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White Paper

Top 10 considerations when validating an autoclave

Validating an autoclave is a daunting and time-consuming task. This white paper details the tricks, tips and traps to such a validation project from how to choose your Control to which load configuration to use for your validation runs.

Consideration 1.

Choosing the right sterilisation cycle to implement

There are three basic types of sterilisation cycles. Choose the right one according to the type of goods to be sterilised:

Hard Goods (Vacuum)

Suitable for items that are easy to sterilise, because air removal and steam penetration are highly effective on these items.

e.g., open glassware and large diameter piping

A typical hard goods cycle may draw one vacuum prior to introducing steam to reach the desired sterilisation temperature.

Wrapped Goods (Vacuum)

Utilized for items that are difficult to sterilise, because air removal and steam penetration are harder to achieve on these items than on hard goods.

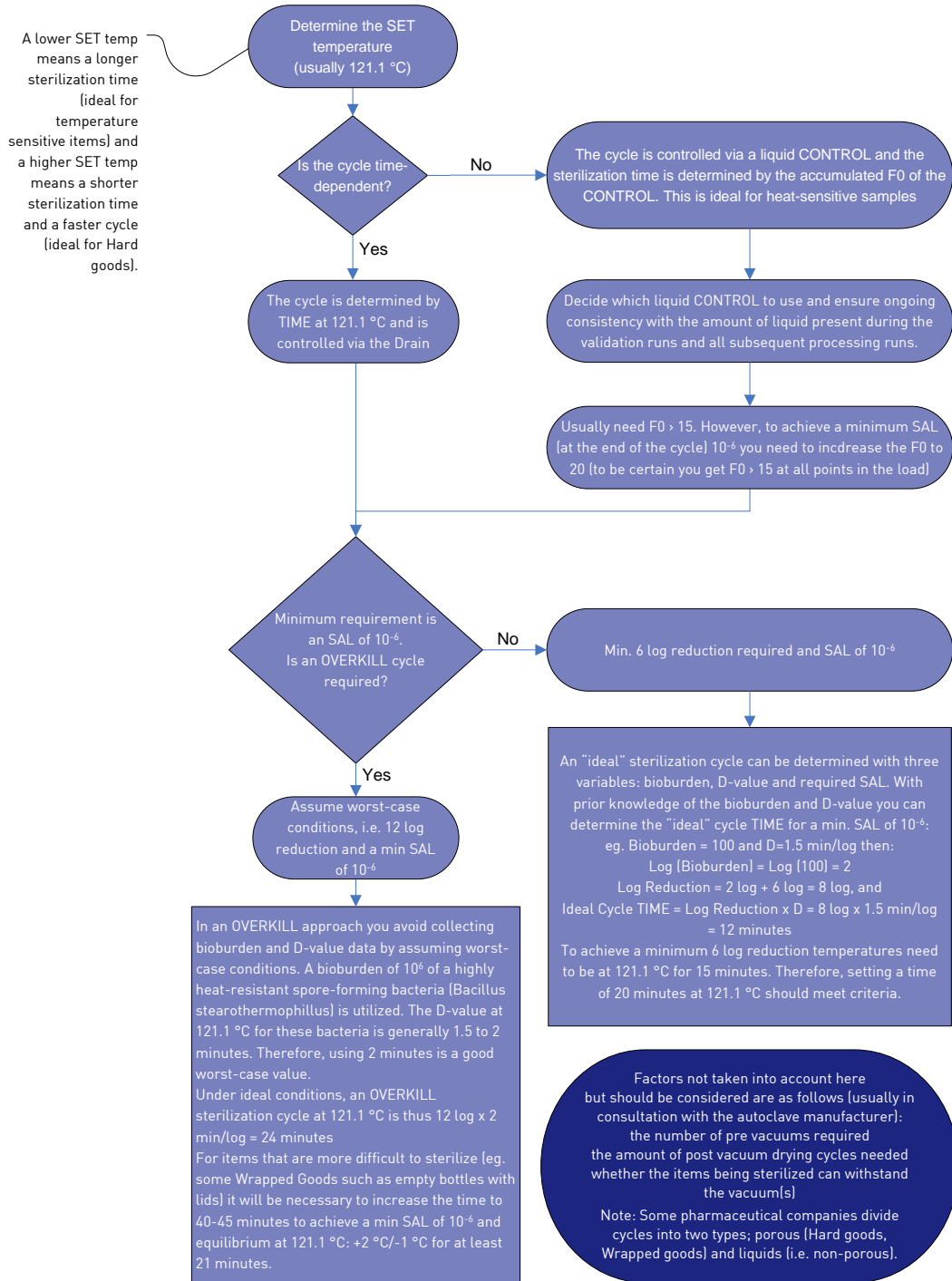
e.g., empty bottles (glass or plastic) with lids, gowns, long hoses/tubes, vent filters, portable vessels with small inlet/outlet ports

A typical wrapped goods cycle may draw three or more vacuums prior to reaching sterilisation. A post-sterilisation vacuum draws the steam from the load items.

Liquids (Non-vacuum)

Items that contain liquids generally cannot have a deep vacuum pulled or the liquid will be drawn out of them. Autoclave cycles for liquids generally heat up and cool down without a vacuum. Steam, introduced into the top of the chamber, displaces the air. The air is pushed to the bottom of the chamber and is removed.

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The steps involved in choosing the right sterilisation cycle.

Consideration 2.

Which load configurations to use

A variable to consider is whether to use fixed load or variable load configurations. There's a trade-off here between validation effort and operational flexibility – do you want to validate a wide range of load configurations to increase Production's flexibility in loading the autoclave? Here are some typical load configurations to consider:

A **fixed load/fixed position configuration** means that any load to be sterilised will be placed inside the chamber in exactly the same way for every processing run. A diagram of the load configuration should appear in the Standard Operating Procedure (SOP) so that operators can reproduce the load for every processing run. This situation requires the fewest validation runs (3), but offers no flexibility in load configurations.

A **fixed load/variable position configuration** means that the location of the load items in the autoclave can vary. Only a list of the items that can be in a load is required for the SOP. The validation runs must demonstrate positional equivalence by rotating the items from location to location during the test runs. If positional equivalence is proven after three validation runs, then you can stop. A fixed load/variable position configuration gives operators flexibility in loading the autoclave. This saves time when loading large loads of numerous items of different types.

A **variable load configuration** means that different combinations of items and/or numbers of any item(s) can be placed into the chamber. The validation runs must demonstrate that the cycle is adequate for both a maximum 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 sterilise .

Consideration 3.

Choosing the right Control for liquid cycles

The choice of the Control used when sterilising liquids determines whether the load you are sterilising will pass all the acceptance criteria.

More than one liquid Control may be needed to validate all the different types of bottles and liquids requiring sterilisation. Consider the following when choosing the Control for liquid cycles:

- The size of the bottle and its fill volume – the larger the bottle and the greater its volume, the harder it is to sterilise.
- The thickness of the glass –thicker glass is more difficult to sterilise than thinner glass.
- The viscosity of the liquid – the greater the viscosity, the slower the heat-up time, and the harder it is to sterilise.

The liquid Control will ideally be the one that is the most difficult to sterilise (worst-case) and will be located at the coldest spot in the chamber (lower level near the front door or directly above the drain).

Don't be tempted to use a Control that is dramatically different from the composition of the load. If the liquid Control takes too long to reach the sterilisation temperature, then the protein composition of the media in the rest of the load (which may have exceeded

the desired temperature by the time the Control reaches the sterilisation temperature) may be denatured.

If in doubt, perform preliminary studies using different liquid Controls to obtain information on the load's heat-up times and F0-values.

The number of validation runs required for different types of liquids and bottles can be reduced by grouping liquids with similar viscosities, bottle sizes and fill volumes. Each liquid Control will have a unique maximum and minimum load configuration associated with it.

Use procedural controls to ensure that the choice of liquid, bottle size and fill volume used for each Control, and its location in the chamber, are maintained during the validation runs and subsequent processing runs.

Case study of load configurations and choice of Control:

Let's assume that a laboratory prepares four different media types in three different bottle sizes in the following configurations:

Table 1. Media types and bottles sizes for loads in the autoclave

Media	Max/Min Load ¹	100 mL bottle	500 mL bottle	600 mL bottle
Media A	fixed at 50		400 mL fill	NA (not applicable)
Media A	Max: 160 Min: 40	50 mL fill	NA	NA
Media B	Max: 144 Min: 9	NA	400 mL & 500 mL fill	500 mL & 600 mL fill
Media C	Max: 144 Min: 9	NA	400 mL & 500 mL fill	NA
Media D	Max: 144 Min: 9	NA	400 mL & 500 mL	NA

Media A is the most viscous fluid of those listed. It is prepared in two very different bottle sizes: 500 mL (with a fixed load of 50 bottles in the autoclave) and 100 mL (with a variable load of 40-160 bottles in the autoclave). This means that Control 1 is a 500 mL bottle filled with Media A (400 mL) for the fixed load (three studies); and Control 2 is a 100 mL bottle filled with Media A (50 mL) for the variable load (six studies: three at maximum load and three at minimum load).

All remaining media types (also known as Broths) have similar viscosities and are in similar bottle sizes with fill volumes ranging from 400 mL to 600 mL. This means that Control 3 is a 600 mL bottle filled with Media B (600 mL)² for the variable load covering the three different Broths, i.e., six studies: 3 x 144 (max) and 3 x 9 (min) using 600 mL bottles.³

¹ Max/Min Load is the number of these bottles that can be loaded into the autoclave per run.

² The choice of Control in this case was based on the largest bottle with the largest fill volume.

³ To complete the study, a mixture of Media B and Media D was spread evenly across the load to capture data across the two different media types and, therefore, show compatibility between the two.

Note: Each validation load consisted of the relevant media type only in the locations where thermocouples, Biological Indicators (BI) and Chemical Indicators (CI) were to be placed. The rest of the load was the same sized bottles filled with the required volume of water.

Consideration 4.

Determining which load items are the most difficult to sterilise and which location(s) within the items represents the worst-case conditions

With a large load containing a wide variety of different types of items, the number of possible test locations within items seems to approach infinity. It also can be difficult to get the thermocouple and indicators (BI & CI) into the item without affecting the item's ability to be sterilised and/or ruining the item (a concern with expensive items).

We must evaluate each item on a case-by-case basis and determine how to best challenge the item. Often the item must be sealed somehow to return it to a state that represents equivalency with respect to steam penetration.

Some examples:

Q. What is the most difficult point to sterilise in a hose of uniform diameter?

A. In the centre of the length of hose.

Q. How do you get a 3 m length of thermocouple into the middle of a 20 m hose?

A. By cutting a slot in the middle of the hose and inserting the thermocouple through the slot, making sure to seal the slot with silicon. If you don't seal it, you will not be challenging the hose properly. Alternatively, get two 10 m lengths of hose (if available) and join them with a connector, after inserting the thermocouple through the connector. Using this method doesn't ruin a 20 m length of hose.

Q. What is the worst-case location within a bottle, flask or cylinder?

A. In the centre near the bottom (but not touching the floor).

Q. How do you hold a thermocouple in position inside a sealed bottle?

A. Choose a piece of Silastic tubing with an internal diameter (ID) that is narrow enough to hold the thermocouple probe without letting it slip through. Drill a hole in the bottle's lid the same size as the outer diameter (OD) of the tubing. Push the tubing through the hole, into the bottle. Now push the probe into the tubing. Slide the probe through the tubing until it reaches the desired position in the bottle. Make sure it is not touching the wall of the bottle. For bottles with rubber stoppers, make a small hole in the centre of the stopper, sufficient to push the thermocouple through.

Consideration 5.

Wired temperature thermocouples are cumbersome and don't always give accurate data

The list below highlights some considerations when using wired thermocouples:

- Some loss of steam (leakage) will occur when the wire's outer plastic protector has been cut and air or steam can pass through it. This may result in a failed leak test.
- Validator thermocouples inside the chamber will draw condensate and will need a slice/cut made in their outer protective layer, to ensure that any fluid is released. If condensate passes through the wires and into the electronics, the thermocouples will be destroyed.
- The thermocouple may be difficult to place into the item without adversely affecting the item's ability to be sterilised and/or ruining the item (a concern with expensive items).
- Wires can get caught (and be damaged) under the autoclave's wheels when moving loads into and out of the chamber
- It is difficult to place wires inside sealed bottles without (i) touching the inside wall, and (ii) compromising the bottle's ability to be sterilised.
- You may be limited by the number of wires you can place through the autoclave's inlet.
- The resistance of the thermocouple in some locations in the chamber can change, leading to inaccurate and/or unreliable data even though the pre/post calibration verifications meet specifications.

Case Study

This example looks at nine thermocouples placed into a loaded chamber. They were evenly spaced from one another at the top, middle and bottom levels and at the front, centre and rear of the chamber. All were in the chamber and subject to Phase II heat distribution requirements.

The study used wired thermocouples in a loaded chamber for a 40-minute cycle at 121.1 °C. The chamber's maximum pressure of 2.16 bar (at any time) was equivalent to 122.7 °C. One probe (top front LHS) constantly reached temperatures between 123.3 °C and 123.5 °C. All other probes were within the required limit of 120.1 °C +2 °C/-1 °C at temperatures from 122.4 °C to 122.7 °C.

The temperature differential started during heat-up and remained during the sterilisation and post vacuum cooling phases. The Equipment Engineer and the Manufacturer agreed that the temperature reading at this position was inaccurate and unreliable. The thermocouple reading was inconsistent with the steam pressure indication and the other thermocouple readings. Consequently, no useful data were collected at that point.

Consideration 6.

Determining the acceptance criteria

An example:

You run your validation studies, only to realise that you cannot meet one of the acceptance criteria. But, was it really needed in the first place?

It's important to understand the aim of the autoclave cycle and what its parameters are. For example, is it for sterilisation or decontamination? Is the load heat sensitive, or can it be subjected to an overkill cycle? Is it a porous load (hard/wrapped goods), or is it a liquid?

Most Validation departments have a Standard Operating Procedure (SOP) detailing the validation requirements for sterilisation processes. Included in that is a complete list of all the acceptance criteria.

Each phase of the autoclave cycle is likely to have different acceptance criteria:

Phase I – Heat distribution (empty chamber)

Phase II – Heat distribution (loaded)

Phase III – Heat distribution (loaded) cold spot determination within

Phase IV – Heat penetration

There may also be different requirements for Phases III and IV if you are sterilising liquids (non-vacuum) vs. porous items (vacuum), e.g., $F_0 > 15$ at the end of sterilisation (liquids only).

Typical acceptance criteria are as follows:

- All porous cycles require min SAL 10^{-6} at the end of sterilisation. All porous items are subject to at least one post-vacuum cycle which removes steam from the chamber (Phases III & IV).
- All liquid cycles require a min SAL 10^{-6} and a min $F_0 > 15$ at the end of the cycle, because they do not use vacuum and are subject to natural cooling (Phases III & IV).
- Throughout the sterilisation phase all temperatures are within a 3 °C range (Phases II, III & IV)*, e.g., 121.1 °C -1 °C/+2 °C.
- Throughout the sterilisation phase all temperatures in the chamber are within 1.0 °C of the chamber's mean temperature (Phase II).
- The steam's temperature corresponds to its vapour pressure (Phases II, III & IV).⁴
- Timed measurements are to be controlled to an accuracy of $\pm 1\%$.*
- Required pre-certification and post-certification of the data logger ensures that the temperature measurement system is accurate to within ± 0.5 °C.
- The load is visually dry at the end of the cycle (porous cycles only).

⁴ Sterilisation of Medical Devices – Validation and Routine Control of Sterilisation by Moist Heat: European Standard EN 554 (1994).

- All autoclaved Biological Indicators (BIs) are negative and the control is positive following incubation (Phase IV).

Consideration 7.

Adequately documenting the validation test runs

Documenting what was done during the validation test runs is all about knowing what needs to be documented and how to present it. This documentation must be clear, consistent between runs and transparent, and must conform to all GMP requirements. It must be complete and must include the following items:

- a diagram showing the location of all load items within the autoclave chamber
- the precise location/number of each thermocouple, BI and CI within each item
- the printout from the data recorder
- the printout or chart from the autoclave
- the time the sterilisation period began and finished (per the data recorder time)
- the time difference between the autoclave controller and the validation temperature monitoring device
- the results of each BI and CI

Label each document with the equipment ID, load description, date, test run number and cycle start/end time.

If you fail to generate good documentation while conducting the validation test runs, you will not be able to analyse the data when putting together the report. Inadequate or poor quality data to support the validation process will not survive the scrutiny of an auditor.

Tip:

Be cautious about the acceptance criteria you employ to verify the accuracy 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 the temperature tolerance after the runs.

Consideration 8.

The frequency of thermocouple accuracy verification

If you are performing a large number of test runs (e.g., over the course of several weeks), you need to think about the points at which you will verify the thermocouples' accuracy. This could be done after every run and/or 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 their failure to meet the verification acceptance criteria. Verifying after every run, however, adds considerably to the length of time required to complete the testing. Performing the verification every three runs or every few days is a reasonable compromise.

As noted in Consideration 6, the acceptance criterion you employ to verify the thermocouples' accuracy should allow at least one or two thermocouples to fail.

Consideration 9.

Having adequate time and access to the autoclave to complete the validation

It's easy to under estimate the length of time it takes to validate an autoclave, and how much access you need to it during the process.

For example, it can take up to four hours to set up your run, i.e., prepare the load, place probes, BIs and CIs into the load, etc. If Production needs to use the autoclave and you need to remove your probes, BIs and CIs, then you need to start all over again, effectively losing a day. Work with the Production department when planning the Validation project to ensure that you have adequate access to the autoclave.

Another approach is to combine Phase II and III (Heat Distribution) with Phase IV (Heat Penetration) studies to save time.

Combining these three phases could reduce the time it takes to complete the validation project; however, you need to consider the following when doing this:

1. You will need to place probes into the chamber and into load items at the same time. Can you fit all the probes through the autoclave's inlet? If not, then you need to either validate each phase separately, or reduce the number of probes.
2. Combining these three phases greatly increases your preparation time. If you are working on a tight schedule (e.g., on a construction site where you need to evacuate at a certain time), you may not have time to complete the study. If this happens, then it may take more time to perform the work than if you had done each phase separately.
3. If you are under time pressure, there is a greater chance that you will miss something or make a mistake.
4. There are more data to consider. If you check only a few critical requirements before proceeding to the next study, you may miss something that did not meet an acceptance criterion. This may mean that all subsequent studies are at risk, because the data cannot be verified. For example, a probe may be falsely reading too high.
5. Allow enough time for the report to be completed.
If you are validating a new autoclave, then you need to allow enough time for:
 - (i) writing a validation plan
 - (ii) writing the commissioning and IQ protocols
 - (iii) preparing the OQ/PQ protocols
 - (iv) performing OQ/PQ studies
 - (v) writing the OQ/PQ reports, preparing folders, etc.

If you are performing a large number of test runs (e.g., over the course of several weeks), then you need to ensure that enough time has been allocated to prepare the folders and write the reports. Allow one day to do a run and another day to analyse the data, i.e., two days per study. Also allow time to write protocols and reports, and to have them reviewed and approved by other people (if appropriate). If you are developing the validation cycles, then this will also take time

Consideration 10.

Have the right procedural controls in place to ensure ongoing consistency and correct operation

Congratulations, you have just finished validating a new autoclave for a number of different cycles and load configurations. Now, what controls need to be in place to ensure that the validated loads are used consistently?

- A Standard Operating Procedure (SOP) for the new autoclave should be prepared. It must include clear guidelines for each of the validated cycles, including diagrams of the load configurations. Test the procedure's clarity by asking a typical operator to follow the instructions with a dummy load.
- Each operator who uses the autoclave should be trained and tested on the SOP.
- Logbooks should be in place for each cycle.
- Use a risk-based approach to determine the troubleshooting guidelines to include in the SOP. The manufacturer's documentation and website may detail things that commonly go wrong.
- An ongoing requalification program for the autoclave and the loads is required. The frequency can be 6, 12 or 24 months.

Glossary

Term	Meaning
Biological Indicator (BI)	Spore strips or vials with a verified microbial count that are placed within the load to challenge the autoclave's performance
Chemical Indicator (CI)	Commercially available indicators that indicate exposure to steam
decontamination	Articles are free of microbial contaminants but are not suitable for use in a Grade A sterile area.
F0 value	This is the number of minutes required to kill a specified number of microorganisms with a Z-value of 10 °C at a temperature of 121.1 °C.
load	The items placed into the autoclave to be sterilised
overkill	A 12-log reduction in the number of microorganisms with an SAL of 10 ⁻⁶
SAL	Sterility Assurance Level. An SAL of 10 ⁻⁶ is a one in one million probability of a single viable microorganism being present.

Term	Meaning
SET temperature	The temperature at which sterilisation occurs. This is the temperature the autoclave is held at for a specified period of time to reach the required sterilisation level.
sterilisation	<p>The destruction of all living microorganisms such as pathogenic or saprophytic bacteria, vegetative forms and spores.</p> <p>Articles that have been sterilised can be used for aseptic purposes in all areas, including areas classified as Grade A sterile areas.</p>
thermocouple	A temperature measuring device

About PharmOut

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PharmOut specialises in GMP compliance, validation and continuous improvement consulting and training.

Some of the company's customers include Abbott Laboratories, ASTRAZENECA, Bernafon, CSL, Fonterra, GSK, Hospira, Invetech, Intertek Caleb Brett, Mayne Pharma, Pharmatel Fresenius Kabi and Probe Analytical Laboratories.

How PharmOut can help

We offer a range of services to help manufacturers achieve regulatory compliance.

Validation services

PharmOut offers both consulting and contracting services to support the validation of manufacturing equipment and computer systems.

Our consulting services can help you with high-level decisions, such as taking a risk-based approach to determine which of your computer systems and equipment to validate, and determining the documentation needed to support decisions about what to validate.

We can also perform an audit to find out if your systems are compliant with global GxP regulations. If they aren't, we can give you practical solutions for achieving compliance and passing an audit by a regulatory body.

Our contract offering supplies people with the knowledge and experience to do the hands-on validation. This includes:

- Writing a Validation Master Plan
- Determining what to test
- Creating the documentation needed for testing
- Writing a requirements traceability matrix

Plus, we can do the testing —which you probably don't have the resources to do.

