Market Forge Sterilmatic© Autoclave Calibration and Use

STEPS	COMMENTS
Materials	
Calibration	
1. Interval timer **2.** *Geobacillus stearothermophilus* spore suspension (SterilAmp® II) **3.** Multi-Block Heater Model 2050 Incubator set at 55-60 °C **4.** Autoclave Minimum/Maximum thermometer	**WARNING: The autoclave and its contents reach high temperatures that will cause severe burns. Residual pressure may be released after cycles are complete. Autoclave gloves are required during loading and unloading.** **WARNING: The Minimum/Maximum thermometer is a mercury thermometer. The thermometer must be shaken down prior to each use in order to get an accurate reading of the temperature during autoclaving. Use care when shaking down the thermometer in order to avoid breakage. In the event the thermometer breaks, contact the Biology Section Safety Office immediately.**
Daily Use	
1. Heat-indicating tape **2.** Deionized (DI) water	
Safety	See http://depnet/burlabs/safety.htm (Intranet).
1. Lab hood or chemical ventilation system **2.** Lab coat **3.** Latex gloves **4.** Safety glasses **5.** Autoclave gloves	Proper laboratory protection is required when working with chemicals and unprocessed samples. CAUTION: Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used.
Pollution Prevention and Waste Management	See http://depnet/burlabs/safety.htm (Intranet).
Documentation	
1. Record the calibration and use information in the Laboratory Information Management System (LIMS) Autoclave log.	Select **LIMS/Laboratory/Technical/Laboratory Logbook/Autoclave Log**
2. For each run of microbiology materials, record in *Autoclave Log*: run duration, exposure time, maximum pressure and temperature (use a max/min thermometer) reached during the run, and the type of materials autoclaved (batch #s if available). Also add whether the QC criteria were acceptable, type of QC done (diack control/ autoclave tape), pass or fail.	
3. Monthly QC using spores suspension of *Geobacillus stearothermophilus* shall be recorded in the *Autclave QC Log*. Include the date of test, results (pass or fail,) and analyst initials.	Select **LIMS/Laboratory/Technical/Laboratory Logbook/Autoclave_UV_QC_Log**

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Methods	
Quarterly Calibration	
1. Once every 3 months, check autoclave timer accuracy for a 5-minute period using an interval timer. Set the interval timer for 15 minutes and the autoclave timer for at least 15 minutes. Observe the autoclave timer reading at the end of this interval. This procedure can also be done using the timer accuracy 45 minutes. The autoclave timer should not deviate by more than 30 seconds for every 5 minutes. Record the time from the interval timer in the Maintenance Log.	Select **LIMS/Laboratory/Technical/Laboratory Logbook/Maintenance_Log.** Make sure to record both minutes and seconds from the interval timer for the timer check. If the timer check fails, manually time every cycle until the autoclave is serviced.
Monthly Calibration	
1. Monthly, test the sterilizing effectiveness of the autoclaves with *Geobacillus stearothermophilus* spore suspensions.	Spores of the thermophilic bacteria, which are unable to grow at room temperatures but do grow when incubated at elevated temperature (55–60 ˚C), are used to monitor the effectiveness of steam sterilization.
2. 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.	
3. After autoclaving, remove SterilAmp® II from the beaker (leave it in centrifuge tube) and place in the multi-block heater at 55–60 ˚C for 48 hours.	Make sure to check and record the multi block heater temperature twice daily in the *Temperature Log* in LIMS Logbook.
4. Incubate a non-autoclaved SterilAmp® II along with the autoclaved one to serve as a control.	
5. After 24 hr incubation, observe for growth and acid production as indicated by the bacteria changing the solution from purple to yellow. If no change, check again at 48 hr.	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 below)
Daily Use	
1. Make sure the drain valve is closed (drain knob is located in front of the autoclave). Fill bottom of sterilizer chamber with 4-5 L of DI water or until water reaches just below the edge at bottom of door opening.	

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2. Load sterilizer. All solid containers or instruments must be placed so that water or air will not be trapped in them. Place heat-indicating tape on the material being sterilized to identify them. This chemically impregnated tape changes from light brown to dark brown after 15 minutes autoclaving at 121 ˚C and 15-psi pressure.	
3. Close door and secure in position.	
4. Determine correct sterilization time by referring to *Minimum Sterilization Time Chart* at the right.	TIME AND TEMPERATURE FOR AUTOCLAVE STERILIZATION Material — Time at 121ÞC Carbohydrate-containing media (lauryl tryptose, BGB broth, etc.) — 12–15 min Contaminated materials and discarded cultures (waste) — 45 min Membrane filter assemblies (wrapped), sample collection bottles (empty) — 15 min Dilution water, 99 mL in screw-cap bottles — 15 min Rinse water volumes of 0.5 to 1 L — 30 min Rinse water in excess of 1 L — Adjust for volume; check for sterility
5. Check temperature and pressure gauge for proper operation while autoclave is running.	Temperature of autoclave should reach 121˚C and 15 psi pressure.
6. Turn timer to the desired length of sterilizing period. This turns power on and starts the cycle after the proper pressure-temperature combination has been reached. The amber pilot light indicates that the unit is on.	The timer is located at upper right front of sterilizer. Indicator light may not initially remain on while unit is reaching proper pressure and temperature.
7. When the exposure cycle is completed, the power will turn off and the exhaust valve will automatically open. **WARNING: Contents under pressure.** Wait until steam and pressure are released from the exhaust before releasing handle. When the chamber pressure gauge reads zero, release the handle slowly, wearing autoclave gloves. Keep hands and face away from opening. With the door slightly ajar, allow a few seconds for the steam to escape from chamber before opening door completely.	The chamber pressure gauge is located at the top of the control housing. **WARNING**. HOT steam may escape rapidly from the space between the door and the chamber. **KEEP HANDS AND FACE OUT OF THE WAY.** New employees must be trained on the use and hazards of the autoclave.

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8. Sterilizer chamber must be cleaned and drained daily. Wash wetted portion of the cylinder thoroughly by adding a mild detergent. After washing, a thorough rinse with clean, soft water is imperative, leave door open overnight.

Verification of new QC reagents and materials

1. Each new batch of *Geobacillus stearothermophilus* and new batch of strips or tape will be evaluated for acceptability with QC Autoclave program. Each batch will be evaluated and compared to the currently acceptable stock. Documentation shall be entered in the QA log as specified above.

Corrective Action

1. In the event the autoclave fails to meet the correct run duration, pressure, or temperature, the autoclave is red-tagged and another cycle is run. If the parameters are still out of range, a service call will be scheduled. The instrument is immediately taken out of service until all requirements are met in accordance with QC criteria.

References

1. Standard Methods Online Edition (1997), Section 9020B

2. Bureau Quality Manual http://www.dep.state.fl.us/labs/library/progplan.htm

3. Health and Safety Plan http://depnet/burlabs/safety.htm

Appendix of Changes

09/10/2010 Replaced materials with new brand of biological indicator and new incubator.

01/31/2011 Updated corrective action and incubation temperature for QC checks.

01/18/2012 Updated reference to Quality Manual.

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