Practical Guide to Autoclave Validation

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In addition to potential business liabilities, there can be significant costs associated with an autoclave validation process. The practical experience that this article is based on may provide assistance in ensuring an effective, efficient validation process for steam sterilization.

Introduction

This article is based on practical experiences gained by the author while conducting hundreds of validation test runs on dozens of autoclaves of varied manufacture. It is primarily intended that personnel who perform validation testing on autoclaves may benefit from these experiences, and that it will assist in ensuring a high level of compliance in the validation process. The article also may be of benefit in selecting an appropriate validation strategy and/or cycle. Personnel unfamiliar with steam sterilization principles or autoclave validation could use the material as a basic training tool and it may be a good refresher for more experienced personnel. A list of definitions and references are provided at the end of the article.

Sterility Assurance Level

The level of microbial inactivation can be described by an exponential function, "Sterility Assurance Level" or SAL. For example, a SAL of 10^{-6} means that the probability of a single viable microorganism being present on a sterilized item/product is one in one million after the item has undergone a sterilization process. A SAL of 10^{-3} means that the probability of a single viable microorganism being present after sterilization is one in one thousand.

The SAL required is determined by the intended use of the item/product. Sterilization processes associated with parenterals and medical devices that pose a significant risk in terms of the probability and severity of an infection (e.g., implants, sterile fluid pathways, products intended to come into contact with compromised tissue) generally have been sterilized to an SAL of 10^{-6}. Medical device products not intended to come into contact with breached skin or compromised tissue are generally sterilized to a SAL of 10^{-3}.

The remainder of this article is written assuming that a SAL of 10^{-6} is required.

Log Reduction

Achieving a 1-log reduction means to decrease the microbial population by a factor of 10. The bioburden is the number and type of viable microorganisms contaminating an item. A sterilization cycle that provides a SAL of 10^{-6} effectively means that the microorganisms that "could" be present (i.e., bioburden) are killed, and an additional 6-log reduction safety factor has been provided. The following provides an example of a cycle achieving a SAL of 10^{-6}.

- Bioburden (worst case) = 134 CFU (colony forming unit).
- To reduce the microbial population from 134 to 1 = log (134) = 2.13 (i.e., a 2.13-log reduction is required to reduce the population from 134 to 1).
- Applying an additional 6-log reduction will theoretically reduce the microbial population from 1 to 0.000001. This provides a SAL of 10^{-6} or a one in one million probability of a single surviving microorganism.
- Total log reduction = 2.13 + 6 = 8.13. Therefore to provide a SAL of 10^{-6} with a bioburden of 134 CFU requires a sterilization cycle that provides an 8.13 log reduction.

Figure 1. Empty chamber temperature mapping (Photograph provided courtesy of Kuhlman Technologies Inc.)

Thermal Resistance Characteristics

The thermal resistance of specific microorganisms is characterized by "D-values" and "Z-values." A D-value is the time in minutes, at a specific temperature, to reduce the surviving microbial population by 1-log. A Z-value is the temperature change required to result in a 1-log reduction in D-value.

Other time measurement variables pertaining to thermal resistance are "F-values" and "F_o-values." An F-value is the number of minutes to kill a specified number of microorganisms with a specified Z-value at a specific temperature. An F_o-value is the number of minutes to kill a specified number of microorganisms with a Z-value of 10°C (50°F) at a temperature of 121.1°C (250°F).

Common Misconception and Equivalent Sterilization Time

It is not uncommon to encounter the concept that "121.1°C (250°F) is the temperature required for steam sterilization." This understanding is not entirely correct. Extensive empirical studies were conducted and one of the critical variables (temperature) was pre-selected. It is not surprising that the temperature selected was an obvious round number in the temperature range of interest (250°F). The F_o-value equation can be used to determine the relative sterilization time at other temperatures as per the following (with Z-value = 10°C):

$$F_o = 10^{(T - 121.1)/10}$$

where T = temperature (° C) and F_o = equivalent sterilization time (min.)[1]

Table A provides some examples and the relationship follows in graphical form in Figure 2.

As is demonstrated by the data above, sterilization can be achieved using any of these temperatures. The lower the temperature the longer the sterilization cycle required. This is an important concept to consider because there are occasions where the temperature needs to be carefully selected. An example is a liquid that cannot withstand high temperatures. Ideally, the highest temperature that the load can withstand is selected, since this will provide the shortest possible cycle.

Variables Required to Determine an Ideal Sterilization Cycle

An "ideal" sterilization cycle presumes an ideal sterilizing environment (i.e., saturated steam with no air). The ideal cycle can be determined with the following three variables: bioburden, D-value, and required SAL. The following provides some examples:

a) Given: Bioburden = 75 CFU, D-value = 0.5 min./log at 121.1°C, Required SAL = 10^{-6}
   Then: Log (75) = 1.88
   Log Reduction required = 1.88 log + 6 log = 7.88 log
   Ideal Cycle at 121.1°C (250°F) = (7.88 log)(0.5 min./log) = 3.94 minutes

b) Given: Bioburden = 1,215 CFU, D-value = 1.6 min./log at 121.1°C, Required SAL = 10^{-6}
   Then: Log (1215) = 3.08
   Log Reduction required = 3.08 log + 6 log = 9.08 log
   Ideal Cycle at 121.1°C (250°F) = (9.08 log)(1.6 min./log) = 14.53 minutes

Overkill Approach

Determining the bioburden and D-value for all items to be sterilized in a load can be quite time consuming and costly. As a result, for items that are not heat sensitive, an "overkill" approach is generally employed.

An overkill approach avoids collecting bioburden and D-value data by assuming worst-case conditions. A bioburden of 10^6 of a highly heat resistant spore forming bacteria (Bacillus stearothermophilus) is utilized. The D-value at 121.1°C for these bacteria is generally slightly above 2 minutes, and therefore using 2.5 minutes is a good worst-case value.

With a bioburden of 10^6, to achieve a SAL of 10^{-6} requires a 12 (6 + 6) log reduction. Under ideal conditions, the length of an overkill sterilization cycle at 121.1°C is therefore (12 log)(2.5 min./log) = 30 minutes.

Bioburden and D-Value Approach

For items that are heat sensitive and cannot withstand an overkill approach, it is necessary to collect bioburden and possibly D-value data. This will dramatically shorten the sterilization cycle required. For example, if the bioburden is low (e.g., 10 CFU) and even moderately resistant (e.g., D-value = 0.5), an ideal 30-minute overkill cycle at 121.1°C can be replaced by an ideal cycle of 3.5 minutes (7 log x 0.5 min./log). Alternatively, the sterilization temperature could be reduced

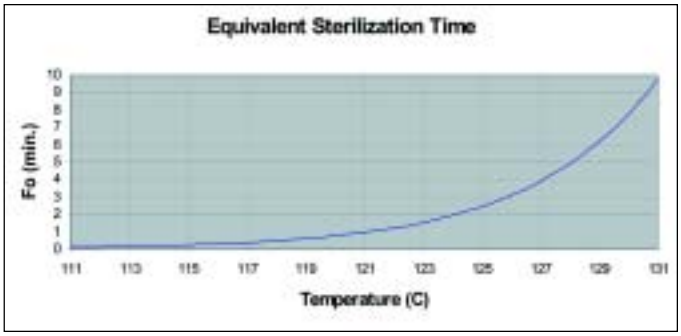

Figure 2. Equivalent sterilization time.

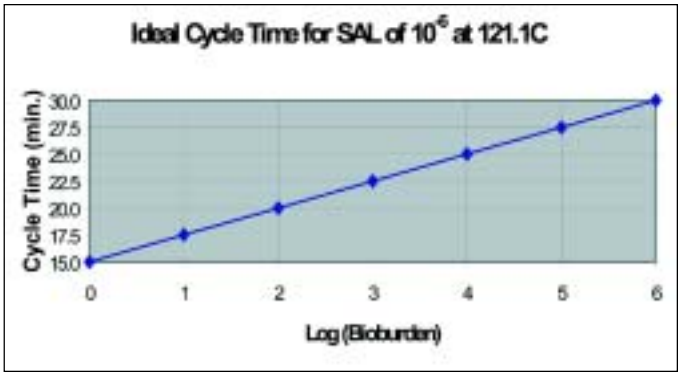

Figure 3. Ideal cycle time.

Temperature	F_o	Equivalency to 121.1°C (250°F)
115°C (239°F)	0.25 min.	1 minute at 115°C provides the same lethality as 0.25 minutes at 121.1°C
120°C (248°F)	0.78 min.	1 minute at 120°C provides the same lethality as 0.78 minutes at 121.1°C
121.1°C (250°F)	1 min.	1 minute at 121.1°C provides the same lethality as 1 minute at 121.1°C
122°C (251.6°F)	1.23 min.	1 minute at 122°C provides the same lethality as 1.23 minutes at 121.1°C
125°C (257°F)	2.45 min.	1 minute at 125°C provides the same lethality as 2.45 minutes at 121.1°C

Table A. Equivalent sterilization time.

to 112°C and yet only require slightly less than a 30 minute ideal cycle. It may be a significant advantage to reduce the sterilization temperature and/or time.

A compromise approach may sometimes be utilized where bioburden data is collected, but D-value studies are not performed. A worst case D-value of 2.5 could then be employed. This approach will provide a somewhat shortened cycle and avoids the time and cost of D-value studies. Following our example with a bioburden of 10 CFU, the ideal cycle at 121.1°C can be shortened from a 30-minute overkill cycle to a 17.5-minute cycle (7 log x 2.5 min./log).

Figure 3 shows the sterilization time required at 121.1°C for an ideal cycle to achieve a SAL of 10^{-6} at varying levels of bioburden (D-value = 2.5 min.).

Vacuum and Non-Vacuum Cycles

Previously, this article has addressed "ideal" cycles that presume an ideal sterilizing environment. In terms of the length of cycle required, one can only approach ideal cycles for items that are easily sterilized. Most often, items/loads with less than ideal conditions are encountered.

There are three basic types of cycles as follows:

a) Hard Goods (Vacuum):
Suitable for items easy to sterilize since air removal and steam penetration are highly effective. Examples are many types of glassware and large diameter piping. A typical hard goods cycle may draw one vacuum prior to introducing steam, reaching the desired sterilization temperature, and beginning the sterilization dwell period. A typical pressure vs. time graph for a hard goods cycle is shown in Figure 4.

b) Wrapped Goods (Vacuum):
Utilized for items difficult to sterilize since air removal and steam penetration are harder to achieve. Examples are gowns, long lengths of tubing, and tanks/vessels/apparatus with small inlet/outlet ports and/or vent filters. A typical wrapped goods cycle may draw three or more vacuums prior to reaching the desired sterilization temperature and beginning the sterilization dwell period. A post sterilization vacuum also is usually drawn to evacuate the steam from the load items. Often the length of time to pull and release the vacuums exceeds the length of the sterilization dwell. A typical pressure vs. time graph for a wrapped goods cycle is shown in Figure 5.

c) Liquids/Gravity Displacement (Non-Vacuum):
Items that contain liquids generally cannot have a deep vacuum pulled or the liquid will be drawn out of the item. Liquid cycles generally just heat up and cool down and do not utilize vacuums. These items may require a lengthy cycle time especially where the liquid volume is large because the length of time required to heat up and cool down the liquid may be considerable. Another term for a liquid cycle is "gravity displacement" as the air is displaced by gravity (i.e., removing air by introducing steam into the top of a chamber and displacing the air, which is heavier than steam, by removing the air from the bottom of the chamber). A typical pressure vs. time graph for a liquids cycle is shown in Figure 6.

Basic Validation Approach

Installation Qualification (IQ)

The IQ process is intended to demonstrate that the autoclave as installed meets all specifications, is installed properly, and that the supporting programs needed for ongoing operation (e.g., standard operating procedures, maintenance program, etc.) are in place.

An IQ may include the following checks:

- Mechanical Equipment Specifications (chamber, valves, traps, strainers, filters, regulators, vacuum pump, heat exchanger, condenser, etc.)
- Control and Instrumentation Specifications (programmable logic controller, operator interface, printer/recorder, control valves, transducers, pressure and temperature transmitters, resistance temperature devices, switches, level sensors, interlocks, photocells, etc.)
- Site Specifications/Utilities (power, grounding, surge protector, uninterruptible power supply, breakers, water, air, clean steam, plant steam, drain, shutoff / isolation valves, electrical disconnect switches, etc.)
- Drawings Verification (P&ID, mechanical, electrical)
- Construction Materials/Materials in Product Contact
- Approval Documentation (e.g., pressure vessel, electrical, etc.)
- Change/Spare Parts
- Bill of Materials
- Vendor Specification Sheets
- Purchase Orders
- Factory Performance Tests
- Commissioning Report
- Preventive Maintenance Program
- Standard Operating Procedures (operating, maintenance, calibration)*
- Operating and Maintenance Manuals
- Piping Installation Verification (slope, dead legs)
- Weld Inspection/Surface Roughness Documentation/Metallurgical Documentation
- Control System Documentation (system configuration/block diagram, flow sheets, display/report layouts, required interlock considerations, general process limits, conditions for operating over range, hard copy and electronic application code listing, timing diagram, system security, input/output point listing, data monitoring, alarms, software inventory

and version, software configurations, parameter listings, software development and testing records, change control, vendor qualification, modular software development documents, detailed module functional specifications, etc.)
- Instrumentation and Input/Output Dry Loop and Wet Loop Checks**
- PID Tuning**
- Instrument Calibrations**

* Operating Procedures can only be finalized after Performance Qualifications tests are completed when validated load configurations and cycles are known.

** Note: in some approaches, these checks are captured as initial Operational Qualification activities.

Operational Qualification (OQ)

The OQ process is intended to demonstrate that the components of the autoclave operate properly and that the autoclave is deemed ready for performance or load testing.

An OQ may include the following checks:

- Operational Tests (operator/supervisory/maintenance modes, doors, abort and emergency stop, alarms, programmable parameters, menu navigation, security, power-up and shutdown, operator interface display checks, interlock override control, procedure select/start control, step advance control, switch and interlock tests, etc.)
- Power Loss Recovery Test
- Source Code Review
- Filter Sterilization
- Leak/Air Removal/Steam Penetration/Vacuum Hold Test*
- Jacket Mapping
- Saturated Steam Check
- Empty Chamber Tests

* The Bowie Dick test is designed to test air removal, the absence of air leaks and steam penetration into a porous load. It uses a test pack of fabric with specific dimensions or there are commercial, use once packs available. It has been widely employed in Europe. In North America, a Vacuum Hold Test has often been employed. European Standard EN 554 specifies that if a sterilization process includes air removal from the product, a steam penetration test shall be carried out at the commencement of each day the autoclave is used. Although a vacuum hold test may be less sensitive than a Bowie Dick test, the author assumes that a vacuum hold test can be considered as a satisfactory alternative if strict acceptance criteria are applied. This assumption is based on steam penetration/lethality in the worst case load items being demonstrated and that the vacuum hold test therefore demonstrates absence of leaks and that the validated conditions that resulted in lethality are being met on an ongoing basis.

Empty Chamber Distribution Tests (Figure 1)

The basic objective is to show the chamber provides a uniform sterilizing environment. In the opinion of the author, "cold spots" in autoclaves are rarely encountered. Sometimes "cold thermocouples" are misinterpreted as cold spots (refer to following section "Tips").

Three consecutive successful runs are performed for each cycle type with typical acceptance criteria as per the following:

- Throughout the dwell time, all temperatures measured in the chamber are within a 3°C band (sterilization temperature + 3°C).[2] Note: the dwell set-point -1°C/+2°C is often used.
- Throughout the dwell time all temperatures measured in the chamber do not fluctuate by more than 1°C.[2]
- Throughout the dwell time, all temperatures measured in the chamber do not differ from each other by more than 2°C.[2]
- The steam is at a temperature corresponding to its vapor pressure.[2]
- The interval of time between the attainment of the sterilization temperature in the hottest and coldest parts of the chamber does not exceed 15 seconds for chambers of not more than 800L and not to exceed 30 seconds for larger chambers.[2]
- Timed measurements shall be controlled to an accuracy of ±1%.[2]
- Required pre-certification and post-certification of the data logger ensures that the temperature measurement system is accurate to within ±0.5°C.
- The vacuum hold test should achieve a vacuum level of 2.5 psia (with vacuum pump) and maintain the vacuum (without further vacuum being initiated) within 0.4 psi over a period of five minutes.

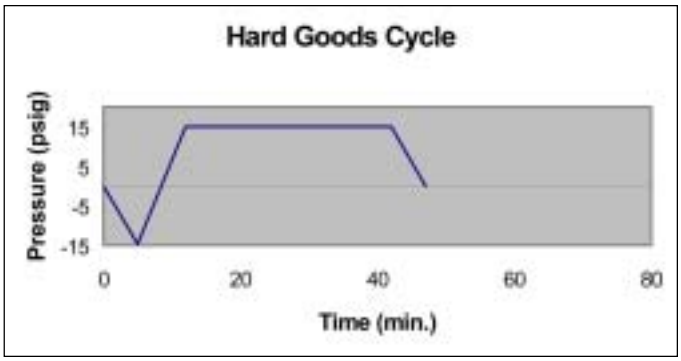

Figure 4. Hard goods cycle.

Figure 5. Wrapped goods cycle.

Performance Qualification (PQ)

Loaded Chamber Steam Penetration Tests

Loaded chamber steam penetration runs are then conducted on every load. Note: this is a very time consuming process, especially if you have a significant number of items to be sterilized. It is necessary to determine which load items are the most difficult to sterilize and which location(s) within the items presents the worst-case conditions.

There are two commonly used methods for determining the worst-case items/locations, thermocouples, and steam integrators. Steam integrators are commercially available strips that provide a quantitative indication of the exposure to steam. The

amount of steam exposure can be determined by measuring the movement of a chemical indicator on the integrator strip. The author recommends utilizing steam integrators since they are designed to measure steam exposure and thermocouples can result in misleading data (i.e., measuring temperature without taking into account whether there is any air present).

Determining which load items are the most difficult to sterilize and which location(s) within the items presents the worst-case conditions can be a daunting task. With a large load containing a wide variety of different types of items, the number of possible test locations seems to approach infinity. It also can be difficult to get the thermocouple and/or steam integrator into the item without adversely affecting the item's ability to be sterilized and/or ruining the item (a concern with expensive items).

One must evaluate an item on a case-by-case basis and determine how best to challenge the item. Often the item must be sealed somehow to return the item to a state that represents equivalency with respect to steam penetration. No attempt will be made to provide an exhaustive commentary here, but rather provide a few basic techniques for answering questions that inevitably arise:

- What is the most difficult point to sterilize in a hose of uniform diameter? Common sense can somtimes assist, dictating in this instance that the most difficult to sterilize point is in the center of the hose.
- How do you get a 10-foot length of thermocouple and/or steam integrator into the middle of a 50-foot hose? You can put a slice/cut into the middle of the hose and insert the thermocouple/integrator through the slice. Note: the cut must be sealed or you will not be challenging the hose properly. You can use silicon to seal the cut. Alternatively, if two 25-foot lengths of the hose are available you can join the two lengths with a connector and insert the thermocouple into the connector. The connector then must be sealed. The advantage here is that you don't ruin the 50-foot hose. The connector technique can be used for small diameter tubing where the hose is too small to insert a thermocouple and/or steam integrator.
- What is the worst-case location within a bottle, flask, or cylinder? This has been shown to be in the center, near, but not at the bottom.
- How can you minimize the number of runs required to challenge a load? Using steam integrators can help minimize the number of runs required to challenge a load. There are a limited number of thermocouples available, but as many integrators as desired can be placed in the load.

Load Configurations

Another variable of concern is whether fixed load configurations or flexible load configurations are desired. A fixed load configuration means that the load to be sterilized will be identical for all future processing runs and that the load is placed in the chamber in exactly the same way for all future processing runs.

In the opinion of the author, the location of an item in the chamber does not influence its ability to be sterilized (assuming that the location change does not involve a change in load density). This observation is based on the experiences of the author in conducting hundreds of validation test runs on dozens of autoclaves of varied manufacture. However, one should proceed as if the location within the autoclave is a variable of concern. One can eliminate this variable by rotating the items within a load from run to run and thereby attempt to demonstrate positional equivalency.

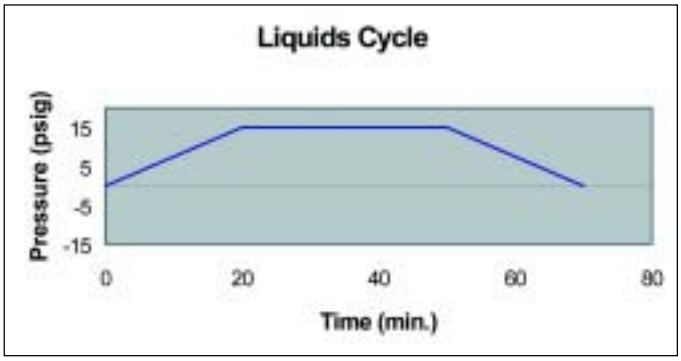

Figure 6. Liquids cycle.

For most loads, again in the opinion of the author based on experience, the number of items in the chamber does not influence an item's ability to be sterilized (unless the load becomes so dense that steam penetration/circulation becomes an issue). One should proceed as if this is a variable of concern. You can successfully validate a load while encompassing this situation by performing minimum and maximum load studies.

The following provides an example of fixed vs. flexible load configurations:

- Example load:
  - three (3) flasks
  - four (4) graduated cylinders
  - 24 plastic bottles with vent filters

- Fixed Load/Fixed Position:
  In this situation, all of the load items are placed in the autoclave, each time in the same position for each item, and a diagram of the load configuration is available in the procedures so that the operators can reproduce the load for every processing run. This situation will require the least validation runs, but offers no flexibility in load configuration.

- Fixed Load/Variable Position:
  In this situation, all of the load items are placed in the autoclave, but the location of the item in the autoclave can vary and only a list of the load items is required for the procedures. The validation runs must demonstrate positional equivalency by rotating the items from location to location during the test runs. It may be possible to accomplish this with the same number of validation runs as above and offers the operators some flexibility in loading the autoclave. This can be an advantage especially for large loads containing numerous items.

- Variable Load/Variable Position:
  In this situation, any or all of the load items (i.e., any combination of from 0 to 3 flasks, from 0 to 4 cylinders, from 0 to 24 bottles) can be placed in the autoclave in any position in the autoclave and only a maximum load list is required for the procedures. The validation runs must demonstrate positional equivalency by rotating the items from location to location during the test runs. The validation runs also must demonstrate that the cycle is adequate for both a maximum

load and minimum load configuration. The minimum load tests are done with only one item in the autoclave, that item being the load item demonstrated as being the most difficult to sterilize. This method will require the greatest number of validation runs, but offers the operators a great deal of flexibility in loading the autoclave. This can be a significant advantage in many situations.

Loaded Chamber Biological Challenge Tests

After determining the worst-case items and worst-case locations within items, these items are then challenged with biological indicators (spore strips and/or vials for placement within liquids). A thermocouple should be placed along with each indicator, as the temperature data will be required to extrapolate the cycle to achieve the SAL of 10^{-6}.

Tests are conducted until a cycle time results in three consecutive runs where the biological indicators show no growth. If it is important to achieve the shortest possible cycle, this process can consume a great deal of time as to determine the success/failure point likely requires obtaining failed test results along with successful test results. In addition, it takes time to determine whether the indicators exhibit growth (after two days of incubation you can be reasonably confident whether there is growth or not in most cases). If a few minutes of possibly unnecessary time added to the cycle is not a significant issue, it can be advantageous to attempt to predict a cycle time that you feel will pass. This can save considerable time and validation costs.

Once one has achieved three consecutive runs resulting in no growth and therefore demonstrating a 6–log reduction (assuming you were using indicators of 10^6 spores/strip), the following equations/example show how to extrapolate the full cycle required to achieve the SAL of 10^{-6}:

$La = [12 \times (Fo/R)] - Fo$

where La = the additional lethality (F_o) required
12 = used to extrapolate a 12-log reduction
Fo = the minimum accumulated F_o value from the biological challenge runs at the end of the cycle
R = the log reduction demonstrated (i.e. log [spore population])

$Fi = 10^{(T-121.1)/10}$

where Fi = the instantaneous F_o value
T = the minimum temperature expected during the additional lethality period (Note: this temperature should be taken as the temperature achieved at the end of the dwell period at the challenge location where the minimum accumulated F_o value resulted)

$Ta = La/Fi$

where Ta = the additional time required

$C = Ta + D$

where C = total dwell period time required
D = the dwell period time which resulted in the demonstrated reduction

Example Calculation:

The biological challenge runs were performed using spore strips that were enumerated at 1.21×10^6 spores/strip.
Therefore R = log (1,210,000) = 6.08

The minimum accumulated F_o value (at the end of the cycle) from the biological challenge runs was 30.2 minutes.
Therefore Fo = 30.2 minutes

$La = [12 \times (30.2/6.08)] - 30.2 = 29.4$ minutes

The temperature in the coldest item at the end of the dwell period was 119.4°C
Therefore T = 119.4°C

$Fi = 10^{(119.4-121.1)/10} = 0.676$

$Ta = La/Fi = 29.4/0.676 = 43.5$ minutes

The biological challenge runs were conducted with a dwell period of 45 minutes. Therefore D = 45 minutes

C = 43.5 + 45 = 88.5 minutes (note: this number should be rounded up)

Therefore the dwell period must be 89 minutes to achieve a 12-log reduction.

Three consecutive successful biological challenge runs are performed for each load with typical acceptance criteria consistent with the empty chamber distribution test acceptance criteria and all biological indicators used during the test cycle must show negative growth.

Tips

1. If you are going to draw a vacuum(s), ensure that the load items can withstand the vacuum(s). You don't want to be the person who has to report that the new $10,000 tank is now as flat as a pancake.
2. Rotate thermocouples from run to run. This avoids misinterpreting thermocouples that read slightly lower temperatures (i.e., cold thermocouples) as cold spots or cold items.
3. Label the thermocouples by number using a small strip of autoclave tape. This will greatly assist with ensuring that you are properly recording what thermocouple was placed in each location and will save validation time.
4. If you are performing a large number of test runs (e.g., over the course of several weeks), strike a compromise between post-calibration verification of thermocouples after every run and at the end of the entire testing period. If you wait until the end of the testing period, you run the risk that all of the runs are of no value due to not meeting the verification acceptance criteria. If you verify after every run, you will add considerably to the length of time required to complete the testing. The author has found that performing the verification every few runs or every few days is a reasonable compromise.
5. Be cautious with the acceptance criteria you employ for post-calibration of thermocouples. If the criterion is too tight (e.g., all thermocouples must meet the acceptance criteria), you may lose a lot of runs if one or two thermocouples cease functioning or are outside of the temperature tolerance after the run(s).
6. Take great care with documenting the validation test runs. The documentation should include: a diagram showing the location of all load items within the autoclave chamber, the items containing thermocouples, integrators and biological indicators, the precise location/number of each thermo

> ***...steam sterilization validation remains a significant issue to regulatory bodies, particularly for processes associated with high risk in terms of the probability and severity of an infection.***

couple, integrator and biological indicator within each item, the printout from the data recorder, the printout or chart from the autoclave, the time that the dwell period begins and ends (as per the data recorder time), and the results for each integrator or indicator. Each document should be clearly labeled with the date, test run number, etc. If you fail to generate good documentation while conducting the runs, you will not be able to recover when analyzing the data/ putting together the report, and you will end up with inadequate or poor quality data to support the validation process.

7. A thermocouple should always be placed beside the drain temperature sensor (usually a drain temperature sensor is used to control the temperature within the autoclave).

Cautions

1. If you are using a non-vacuum cycle to sterilize a non-liquid load, you are taking a significant risk. Some regulatory bodies will simply not allow processing of non-liquid loads with non-vacuum cycles.
2. Some regulatory bodies are extremely concerned that all points within the load achieve sterilization temperature when starting the dwell period. This may mean that you are not drawing enough vacuums or that modifications to the items being sterilized are necessary to allow more efficient steam penetration.
3. If you are not using biological indicators to validate your cycle, you are taking a significant risk. Using temperature data alone means that you are assuming ideal conditions where it is not justified.
4. If you are placing a small quantity of water within load items to assist with sterilization, you must have appropriate procedural controls in place to ensure ongoing consistency with the amount of water present during the validation runs and all subsequent processing runs.

Summary

The requirements to validate steam sterilization processes have been documented for many years. For example, perhaps the most historically significant reference guide, the PDA Technical Monograph No. 1 Validation of Steam Sterilization Cycles was published in 1978. Nonetheless, steam sterilization validation remains a significant issue to regulatory bodies, particularly for processes associated with high risk in terms of the probability and severity of an infection. Failure to adequately address this requirement can place the public at risk and lead to regulatory citations/action.

In addition to potential business liabilities, there may be significant costs associated with the validation process. Large numbers of time consuming and costly test runs may be required, and if appropriate consideration is not given to employing the correct approach, unnecessary ongoing operational costs may result.

It is hoped that the practical experience that this document is based on will provide assistance in ensuring an effective, efficient validation process for steam sterilization and that the end result provides the best possible validated cycle to meet the needs of the specific application.

Definitions

SAL: sterility assurance level.

SAL of 10^{-6}: the probability of a single viable microorganism being present is one in one million.

Bioburden: the number/type of viable microorganisms contaminating an item.

Overkill Approach: a sterilization approach based on assuming worst-case conditions (a bioburden of 10^6 of a highly heat resistant bacteria).

Log Reduction: reduce the surviving microbial population by 1 log or decrease the surviving population by a factor of 10.

12-Log Reduction: the log reduction required achieving overkill and a SAL of 10^{-6}.

CFU: colony-forming unit.

D-value: time in minutes, at a specific temperature, to reduce the surviving microbial population by 90% (one logarithmic reduction).

Z-value: temperature change required resulting in a 1-log reduction in D-value.

F-value: the number of minutes to kill a specified number of microorganisms with a specified Z-value at a specific temperature.

F_o-value: the number of minutes to kill a specified number of microorganisms with a Z-value of 10°C (50°F) at a temperature of 121.1°C (250°F).

1 F_o: the equivalent of 1 minute at 121.1°C (250°F).

Dwell Period: the time period that begins when the autoclave temperature has reached the set-point and ends when the timer has expired.

Worst case items: items in the load which are the most difficult to sterilize (as determined by steam penetration studies).

Worst case location: the location within an item that is the most difficult to sterilize (as determined by steam penetration studies).

Gravity Displacement: a method of removing air by introducing steam into the top of a chamber and displacing the air,

which is heavier than steam, by removing the air from the bottom of the chamber.

Vacuum Cycle: a sterilization cycle that draws one or more vacuums to remove air prior to starting the dwell period.

Pre-vacuum: a vacuum drawn prior to starting the dwell period to remove air.
Post-vacuum: a vacuum drawn after the dwell period has finished to remove steam.

Hard Goods Cycle: a sterilization cycle designed for items for which air removal is not difficult and therefore generally one pre-vacuum is drawn.

Wrapped Goods Cycle: a sterilization cycle designed for items for which air removal is difficult and therefore generally three or more pre-vacuums are drawn.

Liquids Cycle: a cycle designed for liquid loads that generally uses gravity displacement rather than drawing a vacuum.

Bowie Dick Test: a test designed to verify that an autoclave's vacuum phase is removing a sufficient amount of air prior to the introduction of steam into the chamber and tests for air leaks into the chamber.

Empty Chamber Tests: tests with an empty chamber essentially designed to demonstrate that an autoclave provides a uniform sterilizing environment.

Steam Penetration Tests: loaded chamber tests designed to determine the worst-case items and worst-case locations within a load.

Biological Challenge Tests: loaded chamber tests designed to challenge the worst-case locations (within worst case items) with biological indicators to demonstrate the effectiveness of a sterilization cycle.

Steam Integrators: commercially available indicators that provide an indication of exposure to steam.

Fixed Load: a load configuration where the quantity and location of items within the chamber are fixed.

SIP: steam-in-place or sterilize-in-place (often used interchangeably although the level of microbial destruction achieved may differ).

References

1. Validation of Steam Sterilization Cycles: PDA Technical Monograph No. 1 (1978).

2. Sterilization of Medical Devices – Validation and Routine Control of Sterilization by Moist Heat: European Standard EN 554 (1994).

About the Author

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