US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for
Performance Verification of Autoclaves

SOP Number: QC-13-07

Date Revised: 6-15-15

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Title	Performance Verification of Autoclaves
Scope	This protocol describes the procedures for verifying the performance of the autoclaves.
Application	Changes in temperature and pressure within the autoclave but outside the established tolerances may impact the quality and sterility of media and reagents. It is therefore critical to ensure that the autoclaves are operating within acceptable limits (see section 15, #1 and #2)

	Approval Date
SOP Developer:	_____ Print Name: _____
SOP Reviewer	_____ Print Name: _____
Quality Assurance Unit	_____ Print Name: _____
Branch Chief	_____ Print Name: _____

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

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1. Definitions	1. A Kill cycle is a liquid cycle with a duration of 180 minutes to sterilize bio-hazardous waste. 2. A gravity cycle is a dry cycle used for sterilization of dry laboratory materials (e.g., glassware, carriers). 3. Chemical Indicator Strips are engineered to integrate all 3 critical parameters of sterilization (time, temperature and saturated steam) and are certified to perform equal to a biological indicator plus an added safety factor. See section 12.2, a, iii for a discussion of passing and failing results. 4. Biological Indicator Ampule is a Raven Biological PROSPORE Biological Indicator, hermetically sealed, type I borosilicate glass ampule. The ampule is filled with a modified Soybean Casein Digest Broth containing bromocresol purple acid indicator. Each ampule also contains a population (six logs) of *Geobacillus stearothermophilus* spores. 5. Maximum Registering Thermometers (mercury-containing/teflon-coated) are used to verify a maximum autoclave temperature. 6. Additional abbreviations/definitions are provided in the text.
2. Health and Safety	1. Follow procedures specified in SOP MB-01, Laboratory Biosafety. 2. Laboratory personnel have been trained on the proper use of the autoclaves. The autoclaves and materials being removed from the autoclaves are very hot (often greater than 100ºC). Lab personnel should wear lab coats, eye protection and thermal gloves when handling materials being removed from the autoclaves to prevent burns.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Once a year, all of the laboratory's maximum registering thermometers are verified at operating temperatures against a similar maximum registering thermometer that has been certified by an ISO 17025 accredited vendor. See EQ-02, Calibration of Thermometers.
5. Sample Handling and Storage	Biological indicator ampules (sealed spore ampules containing spores in liquid culture media) must be stored according to manufacturer's specifications to insure shelf life. Upon receipt, the biological indicators ampules must be placed in the refrigerator.
6. Quality Control	1. For quality control purposes, the required information is documented on the appropriate form(s) (see section 14). 2. A quality control check of the instruments is performed monthly and is

	recorded on the appropriate form (see section 14). Expiration dates of biological indicator ampules and chemical indicator strips are recorded on the appropriate forms (see section 14).
7. Interferences	1. The maximum registering thermometers should be reset prior to each use as described in 12.2, a, ii. 2. Shake the thermometer until the column registers 110ºC or lower. 3. The thermometer should be allowed to cool to ambient temperature before it is read. Hold thermometer in an upright position for reading, and only after it has cooled to ambient temperature, or you will obtain a falsely high reading. 4. The position of thermometers, chemical indicator strips, and biological indicator ampules is critical to successful quality control measurement. Refer to Attachment 1 for proper placement of thermometers and indicators. 5. Certain media may require a lower (<121ºC) sterilization temperature. For those media, the autoclave will be adjusted accordingly to ensure appropriate sterilization
8. Non-conforming Data	1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports. 2. Failure of any of the quality control indicators (data on autoclave printout, maximum registering thermometer, chemical indicator strip, biological indicator ampule) results in a failed autoclave run. a. Verify that the maximum registering thermometer, chemical indicator strip, and biological indicator ampule were placed in the appropriate location as specified in Attachment 1. b. Verify that the maximum registering thermometer, chemical indicator strips, and biological indicator ampules pass when run in the next cycle (same cycle parameters for time, temperature, and cycle type). If failure continues, consider running a cycle with a different maximum registering thermometer and different lots of indicators. If failure continues, call for service on the autoclave. c. Media autoclaved during a complete (cycle was completed) but failed run may be used if it passes sterility and performance testing (see SOP MB-10). Do not re-autoclave the media (many are heat-sensitive). If media fails sterility or performance, a new batch must be prepared. d. Glassware and non-heat sensitive reagents must be autoclaved again.

	3. An autoclave may go into alarm during a run. a. If an alarm sounds before the sterilization process has begun (e.g., door alarm) and the cycle aborts, attempt to determine the cause of the alarm, resolve it, and restart the cycle. b. If the autoclave goes into alarm but the cycle resumes and is completed successfully, media and any heat-sensitive reagents are checked for sterility and/or performance and may be used if passing. Non-heat sensitive reagents and glassware must be autoclaved again. c. If the autoclave goes into alarm after the sterilization phase has begun and the cycle aborts, media and any heat-sensitive reagents must be discarded. Non-heat sensitive reagents and glassware must be autoclaved again. d. If autoclave goes into alarm during subsequent runs, call for service.
9. Data Management	1. Data will be archived consistent with SOP ADM-03, Records and Archives.
10. Cautions	1. Because autoclaves use high temperatures, it is necessary to exercise extreme caution around the device and its associated plumbing. High-temperature surfaces can be encountered even when the device is not in a sterilizing cycle. 2. For autoclaves #1 and #2, a completed autoclave liquid cycle includes the recommended 10 minute wait period (indicated on the LED screen on the autoclave) once the door has been cracked open. When using these autoclaves, it is recommended that the operator open the door slowly (not greater than one inch) and wait at least 10 minutes prior to unloading.
11. Special Apparatus and Materials	1. Raven Biological Laboratories ProSpore Biological Indicator Ampules with 106 spores of G. stearothermophilus (ATCC #7953) per unit. 2. SPS Medical Chemical Indicator Strips. 3. Incubator with temperature set at 55º C ± 1º C. 4. Autoclave #1 located in room B206, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0105898-25. 5. Autoclave #2 located in room B204, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0108298-11. 6. Autoclave #4 located in room B202, Amsco Lab 250 Laboratory Steam Sterilizer (20×20×38"), Model LG-250, Serial No. 0311511-10. 7. Autoclave # 5 located in room D122, Tuttnauer Prevacuum Steam Heated Autoclave with Vertical Sliding Door and Steam Generator (52×72×51”),

	Model 5596-EP-1V, Serial No. 2311036. 8. Maximum Registering Thermometers (scale range 80-135ºC). See SOP EQ-02 for verifying the accuracy of the thermometers.
12. Procedure and Analysis	Refer to Attachment 1 for a summary of the performance verification practices.
12.1 Sterilization batch number	a. The sterilization batch number consists of two parts: the first seven digits represent the date the batch was sterilized: S-MMDDYY where S=Sterilization, MM=month, DD=day and YY=the last two digits of the calendar year. b. The suffix where the first digit after the dash indicates the autoclave used and the next two digits act as a counter for the number of preparations made on the same date. c. For example, the first batch sterilized on January 8, 2015 in autoclave 1 (Room B206) would have the sterilization batch number S-010815-101. The next batch sterilized on that same day and same autoclave would have a suffix of -102, the third batch sterilized would have a suffix of -103; etc. d. Record the sterilization batch number in the Daily Sterilization Record Information Log Form (see section 14).
12.2 Performance Verification of Autoclave Runs (Per Run Verifications).	a. The following data are collected for every autoclave cycle. i. Autoclave Printout. For each run, record the minimum and maximum temperatures achieved during the "sterilize" portion of the cycle as indicated on the autoclave printer readout on the appropriate form (see section 14). The acceptable temperature range per cycle run is between 120-124ºC, with the exception of certain media (e.g. CTA stabs) which may require a lower sterilizing temperature. ii. Maximum Registering Thermometer. A maximum registering thermometer is used for each autoclave run. Place the thermometer upright in a container and place the container in the autoclave pan along with the items to be processed. Reset the maximum registering thermometer prior to each use by "shaking" the thermometer as you would a fever thermometer. This will force the mercury through the constriction located above the bulb. Record the results from the thermometer on the appropriate form (see section 14). The thermometer should be allowed to

	cool to ambient temperature before it is read. iii. Chemical Indicator Strip. Place the strip flat on top of the container that holds the maximum registering thermometer. Record the results from the chemical indicator strips on the appropriate form (see section 14). Refer to the package instructions for use. A chemical indicator strip is passing if the dark bar on the strip reaches the "steam safe" section indicated at the end of the strip. If the dark bar has not entered the "steam safe" section of the strip, the chemical indicator strip is failing.
12.3 Monthly Performance Verification of Short Gravity and Liquid Cycles	a. On a monthly basis, performance verification is conducted by running a short gravity cycle and a short liquid cycle in Autoclaves 1 and 4. For Autoclave 5, only the Kill cycle is run when the autoclave is needed, otherwise it is shut down and not used (see section 12.4). In addition to recording Per Run Verification data, biological ampules are used. Refer to package instructions for use of ampules. Use the biological indicator ampule, maximum registering thermometer, and chemical indicator strip for monthly QC runs. See Attachment 1. b. For short liquid cycles (autoclaves #1 and #4), place a biological indicator ampule into a test tube containing an appropriate volume of liquid (~10 mL for 20×150 mm test tubes and ~20 mL for 25×150 mm test tubes). Place the test tube containing the ampule in a test tube rack containing 39 other similarly filled test tubes (each rack holds 40 test tubes). Place the tube with the biological indicator ampule as close to the center of the rack as possible. Place the maximum registering thermometer and chemical indicator strip in a beaker or flask and place it near the rack of media. See Attachment 1. c. For short gravity cycles (autoclaves #1 and #4), place the biological indicator ampule, maximum registering thermometer, and chemical indicator strip in an empty beaker in the bin holding the glassware. See Attachment 1. d. Immediately upon completion of the cycle, remove items from the autoclave. e. Remove the ampule from the test tube or beaker and label with: autoclave #, cycle type (i.e., gravity cycle, liquid cycle, kill cycle), and date of run. f. Incubate the ampule as well as one control ampule that has not been autoclaved at 55ºC±1ºC for 48±2 hours and record the results on the

	appropriate form (see section 14). Growth is evident by either turbidity and/or a color change from a purple to or toward yellow. See section 8 for non-conformance and corrective action. g. Record the results for the maximum registering thermometer as per section 12.2. h. Record results for the chemical indicator strip as per section 12.2.
12.4 Monthly Performance Verification of Kill Cycles	a. On a monthly basis, performance verification is conducted by running a Kill cycle in Autoclaves 2 and 4. Performance verification on autoclave #5 is only performed if the autoclave is ever needed, otherwise it is shut down and not used. Use biological indicator ampule, maximum registering thermometer, and chemical indicator strip as per Attachment 1. This may be performed over the course of several days. b. To verify kill cycles, place a biological indicator ampule in the center of an autoclave bag filled with solid waste. Place the maximum registering thermometer and chemical indicator strip in an empty beaker. Place the beaker in the bin with the bag. Run a standard kill load (180 minutes liquid cycle). After completion of the cycle, recover and label the ampule and incubate for 48±2 hours at 55ºC±1ºC along with a control ampule that has not been autoclaved. See 12.3,f for passing result. Record the results on the appropriate form (see section 14).
13. Data Analysis/ Calculations	None.
14. Forms and Data Sheets	Test Sheets. Test sheets are stored separately from the SOP under the following file names: Daily Sterilization Record Log Form QC-13-07_F1.docx Monthly Sterilization Record Form QC-13-07_F2.docx
15. References	1. Bordner, R.H., Winter, J.A., and Scarpino, P.V., eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA 600/8-78-017, Environmental Monitoring & Support Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio. 2. Rice, E.W., Baird, R.B., Eaton, A.D. and Clesceri, L. S., 2012. Standard Methods for the Examination of Water and Wastewater, 22nd Edition. American Public Health Association, Washington, DC.

	3. Lee, C.-H., Montville, T.J., and Sinskey, A.J., 1979. Comparison of the efficacy of steam sterilization indicators. Appl. Environ. Microbiol. 37(6):113-117

Attachment 1: Performance Verification Practices for Autoclaves

Autoclave ID	Room	Performance Verification and Conditions			
		Per Run*	Monthly Quality Check**		
			Short Gravity (45 min)	Short Liquid (15 min)	Kill Cycle (180 min)
#1	B206	*Thermometer/strip located per monthly QC*	*Ampule/thermometer/ strip in empty beaker in the bin holding the glassware*	*Ampule in test tube (with media) in full rack. thermometer/strip in a beaker/flask near media; empty bin (s) on bottom shelf*	N/A
#2	B204	*Thermometer/strip located per monthly QC*	N/A	N/A	*Ampule in the full bag, thermometer/strip in empty beaker, all inside a bin*
#4	B202	*Thermometer/strip located per monthly QC*	*Ampule/thermometer/ strip in empty beaker in the bin holding the glassware*	*Ampule in test tube (with media) in full rack, thermometer/strip in a beaker/flask near media; empty bin on bottom shelf*	*Ampule in the full bag, thermometer/strip in empty beaker, all in a bin on the (bottom) shelf*
#5§	D122	*Thermometer/strip located per monthly QC*	N/A	N/A	*Ampule in the full bag, thermometer/strip in empty beaker, all in a bin*

* Use only maximum registering thermometer and chemical indicator strip per run
**Use ampule, maximum registering thermometer and chemical indicator strip for monthly QC
§ Autoclave 5 is only verified when it is needed, otherwise it is shut down and not used