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Office of Pesticide Programs
Microbiology Laboratory
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Standard Operating Procedure for
Performance Verification of Autoclaves

SOP Number: QC-13-09

Date Revised: 03-01-17

SOP Number	QC-13-09
Title	Performance Verification of Autoclaves
Scope	This protocol describes the procedures for verifying the performance of autoclaves.
Application	Verification of autoclave performance is essential to maintaining the quality and sterility of media and reagents, and to confirm the inactivation of biohazardous waste.

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

Date SOP issued:	
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1. Definitions	1. A liquid cycle is a sterilization cycle in which steam is exhausted slowly at the end of the cycle to allow the liquids to cool without boiling over. 2. A kill cycle is a liquid cycle with a duration of 180 minutes to sterilize bio-hazardous waste. 3. A gravity cycle is a sterilization cycle in which steam is rapidly exhausted at the end of the cycle. Dry time may be added to the cycle. A gravity cycle is used primarily for sterilization of dry laboratory materials (e.g., glassware, plastic ware, carriers). 4. Chemical indicator strips are engineered to integrate all 3 critical parameters of sterilization (time, temperature and saturated steam) and provide a distinct color change when exposed to the sterilization process. 5. Biological indicator ampules (sealed spore ampules containing spores in liquid culture media) are intended for use as a challenge to steam sterilization at 121°C and to confirm the inactivation of biohazardous waste. 6. Sterilization batch number. A distinct number accounting for the date of sterilization, the autoclave used, and a counter for the number of autoclave runs on a given day 7. 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 are 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, 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 must be stored according to manufacturer's specifications to ensure shelf life. The biological indicator ampules must be refrigerated upon receipt and until use.

6. Quality Control	1. For quality control purposes, the required information is documented on the appropriate form(s) (see section 14). Perform a quality control assessment of the autoclaves monthly and record on the appropriate form (see section 14). 2. Record expiration dates of biological indicator ampules and chemical indicator strips on the appropriate forms (see section 14). 3. Quality control checks vary per type of autoclave run; refer to sections 12.3-12.5. 4. If an autoclave undergoes repair, do not use the autoclave to sterilize media until its performance is verified using the monthly verification procedure for a short liquid cycle (refer to section 12.4). a. If autoclave #4 in B202 undergoes repair when the laboratory is commissioned for Select Agent work, do not use the autoclave until its performance is verified using the monthly verification procedure for a kill cycle (refer to section 12.5). 5. Do not use autoclave #3 in B207 to sterilize media using a 15-minute liquid cycle.
7. Interferences	1. Maximum registering thermometers may provide inaccurate readings if not used properly, as outlined in section 12.2b. 2. The position of thermometers, chemical indicator strips, and biological indicator ampules (when applicable) is critical to successful quality control measurement. Refer to Attachments 1 and 2 for proper placement of thermometers, indicator strips, and ampules. 3. Certain media may require a lower (<121ºC) sterilization temperature. For those media, adjust the autoclave accordingly to ensure appropriate sterilization. 4. 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 sections 15.1-15.2).
8. Non-conforming Data	Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports.
9. Data Management	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. 3. Do not overload the autoclave. Allow for steam penetration between materials. Avoid contact of load components with the walls of the chamber.
11. Special Apparatus and Materials	1. ProSpore Ampoule Biological Indicator (Mesa Labs; Lakewood, CO; catalog no. PS-6-50). Each ampule is a hermetically sealed, type I borosilicate glass ampule, filled with a modified Soybean Casein Digest Broth containing bromocresol purple acid indicator and 10^6 spores of *Geobacillus stearothermophilus*. Biological ampules are used to verify the 180-minute liquid cycle and the 45-minute gravity cycle. See section 12.7a for a discussion of passing and failing results. 2. Chemical Indicator Strips (SPS Medical; Rush, NY; catalog no. SSI-100). See section 12.2c,i for a discussion of passing and failing results. 3. Maximum Registering Thermometers (mercury-containing/teflon-coated; scale range 80-135°C) are used to verify a maximum autoclave temperature. 4. Incubators with temperatures set at 36±1°C and 55±1ºC. 5. Autoclave #1 located in room B206, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0105898-25. 6. Autoclave #2 located in room B204, Amsco Eagle 3000 Scientific Series, Model E3031-S-1, Serial No. 0108298-11. 7. Autoclave #3 located in room B207, PRIMUS Model PSS5-AA-MESD, Serial No. 18200. a. Do not use this autoclave to sterilize media using a 15-minute liquid cycle. 8. Autoclave #4 located in room B202, Amsco Lab 250 Laboratory Steam Sterilizer (20×20×38"), Model LG-250, Serial No. 0311511-10. 9. 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, when in use.

12. Procedure and Analysis	1. Refer to Attachment 1 for a summary of the performance verification practices. Refer to Attachment 2 for photographs of quality control indicator placement for monthly performance verification.
12.1 Sterilization Batch Number	a. The sterilization batch number consists of two parts: i. 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. ii. 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. b. For example, the first batch sterilized on January 8, 2017 in autoclave 1 (Room B206) would have the sterilization batch number S-010817-101. 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. c. Record the sterilization batch number in the Daily Sterilization Record Information Log Form (see section 14).
12.2 Verification Per Cycle	Collect the following data for every autoclave cycle. a. 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). i. 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. b. Use a maximum registering thermometer for each autoclave run. Place the thermometer upright in a container and place the container near the items to be processed. i. Record the results from the thermometer on the appropriate form (see section 14). Hold thermometer in an upright position for reading or a falsely high reading will be obtained. ii. Reset the maximum registering thermometer prior to each use by "shaking" the thermometer as would be done for a fever thermometer. This will force the mercury through the constriction located above the bulb. Shake the thermometer until the column registers 110ºC or lower.

	c. 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 strip on the appropriate form (see section 14). i. 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. d. Failure of any of the quality control indicators (data on autoclave printout, maximum registering thermometer, chemical indicator strip, biological indicator ampule (when applicable), tryptic soy broth (TSB) (when applicable)) results in a failed autoclave run. Material in a failed autoclave run must either be re-autoclaved (glassware or biohazardous waste) or remade (media or reagents). i. Verify that the maximum registering thermometer, chemical indicator strip, biological indicator ampule (when applicable), and TSB (when applicable) were placed in the appropriate location as specified in Attachments 1 and 2 and repeat the cycle. ii. If failure continues, call for service on the autoclave.
12.3 Monthly Performance Verification of Short Gravity Cycles	a. On a monthly basis, verify autoclave performance by running a short gravity cycle in autoclaves #1, #2, #3, and #4. Use a biological indicator ampule, maximum registering thermometer, and chemical indicator strip. b. Place the biological indicator ampule and maximum registering thermometer in an empty beaker in the bin holding the glassware. Place the chemical indicator strip on top of the beaker containing the ampule and thermometer. Run a short gravity cycle (45-minute gravity cycle). c. Upon completion of the cycle, remove items from the autoclave and record the minimum and maximum temperatures achieved during the "sterilize" portion of the cycle from the autoclave printer readout, the maximum registering thermometer reading, and chemical indicator strip results (refer to section 12.2) on the appropriate form (see section 14). d. Remove the ampule and label with autoclave #, cycle type (i.e., gravity cycle), and date of run.

	e. Incubate the ampule as well as one control ampule that has not been autoclaved at 55±1ºC for 48-72 hours and record the results on the appropriate form (see section 14). f. Refer to section 12.7a for interpretation of biological indicator ampule results.
12.4 Monthly Performance Verification of Short Liquid Cycles	a. On a monthly basis, verify autoclave performance by running a short liquid cycle in autoclaves #1, #2, and #4. Use freshly prepared tryptic soy broth (TSB), a maximum registering thermometer, and chemical indicator strip. b. Prepare 1 L of TSB and dispense 500 mL into each of two 1 L bottles; record preparation of TSB on the appropriate media preparation sheet. In addition, prepare four 1 L bottles, each containing 500 mL deionized water; no preparation sheet is required for the water. Place the maximum registering thermometer in an empty beaker or flask amongst the six 1 L bottles. Place the chemical indicator strip on top of the beaker or flask containing the thermometer. Run a short liquid cycle (15-minute liquid cycle). c. Upon completion of the cycle, remove items from the autoclave and record the minimum and maximum temperatures achieved during the "sterilize" portion of the cycle from the autoclave printer readout, the maximum registering thermometer reading, and chemical indicator strip results (refer to section 12.2) on the appropriate form (see section 14). d. Remove the two bottles of TSB and label with autoclave #, cycle type (i.e., liquid cycle), and date of run. e. Incubate one bottle of TSB at 36±1°C and the other bottle at 55±1ºC for 3-10 days and record the observations on the appropriate form (see section 14). This preparation of TSB is used only as a quality control check of the autoclave; discard after incubation. f. Refer to section 12.7b for interpretation of TSB incubation results.
12.5 Monthly Performance Verification of Kill Cycles	a. On a monthly basis, verify autoclave performance by running a kill cycle in autoclaves #2, #3, and #4. Performance verification on autoclave #5 is only performed if the autoclave is anticipated to be in use. Use a biological indicator ampule, maximum registering thermometer, and chemical indicator strip.

		b. Place the biological indicator ampule in the center of an autoclave bag filled with solid waste. Place the maximum registering thermometer in an empty beaker or flask in the bin with the bag. Place the chemical indicator strip on top of the beaker or flask containing the thermometer. Run a kill cycle (180-minute liquid cycle). c. Upon completion of the cycle, remove items from the autoclave and record the minimum and maximum temperatures achieved during the "sterilize" portion of the cycle from the autoclave printer readout, the maximum registering thermometer reading, and chemical indicator strip results (refer to section 12.2) on the appropriate form (see section 14). d. Remove the ampule from the autoclave bag and label with autoclave #, cycle type (i.e., kill cycle), and date of run. e. Incubate the ampule as well as one control ampule that has not been autoclaved at 55±1ºC for 48-72 hours and record the results on the appropriate form (see section 14). f. Refer to section 12.7a for biological indicator ampule results.
12.6	Performance Verification of Kill Cycles with Select Agent	a. Prior to commissioning the laboratory, conduct the monthly performance verification of a kill cycle on the autoclave in B202. b. Refer to the Select Agent Registration Biosafety Plan for *Bacillus anthracis*, section 13 (Decontamination of Biohazardous Waste) for quality control measures to decontaminate biohazardous waste associated with select agent. These procedures include using a biological spore ampule with each kill cycle. c. Spore ampule must indicate no growth, see section 12.7a.
12.7	Monthly Performance Results	a. Biological indicator ampule results. The control ampule should show growth: growth is evident by either turbidity and/or a color change from a purple to or toward yellow. The autoclaved ampule should not change color and be clear. If the autoclaved ampule shows growth, repeat the performance verification, verifying that the quality control indicators were placed appropriately. If failure continues, call for service on the autoclave. b. Observations after TSB incubation. After incubation, each bottle of TSB should be free of growth with no turbidity. If either bottle of the autoclaved TSB shows growth, repeat the performance verification, verifying that the quality control indicators were

	placed appropriately. If failure continues, call for service on the autoclave.
12.8 Autoclave Alarms	1. An autoclave may go into alarm during a run. a. If the autoclave goes into alarm before the sterilization process has begun and the cycle aborts (e.g., door alarm), attempt to determine the cause of the alarm, resolve it, and restart the cycle. b. If the autoclave goes into alarm during the sterilization phase and the cycle aborts, media and reagents must be discarded. Non-heat sensitive reagents and glassware must be autoclaved again. c. If the autoclave goes into alarm after the conclusion of the sterilization phase (e.g., exhaust phase), items may be used provided that all quality control indicators pass. c. If autoclave goes into alarm during subsequent runs, call for service.
13. Data Analysis/ Calculations	None.
14. Forms and Data Sheets	1. Attachment 1: Performance Verification Practices for Autoclaves 2. Attachment 2: Placement of Quality Control Indicators for Monthly Performance Verification 3. Test Sheets. Test sheets are stored separately from the SOP under the following file names: Daily Sterilization Record Log Form QC-13-09_F1.docx Monthly Sterilization Record Form QC-13-09_F2.docx Recommended Media Sterilization Procedures QC-13-09_F3.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.

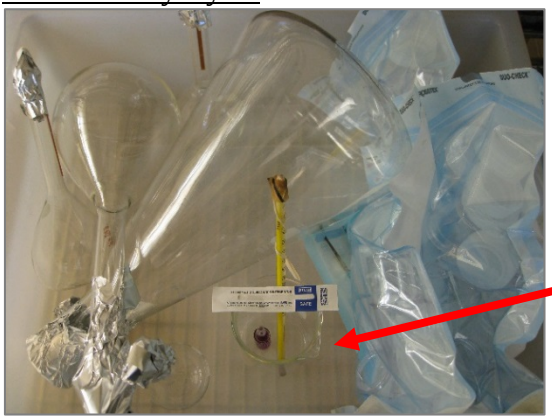
Attachment 1: Performance Verification Practices for Autoclaves

Autoclave ID	Room	Performance Verification and Conditions			
		Per Run	Monthly Performance Verification**		
			Short Gravity: 45 min gravity	Short Liquid: 15 min liquid	Kill Cycle: 180 min liquid
#1	B206	Thermometer/strip located per monthly performance verification instructions	Ampule & thermometer in empty beaker in the bin holding the glassware, strip on top of beaker containing ampule and thermometer	TSB (0.5 L in two 1 L bottles), DI water (0.5 L in four 1 L bottles). Thermometer in a beaker/flask amongst bottles, strip on top of beaker/flask containing thermometer	N/A
#2	B204		Ampule & thermometer in empty beaker in the bin holding the glassware, strip on top of beaker containing ampule and thermometer	TSB (0.5 L in two 1 L bottles), DI water (0.5 L in four 1 L bottles). Thermometer in a beaker/flask amongst bottles, strip on top of beaker/flask containing thermometer	Ampule in center of full waste bag, thermometer in empty beaker/flask, strip on top of beaker/flask containing thermometer; all inside a bin
#3	B207		Ampule & thermometer in empty beaker in the bin holding the glassware, strip on top of beaker containing ampule and thermometer	N/A	Ampule in center of full waste bag, thermometer in empty beaker/flask, strip on top of beaker/flask containing thermometer; all inside a bin
#4	B202		Ampule & thermometer in empty beaker in the bin holding the glassware, strip on top of beaker containing ampule and thermometer	TSB (0.5 L in two 1 L bottles), DI water (0.5 L in four 1 L bottles). Thermometer in a beaker/flask amongst bottles, strip on top of beaker/flask containing thermometer	Ampule in center of full waste bag, thermometer in empty beaker/flask, strip on top of beaker/flask containing thermometer; all inside a bin
#5*	D122		N/A	N/A	Ampule in center of full waste bag, thermometer in empty beaker/flask, strip on top of beaker/flask containing thermometer; all inside a bin

*Autoclave 5 is only verified when it is needed, otherwise it is shut down and not used. **Refer to Attachment 2 for photographs of quality control indicator placement.

Attachment 2: Placement of Quality Control Indicators for Monthly Performance Verification

Short Gravity Cycle



Beaker containing thermometer and ampule with strip on top, inside bin with glassware.

Short Liquid Cycle

Beaker containing thermometer with strip on top, amongst bottles of TSB (2) and DI water (4).

Kill Cycle

Ampule in center of autoclave waste bag.

Flask containing thermometer with strip on top.