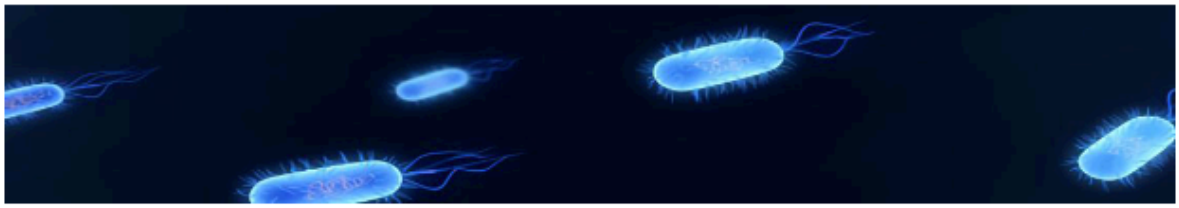
SOP Training Using a Question and Answer Approach

By David A. Porter, Ph.D.



INTRODUCTION

Welcome to the PMF training module covering the SOP “Performance Verification of Autoclaves”. The version in this training module has been updated and is available at

https://19january2017snapshot.epa.gov/sites/production/files/2015-08/documents/ qc-13-07.pdf.

The version addressed in this training module is no longer available, but should serve to illustrate some general aspects of good SOP writing and analysis.

The first thing to do is read the SOP. Put yourself in the shoes of the person who will actually need to follow this SOP. Make note of aspects that are unclear to you. Think about what might be done to make the SOP easier to follow, and more effective as a training tool.

There are a number of questions provided that we recommend you think about prior to clicking the link to an answer (click on the Question # at the top of the question box). Note that many times in science, there is no single correct answer. Often answers come in shades of gray. There is an “Exam” at the end that could be printed, filled out, and submitted as part of a training file. PMF training will always include self-assessments as we consider this to be an essential part of effective learning.

The expectation is that the PMF will add more training to the website on a regular basis.

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

SOP Number	QC-13-07
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:	

TABLE OF CONTENTS

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

Question 1

Why is it necessary to verify the performance of autoclaves?

Question 2

Has the SOP been approved?

Question 3

What is a “kill cycle”, and how was that determined?

Question 4

What species of bacteria is used in the biological indicators in this SOP, and why was that species chosen?

Question 5

Does the position of thermometers, biological indicators and chemical indicator strips matter?

Question 6

How should biological indicator ampules be stored?

Question 7

Where should results be recorded?

Question 8

What qualifies as a passing result for a chemical indicator strip?

Question 9

According to Section 11, how many spores are contained in a biological indicator ampule?

Question 10

What other documents should you review to complete your training in this specific SOP?

Go to Questions

Answer to Question 1

Why is it necessary to verify the performance of autoclaves?

This can be found on page 1 of the SOP:

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)

Go to Questions

Answer to Question 2

Has the SOP been approved?

The approval block (below) has not been signed, so you cannot determine from this copy if the SOP has been approved.

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

Go to Questions

Answer to Question 3

What is a “kill cycle”, and how was that established?

A “kill cycle” is defined as”

1. Definitions	1. A Kill cycle is a liquid cycle with a duration of 180 minutes to sterilize bio-hazardous waste.

What is not clear from the document is how the time of 180 minutes was established. There does not appear to be a reference clearly indicated in which the validation of this time was performed. This would be a fair question to raise to the SOP authors.

Go to Questions

Answer to Question 4

What species of bacteria is used in the biological indicators in this SOP, and why was that species chosen?

> 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.

As you can see, *Geobacillus stearothermophilus* is the species used.

Why was this species chosen? There is no clear reason provided in this SOP. A reference to a chapter such as <1035> Biological Indicators for Sterilization in the United States Pharmacopeia might be helpful. There you can read that the species chosen must possess a high degree of resistance to the mode of sterilization to be evaluated.

Go to Questions

Answer to Question 5

Does the position of thermometers, biological indicators and chemical indicator strips matter?

The following may be found in the SOP:

> 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.

Attachment 1 contains descriptions in tabular format such as:

Monthly Quality Check**

Short Liquid (15 min)
Ampule in test tube (with media) in full rack. thermometer/strip in a beaker/flask near media; empty bin (s) on bottom shelf

Would the inclusion of a picture make this easier to follow, and perhaps improve the SOP for training purposes?

Go to Questions

Answer to Question 6

How should biological indicator ampules be stored?

The following is in the SOP:

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.

Do you think this SOP provides enough specific information as to which refrigerator to use if the lab has more than one?

Go to Questions

Answer to Question 7

Where should results be recorded?

There are a number of references to Section 14 of the SOP in which you find:

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

For use as a training tool, do you think it would be helpful to include copies of these forms as attachments, especially given that they reference the same SOP number?

Go to Questions

Answer to Question 8

What qualifies as a passing result for a chemical indicator strip?

> 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.

What do you do if the strip does not pass?

The SOP contains a section on non-conforming data. The section describes a number of steps to take to evaluate the non-conformance, but it is not clear how management should be involved. You should probably look at the SOP referenced in the section below to see if that issue is clarified.

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

Go to Questions

Answer to Question 9

According to Section 11, how many spores are contained in a biological indicator ampule?

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

It looks like there are 106 spores/ampule. Does this seem correct to you? Would a value of 10^6/ampule be more realistic? If so, should you tell the SOP authors?

Go to Questions

Answer to Question 10

What other documents should you review to complete your training in this specific SOP?

References to the following can be found in the SOP:

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

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.

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.

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.

9. Data Management	1. Data will be archived consistent with SOP ADM-03, Records and Archives.

There may be other documents references in the SOP that are not to other SOPs. Don't hesitate to ask to see these if you think it is necessary to complete your training.

Exam

The exam can contain any questions you would like. They can come from the Q&A sections, or can be completely different. For the purposes of this example, three new questions are presented. For official use, the document can be printed out and completed.

Name: _____
Date: _____
SOP: _____
Trainer: _____

1. Where can you find information about personnel training and qualifications?

2. How often are the registering thermometers verified?

3. How frequently is a quality control check of the instruments performed?

Current PMF Sponsors

Giles Scientific, Inc.
https://www.biomic.com/trinity-v3.html

Special Process Services, LC
https://www.linkedin.com/in/joseph-connaghan-b663929