Heidolph Tuttnauer 3870EL Autoclave Maintenance and Operation

1. SCOPE AND APPLICATION

1.1. This method is based on the Heidolph Tuttnauer 3870EL Operations and Maintenance Manual and Standard Methods for the Examination of Waste and Wastewater 20th edition. 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 Heidolph Tuttnauer 3870EL Operations and Maintenance 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.

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 water

3.11 NIST Traceable Barometer

4. MAINTENANCE

4.1. Daily Maintenance

4.1.1. Clean the door gasket with a soft cloth to assure a tight seal. A mild soap solution can be used.

4.1.2. Check the tray underneath the autoclave and clean/empty prior to running the autoclave, if necessary.

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4.1.3. Double check that enough DI water is present in the water reservoir, located at the top, by twisting the cover counterclockwise to open. Water level should reach the base of the safety valve holder. Replace the cover prior to running. **Note:** It is imperative that only distilled or mineral free water is used. DI water should have a maximum conductivity of <15 µs/cm, or failures can occur. Specific physical characteristics and maximum acceptable contaminant levels in water for sterilizers can be found in Section 3.6 of the Operations and Maintenance Manual. Use of water that does not comply with the standards outlined in the referenced section can invalidate the manufacturer guarantee.

4.2. Weekly Maintenance

4.2.1. Clean the water sensor in the back of the autoclave using a damp cloth. This is an imperative step given that the water sensor ensures the proper level of water in the chamber. Make sure to wipe both the tip and the sides of the sensor.

4.2.2. Check the interior of the autoclave. If it is dirty, take out the tray holder and trays and clean with water. Wipe off any sediment from the chamber bottom using a sponge, and wipe down the interior of the autoclave. Do not use steel wool or any type of material that could scratch the inside of the chamber.

4.2.3. If the reservoir has not been periodically checked and filled, replace the DI water. Additionally, if the autoclave has not been run in over a week, replace the water in the reservoir.

4.3. Monthly Maintenance

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

Note: Microbiology staff performs the monthly and Quarterly calibrations and maintains calibration records.

4.3.1.1. 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).

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

4.3.1.3. After autoclaving, remove SterilAmp® II from the beaker 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

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control. Both AM/PM temperatures will be recorded for the multi-block heater in the LIMS/Laboratory/Logbook/Temperature_Log at the conclusion of the 48 hours.

4.3.1.4. 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.

4.3.1.5. 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.

4.3.1.6. 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 worksheet. After 48 hours of incubation, record any growth/ color change in the Micro-QC worksheet. Make sure to link the Micro-QC worksheet in the comments of the Autoclave_UV_QC_LOG so that it can be tracked.

4.3.2. In order to prevent the safety valve (located in the water reservoir) from being blocked, it must be checked, and steam pressure must be allowed to escape once a month.

4.3.2.1. **Begin the check while the autoclave is cold**. This will prevent the autoclave from getting too hot before it reaches the necessary pressure to activate the safety valve.

4.3.2.2. Start a sterilization cycle, wait until the pressure builds to approximately 200 kPa (29-psi). While wearing autoclave gloves and making sure to keep your face away from the reservoir, remove the reservoir cover. Using a hook or another tool, pull on the ring of the safety valve and lift the safety valve ring for 2 seconds. Reference Section 12.5.1 of the Operations and Maintenance Manual for a diagram of how to perform this step. Note: The 3870EL model has an ASME-approved type safety valve, not PED-approved type.

4.3.2.3. Press the stop key to interrupt the operation and allow the steam to exhaust. Wait until pressure drops, and then open the door of the autoclave.

4.3.3. The water strainer should be cleaned approximately once a month, though the frequency of cleaning can be adjusted as needed according to experience.

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4.3.3.1. **Make sure that the strainer cover isn't hot, the electric cord is disconnected, and there is no pressure in the autoclave before proceeding**.

4.3.3.2. Open the strainer cover and remove the strainer element. Rinse with water, using a brush if necessary. Reinstall the strainer element and close the strainer cover.

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.

NOTE: The autoclave should not be used for microbiology media.

4.4.1.1. 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.

4.5. Other Periodic Maintenance

4.5.1. A qualified technician should perform the following activities biannually:

4.5.1.1. Tighten the screws of the heaters and the electrical connections of the heaters, valves, and connectors in the control box.

4.5.1.2. Replace the air filter at least every 6 months or every 1000 cycles, whichever comes first. Reference Section 12.2 of the Operations and Maintenance Manual for specific instructions.

4.5.2. All annual maintenance should be performed by an authorized technician. Reference Section 12.1.3 of the Operations and Maintenance Manual for a list of all necessary services. 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.

4.5.3. 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. 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

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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.

5. OPERATION

5.1. The Heidolph Tuttnauer 3870EL is programmed with several pre-set cycles that cannot be edited. If these cycles are not acceptable, custom cycles can be created and existing ones can be duplicated, and their parameters changed.

5.1.1. Browse through the menu options on the screen using the up and down keys. To select a category, hit the start/stop button. To go back, click the up and down key simultaneously. This allows you to do things such as click through the autoclave cycles, change parameters where appropriate, and reach the main menu screen.

5.1.2. To change cycle parameters, login as technician from the quick menu screen (reached by cycling through the display screen using the up and down keys). The password to log in is entered from right to left rather than left to right. As entered, it is 1230. However, on the display screen, it should show as 0321, reading left to right. Enter the “Cycle Parameters” category and use the up and down keys to adjust values. Hit the start/stop key to set the value, followed by the up or down key to confirm.

5.1.3. Detailed instructions for logging in, creating custom cycles, duplicating cycles, setting parameters, etc. can be found in Section 11 of the Operations and Maintenance Manual.

5.2. Review and perform all necessary maintenance activities prior to attempting to run the autoclave.

5.2.1. If it is not already on, turn on the autoclave by turning the main switch located under the printer cover.

5.2.2. IMPORTANT. If the autoclave has been cycled prior to use, it is strictly forbidden to lean on the autoclave or to place your hand or any part of your body over the door, or severe injury may occur. Make sure to wear heat resistant gloves and use the tray handle to remove loads from the autoclave.

5.2.3. Open the door to the autoclave by pressing the up button located below the autoclave screen. Please note the following:

5.2.3.1. The autoclave door will not open until the temperature inside the chamber has dropped to a safe level.

5.2.3.2. Because the autoclave is defined as a biohazard autoclave, a failed cycle is treated as having un-sterilized waste in the chamber. For this reason, the autoclave door will not open until a successful cycle has occurred or overridden by the user. To enable opening the door, wait for the slow exhaust

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from the failed cycle to end and perform a new cycle, or see instructions below.

5.2.4. Load the autoclave. 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. Make sure that liquids in glass containers are approximately 2/3 full and that lids to said containers are loose. Place a NIST Traceable Max Thermometer in with the load.

5.2.4.1. Be careful to avoid bending the tubing attached to the temperature probes located at the top towards the front of the autoclave.

5.2.4.2. **NOTE:** All Microbiology instruments/equipment/glassware that go through the autoclave must have a piece of heat-indicating tape before being sterilized. The maximum temperature from a NIST Traceable Max Thermometer, maximum pressure (found on the printer roll; see below), and total time (on printer roll) should be included in the Autoclave Logbook entry.

5.2.4.3. Autoclave use by groups other than microbiology (includes wash party) should be recorded in the official paper autoclave use log rather than the LIMs Autoclave Logbook.

5.2.5. Verify that the printer roll is inserted in the printer. Select the correct cycle using the up and down keys. The program can only be selected while the door is open, so verify that you have chosen the correct one prior to closing the door.

5.2.5.1. The measured temperature and pressure values for all but the sterilization stages are recorded in the printer roll every five minutes; during the sterilization stage, the reading is taken every minute. You will need to note the highest pressure reached during 15 min sterilization cycle by choosing the highest reading from the roll. Total cycle time is the very last time reading displayed, and is found alongside the letter “E,” on the roll, which indicates the exhaust stage. For more specifics regarding printer output, please reference Section 7.1 of the Operations and Maintenance Manual.

5.2.5.2. Instructions and diagrams for replacing the printer roll can be found in section 7.2 of the Operations and Maintenance Manual. Note: do not attempt to disassemble the printer as it can both damage the printer and cause injury.

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5.2.6. Close the chamber door by pushing the door to the wall of the autoclave chamber for approximately 10 seconds until the "System Ready" message appears on the screen. Initiate the cycle by hitting the start/stop key. If the door is not properly closed, the cycle will not start. Once a cycle is started, it can be terminated by using the start/top key. Caution: do not touch the strainer cover, mounted on the exhaust line, during or short after operation to avoid severe injuries.

5.2.7. Once the cycle has ended, verify that there is no pressure in the chamber. The pressure reading will be displayed on the screen. Open the door by pressing the up key; begin by allowing approximately a 1" opening to allow for the removal of any residual steam for a few seconds. Proceed to fully open the door.

5.2.7.1. Reminder: It is strictly forbidden to lean on the autoclave or to place your hand or any part of your body over the door when opening the door. Make sure to wear heat resistant gloves and use the tray handle to remove loads from the autoclave.

5.2.7.2. If a cycle was terminated before the sterilization stage was complete, a "Cycle Failed" message will appear on the screen and the door will not open. Pressing the start/stop key will cancel the message and enable the opening of the door.

5.2.8. Unload the autoclave and visually inspect the contents to ascertain that the load is dry and that the heat indicators have turned to the required color.

6. DATA ARCHIVAL

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

6.1.1. 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.

6.2. The microbiology group should record the use information in *LIMS/Laboratory/Laboratory Logbook/AUTOCLAVE_LOG*. All other groups (includes wash party) should record use information in the official paper Autoclave Use Log.

6.3. 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.

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6.4. 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.

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

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

6.5. 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.).

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

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

7. QUALITY CONTROL

7.1. 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.

7.2. 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.

7.3. 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.

7.4. 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.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

8.2. Refer to Laboratory Quality Manual for sample disposal.

8.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management

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8.4. Read MSDS information for reagent handling and disposal.

8.5. WARNING: The autoclave and its contents reach temperatures that will cause severe burns. Autoclave gloves and common sense are necessary.

9. REFERENCES

9.1. Heidolph Tuttnauer 4870EL Operations and Maintenance Manual

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

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

9.4. 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

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

APPENDIX OF CHANGES

02/01/2021	Included Micro-QC WS instructions.
01/18/2022	Updated the link to the Health and Safety Manual
12/28/2022	Edited section 4.3.1 to further clarify monthly maintenance and sterilization test. Edited link to Laboratory Health and Safety Manual and Hazardous Waste Management.
01/22/2024	Added mention of the autoclave maintenance log to 4.5.2 and annual pressure verification test/leak test in section 4.5.3. Added NIST Traceable Barometer to the list of equipment.

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