Applied Microbiology
&
Biotechnology Laboratory

"sustainable is attainable"

Standard Operating Procedure

AMBL-010-A

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Autoclave Operation and Performance Verification

SOP SUMMARY

This SOP describes the procedure for safe operation of the steam autoclave located in room 239 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

Autoclave make/Model: Market Forge Sterilmatic / STM-E

Location (Building-Room): Building 69 (Engineering), Room 239

Responsible Department: CECMEE

Responsible Individual & Phone: Terry Baxter, 923-523-2008

Responsible Individual & Phone: Adam Bringhurst, 923-523-1164

ENVIRONMENTAL HEALTH AND SAFETY

Hazards Assessment: This procedure involves the use of a steam-generating autoclave that is operated under conditions of high temperature (250°F) and pressure (15 psi) to perform the sterilization of liquids, equipment and biological

cultures and presents a severe burn risk. More specifically hazards associated with using the autoclave include:

- Burns from high-temperature steam exiting the door when opened or from containers of autoclaved liquids after cycle completion
- Burns from high-temperature steam emitted from the steam by-pass vent into the sink
- Burns from touching the autoclaved materials or the autoclave chamber walls and door
- Burns from the hot water reservoir in the bottom of the autoclave or from hot autoclaved liquids
- Injury to fingers, hands or arms when closing and securing the autoclave door
- Bodily injury, dismemberment or death should an explosion occur

Safety Equipment and Engineering Controls: This procedure requires that the autoclave be equipped with a nearby electrical shut-off. During operation, minimize other work in the immediate proximity to the autoclave. If the Fast Exhaust setting is selected, avoid being near the sink when steam is exhausted.

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-toed footwear
- Safety goggles or glasses
- Laboratory coat
- Heat resistant gloves when unloading the autoclave within 4 hours from the end of a cycle
- Gloves (nitrile, PVC or neoprene) when unloading the autoclave during the next day following the ending of a cycle

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 autoclave.	N[1]	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.
This procedure is used to sterilize biological wastes.	N	Sterilized biological waste materials must be disposed according to SOP 005A Laboratory Wastes Disposal.

[1] 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 steam generating autoclave for the sterilization of 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 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 autoclave must be approved according to the procedure described in this SOP.

The procedure and polices described herein are considered applicable to all individuals operating the autoclave.

2.0 Definitions for Terms and Abbreviations Used

a. **Autoclave.** A steam autoclave maintains steam under conditions of high temperature and pressure for an appropriate amount of time thereby sterilizing the materials being autoclaved.

b. **Autoclaved Materials.** Materials that are autoclaved include, but may not be limited to, any waste, media, glassware or instruments that have completed at least one autoclave cycle for the purpose of sterilization. Autoclaved materials that are not considered wastes are labeled as “autoclaved.”

c. **Authorized Operator.** An authorized operator is an individual who has completed the autoclave training and demonstrated proper use and operation of the autoclave to the Responsible Individual.

d. **Biohazardous Waste Materials.** Biohazardous wastes includes any solid or liquid waste that has come in contact with or is known to contain or is suspected to contain pathogenic or otherwise infections materials, genetically modified organisms or genetic materials.

e. **Biological Waste Materials.** Biological wastes typically will include growth cultures known to be free of pathogenic organisms, as well as unused agar plates, slant agar tubes or membrane filter plates containing growth media. These materials, if not used and left unattended, may develop molds or fungal growths and are autoclaved to avoid this condition. Once autoclaved, biological wastes are handled and disposed in the same way as a biohazardous waste material.

e. **Mixed Waste.** Separate or combined biological and chemical waste reagents or growth media that include radiological or radioisotope materials, known amounts or traces of mercury, lead, immunological agents (adjuvants), chemotherapeutic agents, or volatile or semi-volatile organic liquids or solvents. **Autoclaving mixed wastes in the environmental engineering laboratory is strictly forbidden.**

f. **Performance Verification.** Verifying the sterilization performance of the autoclave is to confirm that the autoclave continues to effectively sterilize instruments and media and inactivate biological and biohazardous wastes materials.

g. **Responsible Individual(s).** The responsible individual is the person or persons designated to ensure that the training, performance verification, maintenance, and recordkeeping is being conducted. The Environmental Engineering Laboratory Director and the Laboratory Manager are the designated Responsible Individuals.

3.0 Training Requirements of Authorized Operators

Any individual operating the autoclave in the environmental engineering laboratory regardless of prior experience must successfully complete an autoclave training session conducted by the Laboratory Manager or Laboratory Director. This training requires the individual to complete the following.

- Read this SOP (AMBL 010A *Autoclave 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 autoclave, and
- Conduct a final demonstration of proper autoclave operation under the supervision of the laboratory Director or Laboratory Manager.

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

4.0 Restricted Materials

Certain materials are restricted from being autoclaved 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 autoclaved.

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

Do not autoclave cracked glassware or glassware unable to be autoclaved, and plastics that are not heat resistant, except for disposable plastic petri dishes containing unused growth media or cultures needing to be disposed. When in doubt whether an item can be autoclaved, check the manufacturer's specifications for that item's material.

Also, materials that do not need to be sterilized, are not to be autoclaved.

5.0 Preparing Materials for Autoclaving

It is the environmental engineering laboratory's policy that the autoclave is loaded and unloaded no sooner than 4 hours after the end of a cycle, unless agar-based media is autoclaved prior to preparing pour plates or slant tubes. Plan well in advance of when you need to use the autoclave and coordinate with others who you know are currently and routinely using the autoclave.

Materials that are otherwise not restricted may be autoclaved once they have been properly prepared for sterilization.

When preparing materials for autoclaving, wear personal protective equipment that is appropriate for the material being handled, including working in a fume hood if necessary.

5.1 Preparing Waste Materials

a. All non-liquid biological and biohazardous wastes with the exception of sharps (see 5.1.c below) must be placed in a RED biohazard bag (meeting OSHA and ASTM standards), loosely closed to allow steam penetration. Autoclave tape must be placed on all biohazard bags to verify sterilization.

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.

   First place a stainless-steel pan into the autoclave, then place the prepared containers in this pan to contain any spillage or boil-over that might occur. Do not remove the perforated stainless-steel tray. Place autoclave tape on at least one container to verify sterilization.

c. Sharp biohazardous waste materials are to be placed in a rigid red plastic “sharps container” that can be autoclaved.

5.2 Preparing Equipment, Glassware, and Liquids

a. Place equipment and glassware in a stainless-steel pan that is then placed on top of the perforated stainless-steel tray. Place autoclave tape on the edge of the stainless-steel pan to validate sterilization – do not place this tape on either the glassware or equipment because it will leave an adhesive residue when removed.

b. Place liquids in containers to no more than 2/3 full. Crimp an aluminum foil wrap over the top of the container and keep bottles lids loose to avoid exploding the container and to allow steam penetration.

6.0 Loading the Autoclave

a. Wear lab coat, eye protection, gloves and closed-toe shoes.

b. Water must be added to fill the autoclave chamber to just below the maximum fill level indicated inside on the bottom of the door opening (between 3.5 to 3.8 liters). Make certain that the drain valve is closed and use a 1:1 mixture of tap and deionized water.

c When loading an autoclave within 4 hours from the end of a previous cycle, use the appropriate heat-insulating gloves described above in the Environmental Health and Safety section.

d. When loading an autoclave during the next day after the end of a previous cycle, use the appropriate personal protective equipment described above in the Environmental Health and Safety section.

e. Materials to be autoclaved must be loaded in a way that will allow complete circulation of steam. Materials in the autoclave cannot touch the inside of the autoclave chamber walls or inside of the opened or closed autoclave door. Do not overload the autoclave.

f. Check to verify that autoclave tape has been included with your load. If not, include autoclave tape now.

g. Close the door. Grasp the door handle, and while holding it in vertical position, pull the door down until the bottom of door rests in the bottom of door opening. Rotate the handle downward to engage the lower curved portion under the horizontal bar in the casting at the bottom of the door opening. Push and rotate the handle completely down until the door is securely latched in position and the door is sealed. **VERIFY THAT THE DOOR IS FIRMLY LATCHED!**

h. See the manufacturer’s instructions for additional information.

i. Initiate a new autoclave operation log sheet (see below) with the information required about the load being autoclaved. Autoclave operation log sheets are kept in the autoclave logbook.

7.0 Initiating the Autoclave Cycle

a. Prior to initiating the autoclave cycle, fill out the Autoclave Operations Log located near the autoclave.

b. There are two autoclave exhaust settings available for the environmental engineering laboratory's autoclave: Instruments (Fast) and Liquids (Slow). In this lab, always use the slow setting for liquids.

c. Initiate the autoclave cycle by turning the timer in a clockwise direction to the desired time. The autoclave is intended to be operated intermittently and should never be operated for a cycle more than 60 minutes without ending the cycle and allowing the autoclave 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 Growth Media**	
mL/Container	**Time (Min.)**	**mL/Container**	**Time (Min.)**
≤ 75	25	≤ 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 times for equipment and glassware should be from 15 to 30 minutes.

8.0 Unloading the Autoclave

a. Wear lab coat, eye protection, gloves and closed-toe shoes.

b. Always unload the autoclave within 48 hours after the autoclave cycle has ended.

c. Always verify that the pressure and temperature gages read "zero" before opening the door.

d. Unless the material being autoclaved must be unloaded before the autoclave has cooled, such as when autoclaving agar-based media for pour plates or slant tubes, do not unload the autoclave sooner than 4 hours after the cycle has completed.

e. Open the door. Grasp the door handle and then lift and rotate it to the horizontal position to unlatch the door. Continue lifting the handle to completely open the door.

f. When opening the door before the autoclaved has completely cooled (4 hours), be very cautious of hot steam exiting the door opening and burning your arm. Open the door just enough to allow the steam to exit the autoclave slowly, and then open the door fully when all the steam has dissipated.

g. Use the proper gloves to remove autoclaved materials from the chamber. Retighten lids on bottles that must be tilted to clear the door to avoid accidental spillage.

h. Drain the remaining water from the autoclave after each use.

i. Allow autoclaved materials to cool to room temperature before transporting. Never transport superheated materials.

j. Complete the autoclave operation log sheet (see 6.0i above) and return it to the autoclave logbook.

9.0 Cleaning and Handling Spills or Boil-overs

a. At the end of each day using the autoclave, or as soon as practical thereafter, use a mild detergent and clean the inside of the autoclave chamber walls and rinse. Leave the drain open so that the rinse water drains from the autoclave.

b. Spills or boil-overs must be thoroughly cleaned before the next use.

c. Report all spills 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.

d. Unless the spill has occurred outside of the autoclave, close the autoclave door and place a note on the door indicating "Spill - Do Not Use."

e. The Responsible Individual will consult the Authorized Operator on the removal the contaminated water from the autoclave and its disposal according to SOP 005A *Disposal of Laboratory Wastes*.

f. Once the contaminated water has been removed, thoroughly clean the autoclave.

g. Include a copy of the completed incident reporting form (SOP 002G), which includes the corrective and clean-up actions taken, in the autoclave logbook.

10.0 Verifying Autoclave Performance

a. Routine verification: Routinely verify that the autoclave 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.

Autoclave 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 autoclave*

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

***** Signing above acknowledges that you have completed the training required by SOP 010A and that you understood the contents of SOP 010A and NAU Office of Regulatory Compliance's SOP on *Autoclave Operations and Verification,* and that this "approval to operate" applies only to the autoclave in room 239 of the Environmental Engineering Laboratory, and that you will operate the autoclave in accordance to NAU's policies and procedures and policies described in this SOP. Failure to operate the autoclave properly or a violation of policies on operating the autoclave may result in the loss of your Authorized Operator status and disciplinary action appropriate for the violation.

Autoclave Operations Log

Authorized Operator	Name:	
Cycle Initiated on	Date:	Time:
Materials Autoclaved <u>Non-Routine Verification Test</u> ☐ Biological ☐ Temperature	Waste ☐ Media ☐ Water ☐ Glassware ☐ Equipment ☐ Other ☐	<u>Description</u>
Exhaust Selection	Instruments (Fast) ☐	Liquids (Slow) ☐
Autoclave Unloaded	Date:	Time:
Routine Performance Verification Autoclave Tape Used ☐ Yes ☐ No Tape Changed Color ☐ Yes ☐ No		Cycle Time = _____ minutes

Authorized Operator	Name:	
Cycle Initiated on	Date:	Time:
Materials Autoclaved <u>Non-Routine Verification Test</u> ☐ Biological ☐ Temperature	Waste ☐ Media ☐ Water ☐ Glassware ☐ Equipment ☐ Other ☐	<u>Description</u>
Exhaust Selection	Instruments (Fast) ☐	Liquids (Slow) ☐
Autoclave Unloaded	Date:	Time:
Routine Performance Verification Autoclave Tape Used ☐ Yes ☐ No Tape Changed Color ☐ Yes ☐ No		Cycle Time = _____ minutes