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 in the Biology lab, Room B205, and the 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 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. 3.11 NIST Traceable

3.12 Barometer

METHODS

4.1. Daily Maintenance

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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 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. Login to operating mode by using the username "STERIS" and password "1000". If this is the first login of the day, 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.

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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 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 (Water valve is 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 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 2is 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.

> **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

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never both together. When doing a batch sterilization and the glassware is to be placed on an empty shelf, (e.g., AGP Flasks) then the individual items 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, 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 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 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). 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. To change printer paper, open printer door by pressing button. The door will fall open. Remove any existing paper and open swing door

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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.

4.2.12 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.

4.2.13 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. 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.

4.2.14 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.

4.2.15 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 meanings and corrective action. Generally, an alarm will require a service call.

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 Log in the Biology Lab, Room B205, detailing what was performed, and record the date and analyst’s initials.

4.2.17 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

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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 initial temperature and pressure, then run a cycle with a NIST traceable thermometer to get the final (maximum) temperature. Solve for the final pressure using an online Gay-Lussac's Law calculator. Verify that the pressure is above the 15 psi minimum, and make sure it's not over the expected value by a reasonable amount. Treat the maximum temperature on the autoclave receipt as the expected final pressure value. Log the results in the LIMS/Laboratory Logbook/MAITENANCE_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.

4.3 Monthly Maintenance

4.3.1 Monthly, 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).

4.3.2 Place purple SterilAmp® II in a centrifuge tube and then place 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.

4.3.3 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. Two daily temperatures, at least four hours apart, need to be be recorded for the multi-block heater throughout the

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duration of the incubation period. Record in LIMS/Laboratory/Technical/Laboratory Logbook/TEMPERATURE_LOG.

4.3.4 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.

4.3.5 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.

4.3.6 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. 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/Technical/Laboratory Logbook/AUTOCLAVE_UV_QC_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.

4.5 Weekly Maintenance

4.5.1 For every Wash Party cycle on any given week, apiece of glassware must be marked with the cycle letter and "QC" to indicate that it will be tested for quality assurance reasons by the Bench Biology group. Put this glassware in a conspicuous location in the drying oven so that someone from the Bench group can easily locate it and test it at their convenience.

4.5.1.1 If multiple cycles are run for a Wash Party and multiple pieces of glassware are needed, pick out glassware that stands apart from one another (i.e. an Erlenmeyer flask, a beaker, and a graduated cylinder, as opposed to multiple pieces of the same type). Additionally, the selected pieces of glassware must be an average representative of the autoclaved batch it came from in terms of volume and type of

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glassware.

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 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

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(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

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

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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
https://floridadep.sharepoint.com/dear/Shared%20Documents/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202021.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.

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 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.

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.

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