# AMSCO Renaissance Series 3021 Autoclave Calibration and Use

**(Includes: Autoclave maintenance and water-softener maintenance)**

## 1. SCOPE AND APPLICATION

1.1. This method is based on the AMSCO Model 3021 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 3021 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 'manuals' drawer across from the Biology lab printer next to the sample prep bench area.

## 3. EQUIPMENT/ SUPPLIES AND REAGENTS

3.1. Interval timer

3.2. *Geobacillus stearothermophilus* spore suspension (SterilAmp® II)

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

3.4. Heat-indicating tape

3.5. Autoclave gloves

## 4. METHODS

4.1. Quarterly Calibration

4.1.1. Once every 3 months, check autoclave timer accuracy for a cycle period using an interval timer. Set the interval timer (15 or 45 minutes for gravity cycle, 15 or 30 minutes for liquid cycle). Start the appropriate autoclave cycle. Observe the autoclave timer reading at the end of this interval. Perform this check when you run either a gravity (15 or 45 minutes) or a liquid (15 or 30 minutes) cycle.

**Note**: The AMSCO autoclave has four cycles; two gravity cycles at 15 and 45 minutes and two liquid cycles at 15 and 30 minutes. Each cycle is selected by pressing the appropriate number in the cycle status window. 1 and 4 are gravity, 2 and 3 are liquid. Press twice to start the selected cycle.

4.1.2. Record timer check in LIMS/Laboratory/Laboratory Logbook/MAINTENANCE_LOG. Make sure to record exact cycle time in



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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.

4.2. Monthly Calibration

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

4.2.2. Microbiology staff performs the monthly calibration and maintains calibration records.

4.2.3. Spores of the thermophilic bacteria, which are unable to grow at room temperatures but do grow when incubated at elevated temperatures (55-60 ˚C), are used to monitor the effectiveness of steam sterilization.

4.2.4. Place purple SterilAmp® II in a 2.0 mL plastic centrifuge tube and place tube in beaker. Place beaker in the autoclave and autoclave for 15 minutes.

4.2.5. After autoclaving, remove SterilAmp® II from the beaker (leave in centrifuge tube) and place in the multi-block heater at 55-60 ˚C for 48 hours.

4.2.6. Incubate a non-autoclaved SterilAmp® II along with the autoclaved one to serve as a control.

4.2.7. Pass/Fail criteria: Culture no growth, no color change (remains purple) = pass. Culture 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.

4.2.8. After 24 hours 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.

4.2.9. Record sterilization check in LIMS/Laboratory/Logbook/AUTOCLAVE_UV_QC_LOG.

4.3. Daily Use

4.3.1. The autoclave and the water softener are on a **digital in-line timer** set to come on at 7:00 AM (allowing time for proper ‘warm-up’) and go off at 5:15 PM Monday through Friday. The autoclave should be powered up and ready for use by 8:00 am. Always make sure the autoclave is powered up and ready before use.

4.3.2. The **main power** switch is located behind the autoclave's upper access door-upper left corner of panel. The main power switch remains on continually. The **digital in-line timer** is located on the wall between the autoclaves and the chlorophyll extraction room door. Refer to the in-line timer manual located in the ‘manuals’ drawer to make any changes in preset on/off timer cycles.


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4.3.3. Open the top access door. **Ensure** that the **steam supply** knob is **open** (turned counter-clockwise until it stops). **Ensure** that the **water supply** knob is **open** (turned counter-clockwise until it stops). 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 (turned fully clockwise).

4.3.4. Load the autoclave. All solid containers or instruments 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 brown to dark brown 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.) **NOTICE: Microbiology instruments/equipment must have a piece of tape on each individual piece.**

4.3.5. Close door and secure in position. The door wheel turns clockwise to lock (the wheel will no longer freely turn but will slip approximately one inch when the door is locked) and counter clockwise to unlock the door. Ensure that the door is aligned so that the locking bars do not catch on the outer lip of the chamber.

4.3.6. Ensure that the **values** touch pad is displaying the correct date and time. If the correct time and date are not displayed change per comment. To change date or time: refer to page 9 of the Operators Manual. A) Press and release the **change values** touch pad**.** B) Move the cursor (blinking display value) to the digit you wish to change by pressing right or left direction touch pads. C) Increase or decrease the value (of blinking digit) by pressing the up or down direction pads. D) Repeat steps B and C until correct date and time are set. E) Press **save values** pad to record the new time and date and to advance the control to the ready mode.

4.3.7. **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. These buttons are located below the printer on the **cycle status** touch pad. There are four cycles, each of which is preset for time and temperature:

Cycle 1: Gravity 15 minutes 250 ºF. Dry 5 minutes.

Cycle 2: Liquid 30 minutes 250 ºF.

Cycle 3: Liquid 15 minutes 250 ºF.

Cycle 4: Gravity 45 minutes 250 ºF. Dry 5 minutes


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4.3.8. 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.

4.3.9. 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 pages 27-28 of manual. Paper is AT&T -NCR Thermal Rolls 2 and1/4-inch X 2-inch diameter for TEXAS Instruments Calculators 5015-5040-5050-5220 and purchased through Office Depot.

4.3.10. Open printer door. Grasp and pull (**CAUTION**) remaining paper through the printer. Pull paper **up.** If paper is **pulled down DAMAGE to the printer will occur.** Remove take-up spindle from its drive mechanism by pulling to the left. Remove paper roll from the take-up spindle by pulling it straightforward.

4.3.11. Lower the platen and remove the lower paper spindle by pulling it straightforward. Place new paper roll onto the lower spindle with the paper feeding downward from the back of the paper roll. Replace lower spindle (with new paper roll) back into position by pressing from the front until it snaps into place.

4.3.12. Pull four or five inches of paper out from the roll and tear the corners to make a point. Slide tab of paper roll into printer from the back (with platen still in down position) until it exits from the front of printer. Grasp tab of paper, pull (**CAUTION**) **up** not down 10 to 12 inches of paper, and feed this paper through opening on platen.

4.3.13. Raise platen back up into position and lift out take-up spindle.

4.3.14. Insert tab of new paper roll into slot of take-up spindle and rotate spindle to secure paper in slot.

4.3.15. Press take-up spindle back onto the drive mechanism and rotate to verify paper is secured to take–up roll. Close printer door.

4.3.16. 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.** When using a **'liquids'** cycle, the autoclave will prompt you to **'open the door 1 inch and wait 10 minutes to unload.'** Be very careful unloading hot liquids from the autoclave.


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4.3.17. Allow autoclaved items to cool and then remove them from the chamber.

4.3.18. The last items autoclaved at the end of the day can remain in the autoclave overnight. Open the door a few inches and leave it. Preferably, all items are to be removed and properly stored after autoclave use. Leave the power on.

4.3.19. After the last cycle (of the day) has finished, leave the autoclave on. It will automatically be turned off by the digital in-line timer.

4.3.20. 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 pages 32-38 for alarm message meanings and corrective action. Generally, an alarm will require a service call.

4.3.21. If the autoclave needs service beyond what laboratory personnel can provide: Call 1-800-333-8838 opt 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

4.4. Autoclave Maintenance

4.4.1. Routine maintenance on the autoclave is performed once weekly. The maintenance is limited to draining the “steam” generator to remove contaminants, removing any broken glass from the chamber, and wiping the chamber with a cloth or paper towels. **Important Warning!!!!** Removing broken glass and wiping the interior of the pressure chamber is performed while the chamber is **cold**, before the autoclave is powered up. Draining the “steam” generator is done in the PM or after a run-cycle while the autoclave is HOT!!!

4.4.2. **At the end of the day on FRIDAY**, turn the digital in-line wall mounted timer on/off switch to the off position. The **Main Power** switch is located on the left side of the panel that is behind the top door of the autoclave. The main power switch should remain on always. The digital in-line timer is located on the wall between the autoclave and the chlorophyll extraction room door. Open the timer and locate the on/off switch on the main face of the timer display.

4.4.3. Open the steam valve by turning the **Blue** lever 1/2 of a turn to the left (counter-clockwise). Leave the valve open overnight. The **Blue** steam valve is located behind the bottom door of the autoclave. It is to your front-left, as you face the autoclave, under the pressure gauge.

4.4.4. **The next morning or Monday**, close the steam valve by turning the **Blue** lever 1/2 of a turn to the right (clockwise) until it stops turning.

4.4.5. Open the pressure-chamber door, remove any broken glass, and wipe the interior of the **COLD** chamber with a cloth or paper towels.


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4.4.6. The autoclave should automatically come on at 7:00 AM. Resume standard operating procedures.

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 < 1/3 the tank, add rock salt to just below the level of the drain hose. If the salt level is > 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 testthen the autoclave should be flagged with a DO NOT USE/ OUT OF SERVICE Notice and the service representative should be contacted per STEP 15 of the *daily use section*.

6.3. Should the autoclave not work, the VMR drying oven can be used per Standard Methods 20$^{th}$ 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.

7.5. 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® Model 3021 Operators Manual


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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 http://www.dep.state.fl.us/labs/library/lab_sops.htm

8.5. Laboratory Health and Safety Manual and Hazardous Waste Management https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

## APPENDIX OF CHANGES:

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| 06/18/2008 | Added Scope and Application, Summary of the Method, References and Appendix of Changes. Updated Standard Method reference in comment Acceptance Criteria Step 2. Changed ºC to ºF in comment for Daily Use Step 6. |
| 01/31/2011 | Updated QC check procedure for timer check and spore check. |
| 12/09/2011 | Changed log books to reflect names of electronic log books in LIMS. |
| 11/13/2013 | Reformatted SOP. |
| 02/09/2015 | Formatted for 508 compliance. |
| 09/25/2015 | Updated links. |
| 10/28/2016 | Updated Steris contact information. |
| 10/02/2017 | Updated Links. |


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