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 using the *Geobacillus stearothermophilus* spore suspension is conducted to assess sterilization effectiveness. A quarterly test is performed to verify timer duration.

2.2 All users are **required** to review the AMSCO model 250LS Operators Manual before using the autoclave. The use of this SOP is **acknowledgement** that the user has read the operator's manual. A PDF copy of the manual, *autoclave_operating_procedures,* can be accessed here: X:\Labs\Lab5\Biology\BenchCommon\Equipment\Autoclave.

3 EQUIPMENT/ SUPPLIES AND REAGENTS

3.1 NIST Traceable Timers

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

3.11 NIST Traceable Barometer

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!

4.1.1 Clean the chamber drain strainer by removing it from the drain at the bottom of the chamber. Remove any visible debris and, if needed, clear the screen with a brush, wire, or similar tool. Rinse the strainer under

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running water and then replace it in the chamber drain.

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

4.1.3 Login to operating mode by using the username “STERIS” and password “1000”. If this is the first login of the day, the operating mode should be circumvented by the Flush Generator screen.

> **Note:** The Flush Generator screen is shown only once a day for roughly 15 minutes.

4.1.4 Ensure the generator is at 0 psi and room temperature before flushing by checking the gauges inside the chassis of the autoclave. The access door can be opened by turning the knob and pulling on the top left corner of the front panel.

4.1.5 Open the drain valve by turning the yellow lever at the back of the generator electric box to the left (parallel with the pipe is open, perpendicular with the pipe is closed).

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.

> **Note**: The “Too Long in Jacket Charge” error can occur if the autoclave’s water supply valve is left open for too long after the autoclave is done flushing. If this occurs, silence the alarm, and close the water supply valve.

4.1.8 When finished, the display will advance to the restart generator screen. Close the 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.

> **Note:** If the generator drain valve is not properly closed before running a cycle, the autoclave will fail to build up a charge as the steam will be escaping from the valve. This will result in an alarm and an aborted cycle. **Please be careful to avoid steam burns**!

4.1.9 The generator automatically starts to heat and fill up with steam. Allow 10 minutes for warm-up time once generator starts to fill.

4.2 General Maintenance

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

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operation.

4.2.3 Open the access door front panel by turning the knob and pulling on the top left corner of the panel 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 (The water valve is located below the 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 username 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; 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.

4.2.7 All solid containers must be placed so that water or air will not be trapped in them. Glassware with screw caps should be loosened by approximately a quarter turn to prevent excessive buildup of pressure. 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 should be loaded with either solid items (such as instruments or glassware) or liquids, but not both in the same cycle.

> **Note:** All Microbiology instruments, equipment, and glassware that go through the autoclave must have a piece of heat-indicating tape before being sterilized. A NIST Traceable Max Thermometer must be included with each load that includes the use of media, water, or waste, and the maximum temperature, pressure, sterilization time and total time elapsed must be entered in LIMS/Laboratory/Technical/Laboratory Logbook /AUTOCLAVE_LOG by the Bench Biology staff. All other instances of autoclave usage must be recorded in the paper logbook.

4.2.8 Close the door to secure in position before starting a cycle. Never attempt to

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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 the door. Do not hold foot on pedal, just tap to open then tap to close door. Do not stand close to the door after a cycle has been run and the door is opening, as steam may be released from the chamber when the door is opened.

4.2.9 Ensure that the values touch pad displays 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 or press the mode screen rectangle with circle triangle and square button. Press Supervisors Mode Screen and then 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).

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 Replace the printer roll whenever a colored stripe is visible on one or both edges of the printout paper. Replace the ink ribbon whenever the print readout becomes illegible.

> **Note:** To change the printer paper or ink ribbon, open the swing door and remove the top spool. Open the inner compartment, then remove the ink ribbon and any remaining printer paper. Insert a new roll of paper and feed it into the motor located below. Thread the paper through the ink ribbon and wrap the end around the top spool. Securely replace the ink ribbon, then close the inner compartment and return the top spool. Store the old printer paper secured with a piece of tape with the date of replacement written on it.

4.2.12 When the 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.

4.2.13 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. Press the foot pedal to open the chamber and allow a few seconds for the steam to escape before opening the chamber 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.

4.2.14 Preferably, all items are to be removed and properly stored after autoclave

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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 by ensuring the door is closed when you finish to allow it to cool for morning maintenance.

4.2.15 If the audible autoclave alarm goes off, press the silence alarm button in the top right corner of the screen 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 descriptions and corrective action.

4.2.16 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. If service is conducted, make an entry in the Autoclave Maintenance Logbook located in the Biology Lab, Room B205, detailing what was performed, and record the date and analyst's initials.

4.3 Annual Maintenance

4.3.1 A pressure verification test must be run at least annually. If it is determined that the autoclave has a leak, once it is fixed, a pressure test must be run again regardless of if one has already been conducted within the last year. Leak tests are typically conducted by the autoclave technician during the quarterly preventative maintenance. To conduct a pressure verification test, use a NIST traceable barometer to determine the ambient temperature and pressure, then run a cycle with a NIST traceable thermometer to get the final temperature. Solve for the final pressure using an online Gay-Lussac's Law calculator. Verify that the true final pressure is above the 15 psi minimum, with typical measurements averaging around 20 psi. Treat the maximum temperature on the autoclave receipt as the expected final pressure value. Log the results in the LIMS/Laboratory Logbook/MAINTENANCE_LOG.

Note: Due to a lack of water pressure, the autoclave will sometimes struggle to complete the "vacuum dry" portion of the drying phase. The vacuum pump operates off the water supply to generate suction. If the water pressure is not adequate (>40 psi), this can cause it to get "stuck at 1 minute" for upwards of 10 minutes before it generates a "Too Long in Evacuate Phase" error. If this occurs, check below the timer to ensure it is in the "vacuum dry" phase, then proceed to shut the autoclave off by opening the front panel and flipping the black power switch located in the upper-right section. Once off, flip the power switch back on and wait for the autoclave to wake up. Do not touch anything on the screen, and do not silence the inevitable "Power Failure" alarm. The autoclave should pick up where it left off, skipping the rest of the "vacuum dry" phase of the cycle and moving into a 15-minute drying phase.

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4.4 Monthly Maintenance

4.4.1 Test the sterilizing effectiveness of the autoclave with SterilAmp® II, a *Geobacillus stearothermophilus* spore suspension.

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

4.4.2 Place the SterilAmp® II into a 2 mL centrifuge tube, then place the tube in a glass beaker filled with deionized water. Autoclave the beaker for a 15-minute sterilization cycle. This procedure simulates the actual autoclave sterilization performance on media.

4.4.3 After autoclaving, remove SterilAmp® II from the beaker and incubate in the multi-block heater at 55-60˚C for at least 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. A minimum of four temperature readings must be recorded throughout the 48-hour incubation period, with readings at least four hours apart if recorded on the same day. Record in LIMS/Laboratory/Technical/Laboratory Logbook/TEMPERATURE_LOG.

4.4.4 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.

4.4.5 Record sterilization check in LIMS/Laboratory/Technical/Laboratory Logbook/AUTOCLAVE_UV_QC_LOG. In addition to this, create a MICRO-QC preparation worksheet for the biological indicator test in LIMS/Laboratory/Technical/Prep Worksheet Manager/MICRO-QC. Set the ***preparation start time*** as the ***incubation time*** in the MICRO- QC worksheet. After 48 hours, record in the worksheet of any growth/color change. Link the worksheet in the comment section of Autoclave_UV_QC_LOG when recording the bioindicator results.

4.5 Quarterly Maintenance

4.5.1 Each quarter, use a NIST certified timer to check the timing of all three phases combined for a media run (≤15 min conditioning phase, 15 min sterilization phase and a ≥15 min for exhaust phase). Use an additional NIST certified timer to verify the 15 min sterilization phase independently.

4.5.1.1 Verify that the NIST certified timers have been calibrated annually before use. To calibrate timers, call the National Time Signal (303) 499-7111 and compare the timer for one minute against the National Timer Signal. If no deviation is observed, update the timer calibration

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date in LIMS/Laboratory/Technical/Equipment Editor.

4.5.2 Start a media cycle on the autoclave and at the same time start the NIST timer. Monitor the autoclave and start the second timer once the sterilization phase begins. End the timer for the sterilization phase once you observe the phase change on the screen. End the timer for the entire cycle duration once the cycle is complete. Compare the NIST timer readings to the total cycle duration and the designated 15 minute sterilization period.

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

4.6 Water Softener Maintenance

4.6.1 “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.

4.6.2 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.

4.6.3 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 when in need of salt.

4.6.4 Replace the cover.

5 DATA ARCHIVAL

5.1 Record the calibration information in LIMS/Laboratory/Technical/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 printout. The used rolls are saved in the top cabinet in front

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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 to the 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.

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 non-conformance 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 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.

6.4 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.

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

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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 X:\Labs\Lab5\Biology\BenchCommon\Equipment\Autoclave.

8.2 Big Boss® Water Softener Operators Manual

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

8.4 SterilAmp® II Product Specification \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\Methods & Library Files\TS-117 SterilAmp Technical Report.pdf

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

8.6 Laboratory Health and Safety Manual and Hazardous Waste Management https://floridadep.sharepoint.com/dear/Shared%20Documents/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202021.pdf

8.7 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.

12/20/2021 Re-worded sections 4.1.3-4.1.5 to more accurately reflect the login and flushing processes. Added a section about frequent "Too Long in Jacket Charge" errors after flushing. Re-worded section 4.2.3 for consistency, and 4.2.16 to accurately reflect how to silence the alarm. Added section 4.2.18 due to the frequency of "Too Long in Evacuate" errors when running the autoclave. Added section 4.5 on Weekly Maintenance and how to QC glassware by Bench Biology staff.

12/13/2022 Re-worded section 4.3.1- 4.3.3 to further clarify monthly sterilization test. Corrected link to the operator's manual. Updated link to Laboratory Health and Safety Manual and Hazardous Waste Management.

12/13/2023 Added sterilization time to the list of values to record per cycle in the LIMS Logbook. Mentioned the selected pieces of glassware for weekly QC must be a representative of the cycle. Added further clarifications and fixed formatting mistakes.

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01/05/2024 Added mention of the autoclave maintenance log to 4.2.16 and annual pressure verification test/leak test in section 4.2.17.

01/14/2025 Re-formatted sections 4.2.7, 4.2.12 and 4.2.17 for clarity. Annual maintenance section added to improve readability. Re-worded section 4.4.3 to clarify temperature recording and removed 24-hour check. Changed section 4.5.1 to include a total cycle check and a sterilization check only. Removed weekly maintenance duplicate instructions. Updated references.

01/27/2026 Updated language and formatting. Added annual timer calibration to section 4.5.

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