropriate for the material being handled, including working in a fume hood if necessary.

The inner sterilization pan has limited space for sterilizing materials and care must be taken to avoid over loading the sterilizer with materials to the extent that adequate steam penetration cannot be provided. Additionally, materials are loaded from the top, which means that the vertical placement of materials and the need to keep materials from touching the side wall of the inner pan will limit how much can be sterilized at one time.

## **6.1 Preparing Waste Materials**

- a. All non-liquid biological and biohazardous wastes with the exception of sharps (see 6.1.c below) must be placed in a RED biohazard bag (meeting OSHA and ASTM standards), loosely closed to allow steam penetration. Only one biohazard bag can be sterilized at a time and autoclave tape must be placed on the bag to verify sterilization. Place the bag in a large glass beaker.
- b. All liquid wastes are placed in containers that can withstand the autoclaving process and filled to a level no more than 2/3 full to avoid boil-over.

- For open top containers such as flask or bottle without lids, crimp an aluminum foil wrap over the opening
- For bottles with lids, loosen all lids to avoid exploding the container and to allow steam penetration.

Place autoclave tape on at least one container to verify sterilization.

- c. Sharp biohazardous waste materials are placed either in a rigid red plastic “sharps container” or in a beaker that can be sterilized.

## **6.2 Preparing Equipment, Glassware, and Liquids**

- a. The sterilizer has a limited amount of space for sterilizing equipment, glassware or liquid-filled containers. Do not pack items so close together or against the chamber wall such that the steam cannot penetrate throughout the materials being sterilized.
  - Glass pipettes are placed in a metal pipette canister, which is then placed upright on the perforated stainless-steel tray in the bottom of the sterilizer chamber.
  - Metal equipment, glass rods, or glass tubing are loosely placed in a large beaker.
  - Glassware is placed loosely inside the chamber but are not to be stacked so items do not fall and break.
- b. Place autoclave tape on at least one of the items that will be located in the center of the chamber to validate sterilization – avoid placing this tape on any items that you do not want to have an adhesive residue remaining when the tape is removed.
- c. Place liquids in containers to no more than 2/3 full to avoid boil-over.
  - For open top containers such as flask or bottle without lids, crimp an aluminum foil wrap over the opening.
  - For bottles with lids, loosen all lids to avoid exploding the container and to allow steam penetration.
- d. Agar-based liquids are to occupy no more than 1/2 of the container’s volume. This requirement avoids boil-over as pressure is being released.

## **7.0 Preparing the Sterilizer**

- a. This sterilizer requires a 220-volt power source and is used in Room 241 on the laboratory bench in a location that allows the power cord to reach the 220-volt outlet.
- b. Remove all flammable or potentially flammable materials from the area immediately surrounding the sterilizer. Leave the area around the sterilizer as unobstructed as possible.

- c. Check the sterilizer log sheet to ensure that the sterilizer has been emptied by the previous operator. The lid should be affixed to the top of the sterilizer with two clamps. This also indicates that the sterilizer has been properly shut down and emptied.
- d. Release the clamps and remove the lid. Place the lid on the bench top, upside down and resting on the handle so that the gauge does not touch the bench. Do not place the lid with the bottom side down, otherwise the exhaust line may become damaged.
- e. Remove the inner pan and set it aside.
- f. Verify that the water has been emptied from the sterilizer. If it has, check that the drain valve is closed. If not, move the sterilizer to the edge of the bench so that the water may be drained from the sterilizer into a separate container. Once drained, close the drain valve and move the sterilizer back from the edge of the bench.
- g. Add 4.5 liters of deionized-Reverse osmosis (DI/RO) water. If the sterilizer is filled with either too little or too much water, the water level alarm will sound once it is plugged in and the sterilizer will not operate.
- h. Replace the inner pan and rotate it so that the protective exhaust valve line sleeve on the inside of the pan is aligned with one of the lid clamp locations (see Figure 1). Make sure that the perforated base plate is inside and on the bottom of the inner pan.
- i. Plug in the power cord to the 220-volt outlet and wait approximately 3 seconds to verify the water volume added is correct. If the water level alarm sounds, unplug the power cord and remove the inner pan to correct the water volume. Repeat this step until the water level alarm does not sound and unplug the power cord.
- j. While the sterilizer is plugged in, verify that the operating temperature is set to 121°C (see Figure 2) – **DO NOT CHANGE** this setting. Again, make sure you unplug the power cord after this has been verified.

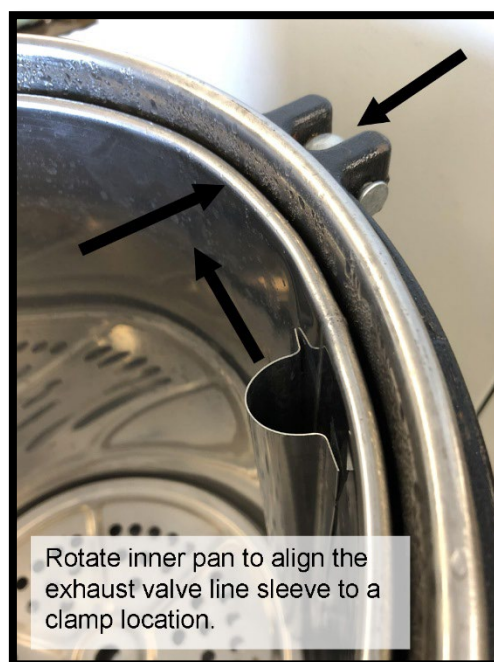


Figure 1. Adjusting the location of the inner pan.

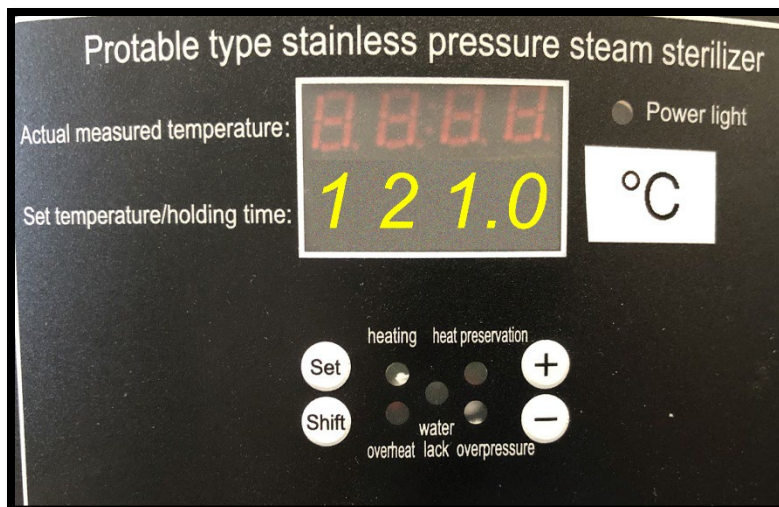


Figure 2. Temperature set point must be 121°C.

## 8.0 Loading the Sterilizer

- Wear lab coat, eye protection, gloves and closed-toe shoes.
- When loading materials into the sterilizer, use the appropriate personal protective equipment described above in the Environmental Health and Safety section.
- Load materials in a way that allows complete circulation of steam and so that materials do not touch the inside wall of the sterilizer's inner pan. Do not stack or load materials in a way that they will fall over. Do not overload the sterilizer.
- Check to verify that autoclave tape has been included with your load. If not, include autoclave tape now.
- Close and clamp the lid. While placing the lid on the top of the sterilizer, direct the exhaust valve line into the protective sleeve on the wall of the inner pan. Center and align the lid to the clamp locations. Secure the lid by sequentially fastening each pair of opposing clamps until all clamps are firmly secured.
- See the manufacturer's instructions for additional information.

## 9.0 Initiating the Sterilization Cycle

- Prior to initiating the sterilizer, fill out the Portable Sterilizer Operations Log located near the sterilizer (see below).
- Plug in the power cord. The sterilizer will begin heating up the water and the front panel (Figure 2) displays the water temperature (on the top) and the set temperature of 121°C (on the bottom). Do not change the set temperature, leave this at 121°C.



- c. Check the previously set holding time (sterilization cycle time) and if necessary, adjust it as follows. Refer to Figure 2 for the button described.
- Press the **[Set]** button to cause the temperature 1-degree position to blink.
  - Press the **[Set]** button a second time to display holding time and cause the 1-minute position to blink.
  - Press either the **[+]** button or the **[-]** button display to increase or decrease the time by 1 minute until the desired time is reached.
  - Press the **[Set]** button one last time to complete the setup and return to the set temperature display (121°C).
- d. The sterilizer will continue to heat the water until a temperature of 105°C is reached (approximately 20 minutes). At this time, an alarm will sound (for 10 seconds) indicating that it is time to release some of the pressurized steam so that cooler air retained in the sterilizer may be exhausted from the sterilizer (see Figure 3).

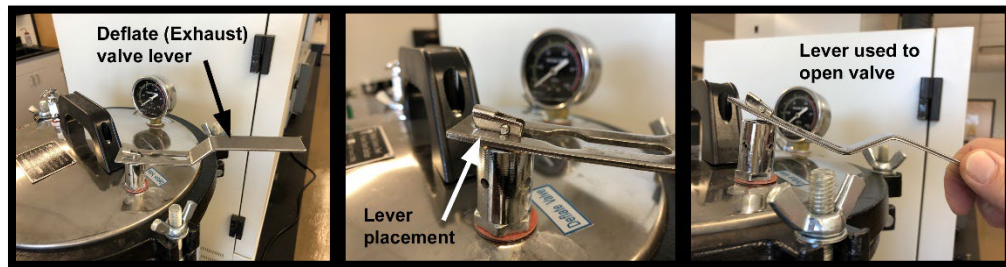


Figure 3. Placement and use of exhaust valve lever for releasing steam pressure.

- Slowly depress and hold the end of the lever down only until the alarm stops sounding. DO NOT release the steam pressure too quickly.
- e. Slowly release the lever and allow the exhaust valve to close.
- f. Wait for the temperature to reach 121°C (approximately an additional 10 minutes). The automatic countdown will begin and the display above the holding time, when selected, now shows the time remaining. You may monitor the time remaining by pressing the **[Shift]** button to change display to show the time.
- g. Do not operate the sterilizer for more than 60 minutes without ending the cycle to allow the sterilizer to cool and be unloaded. Recommended cycle times are as follows.
- 1) Cycle times for liquids are based on the container with the maximum liquid volume and whether the liquid is a waste or a prepared growth media with or without agar.

Liquid Wastes		Liquid Culture Media	
mL/Container	Time (Min.)	mL/Container	Time (Min.)
≤ 75	30	≤ 75	20
76- 250	30	76- 250	20
251 -500	40	251 -500	20
501 - 1000	45	501 - 1000	20
1001 - 1500	50	1001 - 1500	30
1501 - 2000	55	1001 - 2000	30

2) Cycle time for equipment and glassware is generally 30 minutes.

## 10.0 Unloading the Sterilizer

- a. Wear lab coat, eye protection, gloves and closed-toe shoes.
- b. Always unload the autoclave within 48 hours after the sterilizer cycle has ended.
- c. Always verify that the pressure gauge reads “zero” before unloading the sterilizer.
- d. Unless the material sterilized must be unloaded before the sterilizer has cooled, such as when sterilizing agar-based media for pour plates or slant tubes, do not unload the sterilizer sooner than 3 hours after the cycle has completed.
  - 1) It takes approximately 30 minutes for the pressure to reach 0 MPa (1,000,000 Pascal), at which time the internal temperature is still 99°C.
  - 2) The internal sterilizer temperature remains >60°C even after 105 minutes.
- e. Sequentially loosen the wing nuts on each opposing pair of clamps and release the clamps from the lid. Carefully remove the lid from the sterilizer, being cautious of residual hot steam exiting from beneath the lid and burning your arm. Lift the lid enough to allow residual steam to exit the sterilizer slowly, and then remove the lid when all the steam has dissipated.
- f. Place the lid on the bench top, upside down and resting on the handle so that the gauge does not touch the bench. Do not place the lid with the bottom side down, otherwise the exhaust line may become damaged.

- g. Using the proper gloves to remove sterilized materials from the chamber. Retighten lids on bottles before removing them from the sterilizer.
- h. Drain the remaining water from the sterilizer after each use and close the drain valve.

## 11.0 Cleaning and Handling Spills or Boil-overs

- a. Spills or boil-overs inside the sterilizer will be most likely contained by the inner sterilization pan, which must be thoroughly cleaned before the next use.
  - 1)
  - 2) Remove the inner pan from the sterilizer and transfer the spilled liquid to a waste collection container.
  - 3) Clean the inner pan with a mild detergent and warm tap water.
  - 4) Thoroughly rinse the detergent from the pan with tap water followed by a final rinse with deionized-reverse osmosis (DI/RO) water. Set the inner pan aside to dry.
- b. Report all spills occurring inside of the sterilizer using the online Incident Reporting form (SOP 002G). This form notifies the Responsible Individual of the incident. When the spill involves biological or biohazardous materials, the Responsible Individual must also report the spill to NAU's Office of Biological Safety.
- c. Although the liquid will be sterilized, its disposal depends on the actual chemical and biological make up and must be evaluated for disposal according to SOP 005A *Disposal of Laboratory Wastes*. The Responsible Individual will consult the Authorized Operator on the removal the contaminated water from the autoclave and its disposal.
- d. Include a copy of the completed incident reporting form (SOP 002G), which includes the corrective and clean-up actions taken, in the sterilizer logbook.

## 12.0 Verifying Sterilizer Performance

- a. Routine verification: Routinely verify that the sterilizer is performing sterilization by using autoclave tape. This verification is recommended with each run.
- b. Bi-annual (6-month) verification: As specified in *Autoclave Operations and Verification Standard Operating Procedure (SOP) 2017*, which is found on the NAU Environmental Health and Safety web site, a bi-annual (6-month) verification is to be performed by NAU's Office of Biological Safety.

- c. Project-specific verification: A more frequent verification of performance may be specified and conducted by individual projects. The type of verification tests performed is determined by the project as well. In this case the project performs these verifications and submits verification results to the laboratory manager so that these data are available upon request from the NAU Office of EH&S.

## Sterilizer Responsible Individuals and Authorized Operators

### Responsible Individuals

Laboratory Director	Terry Baxter	3-2008	terry.baxter@nau.edu
Laboratory Manager	Adam Bringhurst	3-1164	adam.bringhurst@nau.edu

### Authorized Operator with demonstrated competency to operate sterilizer\*

Name	Operator's Signature	Date Training Completed	Responsible Individual's Initials

\* Signing above acknowledges that you have completed the training required by SOP 010B and that you understood the contents of SOP 010B and NAU Office of Regulatory Compliance's SOP on *Autoclave Operations and Verification*, and that this "approval to use" applies only to the sterilizer in room 241 of the Environmental Engineering Laboratory, and that you will use the sterilizer in accordance to NAU's policies and procedures and policies described in this SOP. Failure to operate the sterilizer properly or violations of policies on operating the sterilizer may result in the loss of your Authorized Operator status and disciplinary action appropriate for the violation.

## Portable Sterilizer Operations Log

<b>Authorized Operator</b>	Name:	
<b>Cycle Initiated on</b>	Date:	Time:
<b>Materials Sterilized</b>  <u>Non-Routine Verification Test</u> <input type="checkbox"/> Biological <input type="checkbox"/> Temperature	Waste <input type="checkbox"/> Media <input type="checkbox"/> Water <input type="checkbox"/> Glassware <input type="checkbox"/> Equipment <input type="checkbox"/> Other <input type="checkbox"/>	<u>Description</u>     
<b>Pressure Release</b>	Exhausted 15-min. <input type="checkbox"/>	2-hr cool down <input type="checkbox"/>
<b>Sterilizer Unloaded</b>	Date:	Time:
<b>Routine Performance Verification</b> Autoclave Tape Used <input type="checkbox"/> Yes <input type="checkbox"/> No Tape Changed Color <input type="checkbox"/> Yes <input type="checkbox"/> No		Cycle Time = _____ minutes

<b>Authorized Operator</b>	Name:	
<b>Cycle Initiated on</b>	Date:	Time:
<b>Materials Sterilized</b>  <u>Non-Routine Verification Test</u> <input type="checkbox"/> Biological <input type="checkbox"/> Temperature	Waste <input type="checkbox"/> Media <input type="checkbox"/> Water <input type="checkbox"/> Glassware <input type="checkbox"/> Equipment <input type="checkbox"/> Other <input type="checkbox"/>	<u>Description</u>     
<b>Pressure Release</b>	Exhausted 15-min. <input type="checkbox"/>	2-hr cool down <input type="checkbox"/>
<b>Sterilizer Unloaded</b>	Date:	Time:
<b>Routine Performance Verification</b> Autoclave Tape Used <input type="checkbox"/> Yes <input type="checkbox"/> No Tape Changed Color <input type="checkbox"/> Yes <input type="checkbox"/> No		Cycle Time = _____ minutes