STERIS AMSCO Series 250LS Autoclave Calibration and Use

(Includes: Autoclave maintenance and water-softener maintenance)

1. SCOPE AND APPLICATION

1.1. This method is based on the STERIS AMSCO Model 250LS Operators Manual, Standard Methods for the Examination of Waste and Wastewater 20th edition, and the Big Boss® Water Softener Operator's Manual. It is used to ensure that the autoclave functions properly in the sterilization of liquids and glassware.

2. SUMMARY OF METHOD

2.1. A monthly QA test utilizing the *Geobacillus stearothermophilus* spore suspension is used to check sterilization effectiveness. A quarterly test is performed to check timer duration.

2.2. It is **required** that the user review the AMSCO model 250LS Operators Manual before attempting to use the autoclave. The use of this SOP is **acknowledgement** that the manual has been reviewed. The manual is located in the Biology lab, Room B205.and as a PDF; https://umanitoba.ca/faculties/health_sciences/medicine/units/immunology/media/Lab_110-250_AUTOCLAVE_Operator_Manual_P387352-364.pdf

3. EQUIPMENT/ SUPPLIES AND REAGENTS

3.1. NIST Traceable Timer

3.2. NIST Traceable Max Thermometer

3.3 *Geobacillus stearothermophilus* spore suspension (SterilAmp® II)

3.4 Multi-Block Heater Model 2050 Incubator set at 55-60 °C

3.5 Heat-indicating tape

3.6 Autoclave gloves

3.7 Safety Glasses

3.8 Lab Coat

3.9 Aluminum Foil

3.10 Deionized (DI) water

4. METHODS

4.1. Daily Maintenance

NOTE: Daily maintenance must be performed each day of use while autoclave is still at room temperature and prior to first cycle run!

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4.1.1. Clean chamber drain strainer by removing drain strainer from drain in chamber bottom. Remove any obvious debris from strainer. If necessary, clear screen in strainer using a brush, wire, or similar tool. Once strainer has been cleared of obvious debris, reverse strainer under running water. Replace strainer in chamber drain.

4.1.2. While chamber door is open and chamber is at room temperature, remove any broken glass or debris and wipe the interior with a cloth or paper towels.

4.1.3. Enter operating mode on display screen. It should display a Flush Generator screen.

Note: The Flush Generator Screen is shown only one time per day.

4.1.4. Generator must be at 0 psig and room temperature before flushing.

4.1.5. Open the access door front panel for the supply valves by turning the knob that has the key in it. Open the drain valve by turning the yellow lever at the back of the generator electric box to the left.

4.1.6. Verify water supply valves to sterilizer and generator are open. These should always remain open. The autoclave will not work if either of these valves are in the closed position.

4.1.7. Press START TIMER. Water will automatically flush through the generator and drain for 5 minutes.

4.1.8. When finished, the display will advance to the restart generator screen. Close generator drain valve by turning the yellow lever to the right and press CONTINUE to advance to operating mode screen. Initial sheet on the Steris next to the appropriate date stating that it has been flushed.

4.1.8.1. Note: If generator drain valve is not properly closed before running a cycle (for ex. program 2 Media cycle 121.0ºC, S=0:15:00) then the autoclave will abort leaving the cycle run uncompleted.

4.1.9. The generator automatically fills to the proper level and starts to heat. Allow 10 minutes warm-up time once generator starts to fill.

4.2. General Operation

4.2.1. The autoclave should always be powered on, and when not in use placed in standby mode by pressing the top left-hand corner button on the touch screen after use. **Always keep door in the up position when not in use.**

4.2.2. The main power disconnect switch, located behind the autoclave's front cabinet panel, controls power supply to the sterilizer and control system. This switch should always remain in the ON position for normal unit operation.

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4.2.3 Open the access door front panel for the supply valves by turning the knob that has the key in it to ensure that the steam supply knob is open (Supply valves are located behind the front cabinet panel; steam valve is above the chamber door). Ensure that the water supply knob is open (Water valve located below chamber door). The steam supply valve and the water supply valve should always remain open. The autoclave will not work if either of these valves are in the closed position.

4.2.4 A touch screen allows users to operate and program the autoclave after entering the user name and password. If machine is in standby mode, touch screen anywhere to start.

4.2.5 Pressing the upper right on the screen enables the control panel. To program machine, select mode button by pressing top right corner rectangle box with the circle, triangle and square shapes inside, then press Supervisor button on the screen and select change date and time or create a new cycle or set unit parameters.

> **Note:** The AMSCO autoclave has four cycles programed for use with the option of adding up to 12 more ; program 1 is a gravity cycle 121.0ºC, S=0:15:00, D=0:15:00 , program 2 is a Media cycle 121.0ºC, S=0:15:00, program 3 is a Media cycle 121.0ºC, S=0:30:00,and program 4 is a Lab Waste cycle 121.0ºC, S=0:45:00,D= 0:05:00. Each cycle is selected by pressing the appropriate number in the cycle status window. Press again to start the selected cycle.

4.2.6 See instructions in Operator Manual to set up a new cycle. The machine can be programmed to shut down and start up at a certain time if in a utility shut down mode. Press the cancel button to restart utilities to run a cycle. Do not change a cycle that has been set up by another user unless there is not room to create the cycle run that you need.

> **Note:** Please do not change the first 4 cycles, those should be generic for all users.

4.2.7 All solid containers must be placed so that water or air will not be trapped in them. Place heat-indicating tape on items being sterilized to identify them. This chemically impregnated tape changes from light to dark black after 15 minutes autoclaving at 121 ˚C (250 °F) and 15 psi pressure. The autoclave is loaded with solids (instruments/glassware) or liquids but never both together. When doing a batch sterilization and the glassware is to be placed on an empty shelf, (e.g., AGP Flasks) then each individual item need not be taped, but tape should be placed on three items located on each of the two shelves, front, middle and rear. (Three items for one shelf, six items for two shelves.)

> **Note:** All Microbiology instruments/equipment/glassware that goes through the autoclave must have a piece of heat-indicating tape before being sterilized. A NIST Traceable Max Thermometer

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must be included with each load and the maximum temperature reached entered in either the LIMS Autoclave Logbook or the paper logbook located with the autoclave. Along with recording maximum temperature, the highest psi pressure during the sterilization cycle and the total time of the entire cycle should be recorded in LIMS Autoclave Logbook for Biology Bench staff.

4.2.8 Close door to secure in position before starting a cycle. Never attempt to open the door if a "Water in The Chamber Alarm" condition exists, call a qualified service technician. To open and close the door, step on the foot pedal to open then again to close door. Do not hold foot on pedal just tap to open then tap to close door. Also, do not stand close to door after a cycle has been run and you are opening the door, as steam may be released from the chamber when door is opened.

4.2.9 Ensure that the values touch pad is displaying the correct date and time. If the correct time and date are not displayed change them to the correct time and date. To change date or time: refer to Operators Manual. Press the mode screen rectangle with circle triangle and square button then press Supervisors Mode Screen press SET Time and Date button. Change Date/Time screen is displayed. Enter Date and Time in boxes. Press the Exit button to return to Supervisor Mode (screen #7) If autoclaving solids: select and press the appropriate gravity cycle button two times. If autoclaving liquids: select and press the appropriate liquids button two times.

4.2.10 Ensure that the hose from the water line is in the drain (floor drain is on the left side of the autoclave). The water line drain hose should remain in the drain always. The water valve should remain at least 1/4-1/2 inch from the vertical off position. Water must run until after the last cycle of the day has been completed. Water must flow into the floor drain to prevent heat damage to the drainpipes as the autoclave discharges into the floor drain.

4.2.11 Use a black waterproof pen and initial the printer paper beside 'operator' and write the item group being autoclaved (glassware/DI water, etc.). The automatic recorder records the date/time/cycle/temperature/pressure/duration for each cycle. If the printer paper edges are red, change the paper: See section 8.2 of the Operator Manual.

4.2.12 To change printer paper, open printer door by pressing button. The door will fall open. Remove any existing paper and open swing door in center of paper. Remove existing ink cartridge, insert new paper roll in compartment behind swing door, insert end of the roll into slit of print mechanism, press paper feed button until 2-3 inches of paper feeds through machine, insert end of paper through new ink cartridge so paper feeds between ink ribbon and plastic housing of cartridge. Install cartridge into position, close swing door. Insert end of paper into removable paper take up spool, return spool and close printer.

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4.2.13 When sterilizer is placed in operating mode the generated printout lists the sterilizer type and manufacture. When a cycle is started, the generated printout lists name of cycle started, time and date the cycle was started, current cycle count (number of cycles run since original start up), operator name, sterilizer id number, default cycle number/type and the programed parameters.

4.2.14 When the autoclave cycle is completed, a message "cycle complete" and "it is safe to open the door" will appear on the touch pad display screen. Carefully, slowly open the door a few inches. Allow a few seconds for the steam to escape from the chamber before opening door completely. WARNING. HOT steam will billow upwards from the space between the door and the chamber. KEEP YOUR FACE OUT OF THE WAY. Be very careful unloading hot liquids from the autoclave.

4.2.15 Preferably, all items are to be removed and properly stored after autoclave use. Leave the power on. The autoclave should enter Utility Shutdown Mode after 5:00pm every afternoon and resume standard operating procedures at 6:00am every morning. If the autoclave is in use after 5:00pm please make sure that it is in Utility Shutdown when you finish in order to allow it to cool for morning maintenance.

Note: At the end of each day (when it is no longer being used), make sure the door of the autoclave is closed so that it can properly enter Utility Shutdown Mode.

4.2.16 If the audible autoclave alarm goes off, press one of the cycle touch pads (gravity, liquid, change value) to silence it. Notify the operator and/or the microbiology lab coordinator. Record the displayed message. Press the reset pad to clear the message. Refer to the Operators Manual, section 7, for alarm message meanings and corrective action. Generally, an alarm will require a service call.

4.2.17 If the autoclave needs service beyond what laboratory personnel can provide: Call 1-800-333-8828, option 2. Tell the representative: who you are, the customer ID (Account #26965 Agreement#1-1194336744/7), that you need autoclave service, and the nature of the problem.

4.3 Monthly Maintenance

4.3.1 Monthly, test the sterilizing effectiveness of the autoclave with a Geobacillus stearothermophilus spore suspension.

4.3.2 **Note:** Microbiology staff performs the monthly and Quarterly calibrations and maintains calibration records. Spores of the thermophilic bacteria are used to monitor the effectiveness of steam sterilization. These bacteria, which are unable to grow at room

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temperature, will grow when incubated at elevated temperatures (55-60˚C).

4.3.3 Place purple SterilAmp® II in a glass beaker with deionized water. Place beaker in the autoclave and autoclave for 15 minutes. This will simulate actual autoclave sterilization performance on media.

4.3.4 After autoclaving, remove SterilAmp® II from the beaker and place in a centrifuge tube and incubate in the multi-block heater at 55-60˚C for 48 hours. Incubate a non-autoclaved SterilAmp® II along with the autoclaved one to serve as a control. Make sure to label which SterilAmp® II has been autoclaved and which is the control. Both AM/PM temps will be recorded for the multi-block heater in the LIMS/Labortory/Logbook/Temperature_Log at the conclusion of the 48 hours.

4.3.5 Pass/Fail criteria: Culture has no growth, no color change (remains purple) = pass. Culture has growth, color change (purple to yellow) = fail. Corrective action is immediately initiated for any failed test (see Quality Control section). A color change indicates that the autoclave did not function properly. The autoclave must not be used until serviced.

4.3.6 After 24-hour incubation, observe for growth and acid production as indicated by the ampule changing from purple to yellow. If no change, check again at 48 hours.

4.3.7 Record sterilization check in LIMS/Laboratory/Logbook/AUTOCLAVE_UV_QC_LOG. In addition to this, create a Micro-QC preparation worksheet for the biological indicator test. Set the ***preparation start time*** as the ***incubation time*** in the Micro-QC worksheet. After 48 hours, record in the Micro-QC worksheet of any growth/color change. Finally, link the worksheet in the comment section of Autoclave_UV_QC_LOG so that it can be tracked.

4.4 Quarterly Maintenance

4.4.1 Each quarter use the NIST certified timer to check the timing of all three phases for a media run (≤15 min conditioning phase, 15 min sterilization phase and a ≥15 min for exhaust phase). Start a media cycle on the autoclave and at the same time, start the NIST timer. Observe the autoclave timer reading at the end of each phase and compare it to the time registered on the timer for each cycle.

4.4.2 Record timer check in LIMS/Laboratory/Laboratory Logbook/MAINTENANCE_LOG. Make sure to record exact cycle time in minutes and seconds. The autoclave timer should not deviate by more than 30 seconds for every 5 minutes. If the timer check fails, manually time every cycle until the autoclave is serviced.

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4.5 Water Softener Maintenance

4.5.1 "Big Boss" brand manufactured by "Water Boss" is the water softener used to reduce hardness of water to appropriate levels for the autoclave. Water softener maintenance is limited to adding rock salt to the unit to replace salt expended by the "softening" process. The water softener manual is in the same drawer as the autoclave manual. Read the manual before attempting any maintenance.

4.5.2 Once weekly**,** remove the top cover of the water softener by lifting the cover. The water softener should contain the proper amount of salt always to prevent damage to the autoclave.

4.5.3 Observe the level of salt in the tank. If the level is less than 1/3 the tank, add rock salt to just below the level of the drain hose. If the salt level is greater than 1/3 the tank, salt doesn't have to be added but can be added as above. **IMPORTANT** Contact the purchasing agent in the FDEP Chemistry Section (currently Sara Armour) when in need of salt.

4.5.4 Replace the cover.

5. DATA ARCHIVAL

5.1 Record the calibration information in LIMS/Laboratory/Laboratory Logbook/AUTOCLAVE_UV_QC_LOG and MAINTENANCE_LOG.

5.1.1 Autoclave "calibration" is performed by Microbiology Section staff. The purpose is to ensure that the autoclave heats long enough to "inactivate" the test species. Do not overwrite any written information. To correct written information: perform a single strike through, record correct information, and initial change.

5.2 Record the use information in LIMS/Laboratory/Laboratory Logbook/AUTOCLAVE_LOG.

5.3 The autoclave automatically prints onto thermal paper the date, time, cycle, pressure, temperature and duration of the cycle. The operator must record initials and what is being autoclaved on the print out. The used rolls are saved in the top cabinet in front of the autoclave.

5.4 To correct raw data entries, place a single line through the incorrect or illegible entry. Write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

5.4.1 The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

5.4.2 If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

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5.5 Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

5.6 Use a black, waterproof pen for all bench sheets and logbooks.

5.7 If a problem occurs during sample processing, a nonconformance report (NCR) is submitted describing the problem and the corrective action.

6. QUALITY CONTROL

6.1 The autoclave is deemed acceptable for use if the monthly QA test utilizing the *Geobacillus stearothermophilus* spore suspension is successful and if the temperature tape indicates that the correct pressure and temperature were maintained during an autoclave cycle.

6.2 If the monthly check fails, the spore test should be repeated immediately. If the repeat test fails the autoclave should be flagged with a DO NOT USE/ OUT OF SERVICE Notice. If at any time, the tape fails to indicate sterilization, then an autoclave cycle should be repeated with tape from the same roll and tape from a different roll. If satisfactory results are not obtained from the second test then the autoclave should be flagged with a DO NOT USE/ OUT OF SERVICE Notice and the service representative should be contacted per STEP 4.2.18 of the *General Operation* section.

6.3 Should the autoclave not work, the VMR drying oven can be used per Standard Methods 20th Edition, p 9-8. (170 °C for two hours). This is for emergency use only and should not be an established routine.

6.4 If the second roll of tape indicates that sterilization temperatures and pressure have been achieved in the autoclave and the initial roll doesn't, it is likely the initial tape was 'bad' and the autoclave should be useable. Discard the ‘bad’ roll of tape.

7. SAFETY/HAZARDOUS WASTE MANAGEMENT

7.1 Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

7.2 Refer to Laboratory Quality Manual for sample disposal.

7.3 Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management

7.4 Read MSDS information for reagent handling and disposal. **WARNING: The autoclave and its contents reach temperatures that will cause severe burns. Autoclave gloves and common sense are necessary.**

8. REFERENCES

8.1 AMSCO® Lab 250 and 110 Series Life Sciences Small Steam Sterilizer Operators Manual https://umanitoba.ca/faculties/health_sciences/medicine/units/immunology/media/Lab_110-250_AUTOCLAVE_Operator_Manual_P387352-364.pdf

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8.2 Big Boss® Water Softener Operators Manual

8.3 Standard Methods for the Examination of Waste and Wastewater, 20th Edition

8.4 Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

8.5 Laboratory Health and Safety Manual and Hazardous Waste Management http://publicfiles.dep.state.fl.us/DEAR/DEAR%20Safety/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202016.pdf

8.6 TNI Standards EL-V1-2016-Rev.2.1

Appendix of Changes:

01/09/2020 This SOP replaces SOP TA-06.18.

12/01/2020 Opening/closing and location of generator drain valve was changed. Mentioned autoclave should be closed before it can perform a Utility Shutdown. Pressure and time of cycle needs to be recorded in LIMS for Bench Biology staff. Micro-QC Worksheet for biological indicator test.

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