Preparation and Review of SOPs Designed to Maximize Scientific Usefulness and Compliance[1]

"Science is more than a body of knowledge; it is a way of thinking."
- Carl Sagan

An SOP is supposed to describe what is to be done, and positive documentation associated with it is supposed to provide evidence that what was said to be done was indeed done. In other words, say what to do, and then do what is said. An effective SOP should contain only as many words as necessary, no more, no less, and should certainly make use of images where such images can replace much verbiage.

There is a danger hiding in this standard view of SOPs. By dogmatically adhering to this view, or approach, to SOPs, the risk hiding in plain sight is that of falling into the "it's always been done this way" trap. I am going to describe an approach to the preparation and review of SOPs that should facilitate their treatment as living scientific documents. By treating SOPs as scientific documents, and by treating those who write and follow them as scientists, I believe that compliance with them will follow naturally.

The SOP chosen for this exercise is "Standard Operating Procedure for Performance Verification of Autoclaves", QC-13-07 (6-15-2015 version) available online from the U.S. Environmental Protection Agency, Office of Pesticide Programs [https://19january2017snapshot.epa.gov/sites/production/files/2015-08/documents/qc-13-07.pdf]. Actual text from the SOP is in **bold black font**, questions that may arise from the text are in blue font (the provided questions are certainly not to be thought of as exhaustive as others may arise from other reviewers and the trainees), possible answers to the questions are in purple font, and suggested modifications to the SOP text offered by the reviewer(s) are in [red font]. Note that for the most part answers should be provided by the authors of the SOP, and therefore mostly placeholders are indicated for the answers. A final aspect of the questions that may be provided is that they often address what the consequences are if the stated directions/conditions are **not** met.

At the end of the SOP example is a discussion of how this preparation and review approach can lead to steady improvement of the SOPs as instructions, as training material, and as a means of capturing institutional knowledge that makes it readily accessible to users and regulators/auditors/inspectors/etc.

SOP Number QC-13-07

Title	**Performance Verification of Autoclaves**
Scope	**This protocol describes the procedures for verifying the performance of the autoclaves.** **How does verification compare to validation?** **[answer]**

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)**

These are literature citations. Where can this literature be found?

[answer]

1. **Definitions**

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

Is it still a kill cycle if it runs for > 180 minutes?

[answer]

How was this length of time determined?

[answer]

2. A gravity cycle is a dry cycle used for sterilization of dry laboratory materials (e.g., glassware, carriers).

Why is it called a gravity cycle?

[answer]

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.

Are there chemical indicators for cycles that don't use steam?

[answer]

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 [italicize Geobacillus stearothermophilus?] spores.

Why was the brand Raven Biological chosen? Are there other acceptable makers of such indicators?

[answer]

Why is this particular species of microorganism indicated?

[*Geobacillus stearothermophilus* is a species known to be highly tolerant of elevated temperatures, thus making it a good indicator of effective sterilization at high temperatures.]

5. Maximum Registering Thermometers (mercury-containing/teflon-coated) are used to verify a maximum autoclave temperature.

Are there other types of maximum registering thermometers?

[answer]

6. Additional abbreviations/definitions are provided in the text.

2. Health and Safety

1. Follow procedures specified in SOP MB-01, Laboratory Biosafety.

Where are the various cross-referenced SOPs indicated in this SOP located?

[answer]

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.

How can we determine which laboratory personnel have been trained on autoclave usage?

[answer]

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.

What constitutes a "similar" maximum registering thermometer?

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~~ ensure] shelf life. Upon receipt, the biological indicators ampules must be placed in the refrigerator.

Where can the manufacturer's specifications be found?

[answer]

What refrigerator should be used?

[answer]

What temperature range for the refrigerator is acceptable?

[answer]

6.Quality Control

1. For quality control purposes, the required information is documented on the appropriate form(s) (see section 14).
Where are the required forms kept?
[answer
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).
Where are expiration dates found?
[answer]
Who performs the quality control checks?
[answer]

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.
Should the thermometer be held any particular way for shaking?
[answer]
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.
Does the thermometer have to be read exactly at ambient temperature? If so, how is it ensured that the thermometer is at ambient temperature?
[answer]
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.
How does one know if a specific medium requires a lower sterilization temperature?
[answer]

8. Nonconforming Data

1. Management of non-conforming data will be consistent with SOP ADM07, 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.

How were those positions established?

[answer]

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.

How does doing this help establish what went wrong during the first cycle run?

[answer]

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 heatsensitive). If media fails sterility or performance, a new batch must be prepared.

If it is OK to use media prepared in a complete but failed cycle, then what was the point of running the cycle with control indicators?

[answer]

d. Glassware and non-heat sensitive reagents must be autoclaved again.

For how many extra cycles, and how does one know when to stop?

[answer]

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.

What if one can't resolve the cause for the alarm?

[answer]

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.

How does this help resolve the reason for the alarm?

[answer]

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.

Why isn't it permissible to re-autoclave the media and heat-sensitive reagents?

[answer]

d. If autoclave goes into alarm during subsequent runs, call for service.

If one weren't able to determine the cause for alarms in the previous cases, shouldn't service be called as well?

[answer]

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.

Are there visible labels for autoclaves # 1 and 2?

[answer]

How much longer than 10 minutes can the wait period be?

[answer]

The final line says to wait at least 10 minutes prior to unloading. Is this the same as the "wait period"?

[answer]

11. Special Apparatus and Materials

1. Raven Biological Laboratories ProSpore Biological Indicator Ampules with 106 [10^6?] spores of G. stearothermophilus (ATCC #7953) per unit.

Is it OK to use a different brand?

[answer]

2. SPS Medical Chemical Indicator Strips.
Is it OK to use a different brand?
[answer]
3. Incubator with temperature set at 55º C ± 1º C.
What would happen if the temperature range exceeds ± 1º C?
[answer]
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.
Is there an Autoclave #3 somewhere?
[answer]
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.
Would it be OK to use maximum registering thermometers with a wider range?
[answer]

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. [perhaps use S-MMDDYYYY format]
b. The suffix where the first digit after the [~~dash~~ year] 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. [consider making this text indented and labelled “i” as opposed to “c” to ensure linkage with section “b”]

d. Record the sterilization batch number in the Daily Sterilization Record Information Log Form (see section 14). [if the suggestion immediately above is taken, this becomes section “c”]

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.

Is the indicated temperature range inclusive or exclusive of the endpoints?

[answer]

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.

[It is possible that younger trainees/operators may have only used digital fever thermometers, thus they may have never “shaken” a fever thermometer.]

What should be done if the maximums indicate on the autoclave printout and the thermometer differ, and how much difference would be acceptable?

[answer]

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. [include pictures of passing and failing strips]

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.

What constitutes "short" gravity and liquid cycles?
Who is responsible for performing the monthly performance verifications?
[answer]

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. [Include a picture of a rack properly prepared?] 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.

How close to the nominal volumes of liquid are required? In other words, what is an acceptable range?
[answer]

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.

Are there any size limitations for the beaker to be used?
[answer]

d. Immediately upon completion of the cycle, remove items from the autoclave.

What are the consequences if the items are not removed "immediately"?
[answer]

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.
Is there an SOP describing how dates should be indicated?
Is the labeling to be done directly on the ampule, or on a label affixed to the ampule?
[answer]
[Include a picture of a properly labeled ampule?]
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.
Are the results to be considered invalid if the incubation temperatures or times were to exceed the indicated range for any period of time?
What is to be done if the control ampule does not show growth?
[answer]
[Could pictures be provided showing turbidity and color changes?]
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.
Who is responsible for conducting the performance verifications?
How many days constitutes “several”? What is an acceptable range of days?
[answer]
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).

How large should the autoclave bag be, and how much solid waste should be included (ranges)?

[answer]

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

Where are the Test Sheets to be stored?

[answer]

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

Are printouts of these references available to read?

[answer]

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	B205	*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

Are there particular sizes of beakers/flasks that should be used?
[answer]
What size/types of bins are suitable for use that are to hold glassware?
[answer]
What constitutes "near"?
[answer]
Is there a range of bag sizes that should be used?
[answer]

Discussion

When preparing and reviewing SOPs, it is important to remember that they serve a myriad of functions. SOPs and positive documentation called for by the SOPs describe what is to be done and provide evidence that what was said to be done was actually done. Effective SOPs also serve as training vehicles for both trainees and trainers, as well as regulators/inspectors/auditors/etc.

The approach used in the sample SOP given above provides a means whereby the requirements described above may be met, as well as providing a means of facilitating the SOPs' existence as a living system. Asking questions during the preparation of the SOPs helps the authors reduce ambiguities, and helps the authors to remember that what seems obvious to them may very well not be to the trainees. Many more experienced microbiologists are seeing in recent microbiology graduates less knowledge in classical microbiology and more in the use of microorganisms as components in molecular biology research. Moreover, it is not uncommon for individuals with virtually no microbiology training to find themselves working in a microbiology laboratory, or in a manufacturing facility where some basic understanding of microbiological principles would be advantageous.

As noted above, the answers would most often be developed by the authors. Sometimes the authors may find themselves actually having to do some research into just why the SOP instructions are written the way they are. This can be helpful in reducing the "we've always done it this way" trap described in the introduction. It can also be an effective means of capturing institutional knowledge.

The approach illustrated with the sample SOP can be thought of as leading to three varieties of the same SOP that could actually be derived from one SOP that contains the questions and answers along with the actual instructions to be followed. The full version containing operational text, questions and answers could be thought of as the trainer's version (like the teacher's version of the text book form days of old). A version containing the operational text and questions could be thought of as the trainee's text (like the student textbook, again from the days of old). A major advantage of providing these to trainees is that it should serve to remind them that science involves questions, and that additional questions that they come up with should also be considered, and may well be added to the next version of the trainer's and trainee's versions (keeping the SOP system "living"). The third version would of course be the operational text (would not contain questions and answers). Trainees/operators brought up with this type of SOP system would know that scientific questioning is always important, and they would know that documents exist with answers to many questions, and this knowledge would be valuable in day to day operations, and in responding to regulator/inspector/auditor questions as well. This would also serve to help encapsulate institutional knowledge, all too easily lost due to employee turnover.

There is a role that third parties, including consultants, can play in this process. A third party, especially one trained broadly in science but not a member of the company preparing the SOPs, will not be indoctrinated as to how the company has done what it does, nor will such a party fully understand, if at all, why the company does what it does. Moreover, because the third party is not directly employed by the company writing the SOPs, there will be little to no fear about asking questions that may be thought of as obvious by the company, or perhaps even uncomfortable for the company. Also, by having a third party reviewing and preparing questions, it is less likely that a regulator/inspector/auditor/etc. will come in and ask such questions that come as a surprise. It is helpful to remember that such people most often are

technically trained, and reasonable, scientific questions deserve reasonable, scientific answers.

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