

# An Overview of the Validation Approach for Moist Heat Sterilization, Part II

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This article illustrates a qualification-validation strategy for moist heat sterilization and briefly discusses the sterility concept and common sterilization principles. In Part II, the authors discuss the qualification-validation procedure, including the installation, operational, and performance qualification phases, when requalification-revalidation is required, and a system for change control.

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**P**art I of this article was published in the September issue of *Pharmaceutical Technology* and provided an update of the validation of moist heat sterilization, reviewing the sterility concept, sterilization principles, development of sterilization cycles, and the measurement of sterilization efficiency. Part II discusses the qualification-validation procedure and the probability of nonsterility of a load during the validation of the steam sterilization process.

## The qualification-validation procedure

As recommended by the American National Standards Institute (1), the qualification-validation of a moist heat sterilization process involves

- qualification of the autoclave by checking its performance against the design specifications
- validation of the process by establishing the actual effectiveness and reproducibility of the cycle in relation to the product and the loading configurations
- assessment of possible changes in the product that could have occurred during sterilization.

A typical qualification program is outlined in the *United States Pharmacopeia (USP)* (2). However, choosing a particular qualification approach depends on the type of autoclave, its actual condition (whether it is new or refurbished), the critical parameters controlling the process, the geometry of the chamber, the type of goods that are going to be sterilized, and the sterility assurance level (SAL) required. Therefore, before the qualification process is started, the user must become acquainted with the autoclave type and its characteristics. It is important to assemble a validation team consisting of at least a user representative and a member of the quality assurance division.

After the decision has been made to qualify an autoclave, the process of collecting information must be performed in a logical manner. Because extensive literature about this topic is available, one should be selective when consulting information sources and retrieve only the type of information applicable to the appropriate sterilizing principle and the operating parameters of the sterilizer being validated.

The first step in collecting information is to consult national standards. If these are not available, then one should consult international standards. Because validation cannot be per-

formed in general, the type of materials that will be routinely sterilized should be considered at all times when setting objectives for validation (3). In accordance with the purpose of the validation, scientifically sound acceptance criteria that can be accurately evaluated by monitoring the physical parameters of the process should be established.

The qualification-validation procedures should be performed using approved protocols developed before the procedures are initiated (4). A description of the autoclave process, cycle types, parameters, and performance specifications must be completed. The protocols should clearly state the objectives of the validation and define the scope of the activities for each stage of the process. Time frames and general acceptance criteria should also be part of the protocols. Each stage of validation must be documented and approved.

Qualification-validation consists of a series of tests, each of which includes an objective, a method, acceptance criteria, and test results. The responsibility for testing must be assigned to either the manufacturer or user, agreed on, and documented.

To perform a proper validation, several items are required such as a temperature recorder (data logger) that can record and accumulate temperature data collected by thermocouples, a calibrated thermometer, and a computer that can analyze raw data and compute  $F_0$  values (5). A laminar-flow hood, biological indicators (BIs), access to an incubator (55–60 °C), a temperature bath, and an ice bath for accuracy verification of the thermocouples also are compulsory elements.

**Commissioning or pre-installation qualification (IQ)–operational qualification (OQ) phase.** As a prerequisite to qualification of both new and refurbished autoclaves, a user requirement specification document containing key equipment requirements should be developed and approved. Referring to this document helps prevent purchases of unsuitable and inappropriate equipment that can be difficult to qualify for the intended purpose. After its approval and authorization, the document must be passed to relevant vendors before receipt of quotes or offers for work.

Commissioning is the first step of qualification (6) and consists of obtaining evidence that equipment has been provided and installed in accordance with its specifications and that it functions within predetermined limits when operated as directed by its instructions. The company installing the unit must deliver and commission the autoclave and must offer technical support to the owner until the autoclave is in the proper condition for use. Performing a proper commissioning saves time and money and provides the necessary confidence in the performance of the equipment.

**IQ–OQ phases.** The IQ and OQ phases can be instituted separately or simultaneously. During the IQ and OQ phases, various features of the autoclave are examined and tested for proper functioning. This procedure includes tests of the chamber design, pressure vessel, and door safety interlock system and inspections of the chamber jacket, steam traps, electrical circuits, pressure and temperature indicators, vacuum pumps, vent filters, and steam supply to the chamber.

Usually the IQ document indicates the location of the documentation for the equipment (which must include a checklist containing specifications and identification of equipment or

components by description, model, and serial number), the instruction manual, the troubleshooting and preventive maintenance schedule, the electrical and piping drawings, and the diagram of the programmable logic controller (PLC).

**Calibration of command and control instruments.** During the IQ phase, the calibration of all critical instruments—process controllers, temperature sensors, pressure gauges, timers, and measuring and recording instruments—should be performed. It is also important to know the degree of accuracy of the instruments used to calibrate the gauges. The accuracy level of the calibrating instruments must exceed the accuracy level of the autoclave's control, measuring, and recording systems (1,7). Likewise, the control, measuring, and recording instruments must have an accuracy level that fits the purpose of the autoclave. When the desired level of accuracy is not known, standards must be consulted.

The performance of calibration test instruments should be traceable to national reference standards. All calibration procedures must be fully documented. Calibrations must be completed before any other validation tests are started. The calibration can be performed either before installation of the unit or in situ after the unit is placed in its operating site. The advantages of calibrating in situ include the ability of a sensor or gauge to provide accurate information about the environment that the unit is monitoring when compared with a known standard (6). This type of calibration is more rugged and provides a more accurate image of the process being monitored.

**Requirements for thermocouples.** To monitor temperatures attained at various locations throughout the chamber, temperature-measuring devices called *thermocouples* are used. The thermocouples are connected to computerized multichannel recording systems that can record and print temperature data. For validation purposes, type T thermocouples are recommended because they are stable throughout a wide temperature range. Depending on the chamber size, 10–20 thermocouples must be used per cycle. Thermocouples should not be placed in the chamber through the door gasket because they can be easily damaged when loading or unloading the chamber, which can also create a leak that will affect the normal working conditions of the autoclave. One should place the thermocouples in the autoclave chamber by means of a feed-through assembly connected to a suitable port. After purchasing a new autoclave, this port must be documented during the IQ phase.

One must also verify the accuracy of the thermocouples to traceable secondary standards at two significant temperatures (e.g., the freezing and boiling point of water) at the beginning and end of each phase of the qualification. This procedure can be performed by immersing the thermocouples and a reference calibrated thermometer in water maintained at the previously mentioned temperatures and monitoring the temperature values of both the thermocouples and the reference thermometer for a certain period of time. Accuracy of the thermocouples should be at least  $\pm 0.5$  °C. Temperature accuracy is especially important in steam-sterilization validation because an error of just 0.1 °C measured by a faulty thermocouple will produce an error of 2.4% in the calculated  $F_0$  value (8). All inaccurate thermocouples should be discarded.

**Table I: Temperature data interpretation for a heat distribution study during exposure phase.**

Function	Run Number	Values within the Run									Values across the Run
		Thermocouple Numbers for Heat Distribution									
		T <sub>5</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>	
Minimum temperature	1	120.43	121.87	121.85	121.37	121.79	121.71	121.53	121.62	121.63	120.43
	2	121.71	121.81	121.82	121.77	121.92	121.81	121.39	121.58	121.78	121.39
	3	121.35	121.91	121.92	121.84	121.75	121.71	121.53	121.62	121.70	121.35
	4	121.85	122.09	122.07	122.01	122.02	122.10	121.66	121.29	121.83	121.28
Maximum temperature	1	123.27	122.02	122.02	121.96	122.16	122.14	121.92	122.05	122.03	123.27
	2	121.91	122.13	122.02	121.98	122.15	122.20	122.02	122.07	122.25	122.24
	3	122.37	122.22	122.14	122.08	122.30	123.45	122.00	122.12	122.16	123.44
	4	122.16	122.44	122.42	122.34	122.35	122.46	122.16	122.83	122.39	122.82
Mean temperature	1	121.76	121.95	121.93	121.87	121.99	121.98	121.73	121.82	121.81	121.89
	2	121.84	121.97	121.94	121.89	122.06	122.02	121.72	121.85	122.03	121.92
	3	121.88	122.05	122.03	121.96	122.15	122.18	121.77	121.85	121.93	121.98
	4	122.00	122.21	122.19	122.11	122.12	122.22	121.89	121.94	122.01	122.08
Mean of the four runs		121.87	122.05	122.02	121.96	122.09	122.10	121.84	121.84	121.95	
Standard deviation for the means	1	0.10	0.12	0.12	0.11	0.06	0.11	0.12	0.08	0.09	0.09
	2										0.10
	3										0.13
	4										0.12
t value	1	—	0.07	0.10	0.28	0.01	0.02	0.71	0.69	0.30	—
	2										0.59
	3										0.15
	4										0.002

One thermocouple must always be located next to the autoclave temperature probe, which is usually placed in the chamber drain. The reason for this practice is that, theoretically, the “cold spot” of a chamber is in the drain. Depending on various chambers’ geometry, sometimes the location of the cold spot in the chamber drain is not confirmed in practice.

The OQ phase consists of a verification of the equipment functionality. A series of checks and tests should be performed after the unit is installed. The proper operation of alarms and safety devices also must be tested. For a prevacuum cycle involving an air-removal phase, the attainability, effectiveness, and consistency in time of vacuum conditions must be tested.

Chamber mapping consists of heat distribution studies throughout the chamber for each sterilizing temperature used. Heat distribution studies determine during processing whether the temperature is uniform and reproducible throughout the empty chamber and localize the cold spots within the chamber. These studies also confirm that the system works within the specified limits throughout the entire range of operational parameters.

The sterilizing temperatures are programmed into the autoclave controls. For each sterilizing temperature being validated, the empty chamber should be monitored three times during a period of one hour, and the reproducibility of the results obtained must be compared. The comparative readings of the reference temperature sensor, the thermocouple near the temperature sensor, and the temperature displayed by the autoclave recorder should be  $\pm 1$  °C. During the exposure phase, a difference no greater than  $\pm 2.5$  °C between the temperature of

the chamber’s coolest spot and the mean chamber temperature is acceptable (8).

**Performance qualification (PQ) phase.** PQ represents the confirmatory phase of the validation program and consists of tests performed with the autoclave chamber under loaded conditions. During the PQ phase, which is sometimes referred to as *process validation*, the following objectives must be attained (1):

- demonstration of the uniformity and effectiveness of the process in inactivating or removing microorganisms to the required safety level
- demonstration of the reproducibility of the process—through the use of sufficient cycles
- demonstration of the compatibility of the process with the items to be sterilized—through the assessment of the influence of the sterilization process on the products.

The PQ phase consists of studies that use thermocouples inserted into the articles being sterilized and studies that use BIs in operationally fully loaded autoclave conditions (2).

Heat penetration studies are considered the most critical component of the entire validation program. These studies are intended to find areas in the loads that are difficult to penetrate or heat. When selecting the monitoring sites, one must take into account the cold spots previously found during the monitoring of the empty chamber.

Heat penetration studies must demonstrate the reproducibility of a cycle in relation to the loads and the effectiveness of the killing effect throughout the chamber and load. Selecting loading configurations must be performed very carefully and should be based on worst-case situations. The maxi-

**Table II: Temperature data interpretation for a heat penetration study during exposure phase.**

Function	Run Number	Values within the Run										Values across the Run
		Thermocouple Numbers for Heat Penetration										
		T <sub>10</sub>	T <sub>11</sub>	T <sub>12</sub>	T <sub>13</sub>	T <sub>14</sub>	T <sub>15</sub>	T <sub>16</sub>	T <sub>17</sub>	T <sub>18</sub>	T <sub>19</sub>	
Minimum temperature	1	120.26	120.91	121.05	120.76	120.69	120.86	120.57	120.67	120.91	121.15	120.26
	2	120.84	121.35	121.04	120.80	121.08	120.73	120.80	120.87	121.07	121.14	120.73
	3	120.62	120.95	121.18	120.96	120.86	120.92	120.45	121.15	120.22	120.87	120.21
	4	120.81	121.48	121.31	121.33	121.19	121.38	121.27	121.20	121.14	121.23	120.81
Maximum temperature	1	123.10	122.33	122.20	122.31	122.66	122.34	122.49	122.52	122.28	121.92	123.10
	2	122.35	121.92	122.11	122.31	121.99	122.40	122.10	122.17	122.04	121.98	122.40
	3	122.83	122.27	122.01	122.04	121.91	122.09	121.85	121.71	122.40	121.84	122.83
	4	122.91	122.14	122.26	122.19	122.32	122.20	122.37	122.27	122.30	122.25	122.90
Mean temperature	1	121.67	121.68	121.69	121.61	121.64	121.60	121.53	121.55	121.54	121.56	121.61
	2	121.53	121.63	121.55	121.53	121.56	121.57	121.56	121.63	121.64	121.63	121.58
	3	121.63	121.62	121.54	121.53	121.45	121.54	121.33	121.52	121.33	121.39	121.49
	4	121.58	121.77	121.81	121.81	121.84	121.83	121.82	121.84	121.81	121.82	121.79
Mean of the four runs		121.60	121.67	121.64	121.62	121.62	121.63	121.56	121.63	121.58	121.60	
Standard deviation for the means	1	0.06	0.06	0.12	0.13	1.66	0.13	0.2	0.14	0.19	0.17	1.04
	2											0.04
	3											0.10
	4											0.07
t value	1	—	0.17	0.57	0.83	0.4	0.69	0.71	0.69	0.83	0.98	—
	2											0.35
	3											0.007
	4											0.00002

imum number of bottles in the loads for the most-critical or used products should be decided. Ideally, three consecutive validation runs should be performed per loading configuration. During testing, if some configurations cannot be successfully sterilized because they are too big, the worst case in regard to the size of the loading configuration should be redesigned. If the results are still unsatisfactory, then the cycle should be re-designed, which usually can be achieved by modifying the exposure time.

Heat penetration results must show the slowest heating points in the load and must ensure that the minimum time and temperature requirements are met. The success of a qualified cycle depends on the determination of the  $F_0$  value measured inside the item located at the coldest spot. It should be confirmed that a minimum prescribed  $F_0$  value is delivered consistently throughout the autoclave chamber.

Microbiological validation, which consists of tests involving BIs, should be performed concomitant with heat penetration studies to verify independent of the temperature data results that the minimum  $F_0$  value is met at the coldest spot of the load. For terminal moist heat sterilization, heat penetration studies must demonstrate an SAL of  $10^{-6}$  or higher. The BIs should be placed in operational, maximum loads at the locations presumed to be the least accessible to the sterilizing agent. One should place the BIs next to the thermocouples and inside the item being tested. They must not be placed directly on surfaces or outside the loads because the heating process is rapid in those areas and the BIs may get killed faster. The number of BIs used per load depends on the size and the complexity of the load.

For statistical significance, tests with BIs that are performed during the PQ phase should be conducted at least three times per loading configuration. After sterilization, the BIs should be incubated for seven days and checked daily for growth. As a part of the documentation, information provided by the manufacturer concerning the spore lot number (i.e.,  $D$  and  $z$  values for the current lot) must be available.

Demonstrating the integrity of the product after sterilization represents another aspect of process validation. The product must be tested for possible physical and chemical changes that may have occurred during sterilization. In this regard, the pH of solutions and the physical condition and appearance of goods before and after sterilization must be verified. For culture media, tests for growth promotion that are recommended by USP should prove whether the culture media show growth following the sterilization process.

**Qualification-validation report.** The final stage of the validation program requires the documentation of all acquired data. The qualification-validation report summarizes the overall results of validation. It includes the calibration certificates for calibrating instrumentation, calibration records, and methods for calibrating the measuring instruments, gauges, and recorders as well as the accuracy verification data of thermocouples (4). Test data such as high-low temperature ranges, average temperatures during exposure time, minimum and maximum  $F_0$  values achieved for every load configuration, run date and time, and autoclave records must be included.

The qualification-validation report is not complete unless it contains evidence checks for the availability of an instrument

**Table III: Accumulated  $F_0$  value interpretation for a heat penetration study.**

Function	Run No.	Thermocouple Number										Mean $F_0$ per Run	Std. Dev. per Run	Std. Error of the Mean	95% Confidence Interval		$t$ Values across the Run
		$T_{10}$	$T_{11}$	$T_{12}$	$T_{13}$	$T_{14}$	$T_{15}$	$T_{16}$	$T_{17}$	$T_{18}$	$T_{19}$				Lower	Upper	
$F_0$	1	25.82	25.04	24.92	23.86	24.50	24.35	23.76	24.42	24.42	24.51	24.56	0.59	0.18	24.14*	24.98*	—
	2	23.95	24.81	24.01	23.50	24.20	23.91	24.25	24.80	24.90	25.12	24.35	0.53	0.16	23.97*	24.73*	0.4
	3	23.85	24.55	24.26	23.96	23.03	24.42	22.02	24.08	22.03	22.69	23.49	0.96	0.31	22.80*	24.17*	0.009
	4	24.68	26.05	26.13	26.14	26.05	26.43	25.65	26.32	25.15	26.06	25.87	0.55	0.17	25.47*	26.26*	0.00008
Mean of the four runs		24.58	25.11	24.83	24.36	24.45	24.78	23.92	24.91	24.13	24.60	24.57	1.08		24.23**	24.90**	
Standard deviation for the means		0.90	0.66	0.95	1.20	1.24	1.12	1.50	0.99	1.43	1.42						
$t$ value		—	0.38	0.71	0.78	0.87	0.79	0.49	0.64	0.62	0.98						

\* The confidence interval per run was calculated considering  $t$  for 0.05 (95%) confidence level = 2.262 (N-1 = 9 degrees of freedom).

\*\* The confidence interval across runs was calculated considering the critical value for 95% level of confidence = 1.96.

logbook, the standard operating procedures (SOPs) used with the autoclave, procedures for preventive and unscheduled maintenance, and recalibration programs. The validation report also should include the user's training records. Training should begin only after the validation has been accomplished to ensure that users will implement already-validated loading configurations.

The qualification-validation report must include the drawings of all loads tested. The location of the thermocouples and BIs must be specified in each drawing. Any specification deviations that were encountered during validation activities and the procedures that followed their discovery should be reviewed. After the validation activities are completed, all data and documents that were accumulated must be revised, approved, and certified by both the owner of the autoclave and the contractor in cases when the validation was performed with third parties or by the manufacturer.

Once the autoclave is qualified and the loading configurations are validated, the following documents must be present in the laboratory at all times:

- diagrams of various loading configurations stamped with their effective dates
- a list of the determined lag times for each loading configuration
- temperature-pressure recorder printouts

- user, calibration, and maintenance SOPs.

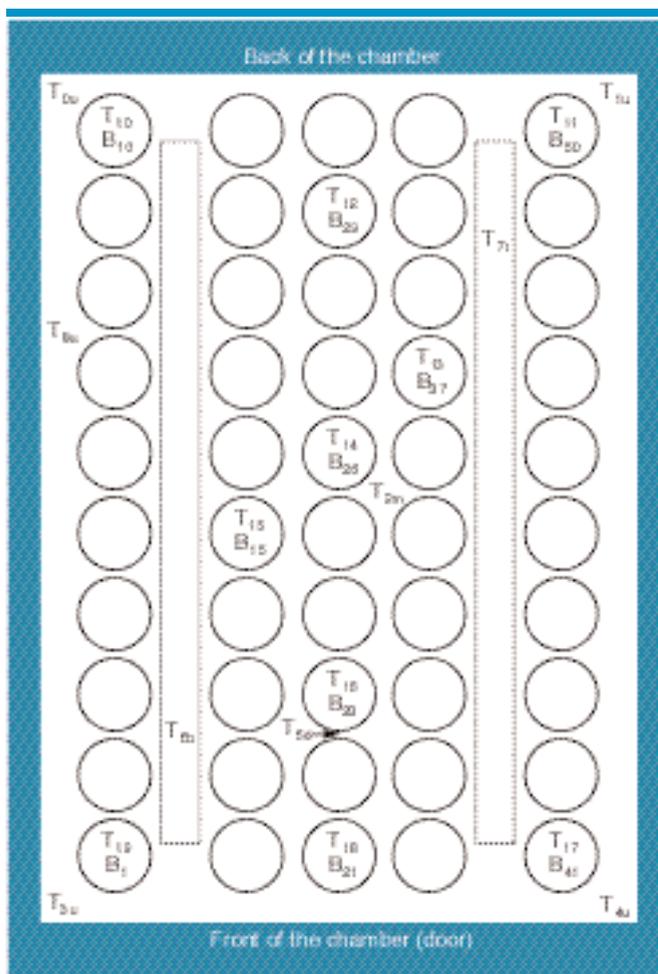
**When requalification-revalidation activities are required.** Once a unit has been installed and qualified, it does not need requalification, unless the unit is reinstalled in another location or has undergone major modifications. Only revalidation is performed periodically. Generally, the extent of requalification-revalidation depends on the nature of the changes that occur and how they affect the already-validated sterilization cycles. The responsibility for deciding which validation activities should be reperformed must be assigned. Validation frequency must be stipulated in the validation documents. The need for possible revalidation should be assessed every 12–24 months, and revalidation should occur whenever major repairs have been performed. Any modifications to control systems should be evaluated to confirm that process conditions delivered to the load are comparable with those originally validated. However, the validation should be repeated whenever new sterilization cycles, loading configurations, or major changes in the sterilization procedure are introduced.

**Change control.** A system for change control should be implemented that establishes when the qualification-validation process should be repeated (1). The nature of the change must be assessed to determine the potential effects on the cycles and load. Changes that could affect sterilization conditions are not allowed to be introduced without documentation. The change-control procedure must follow a change-control SOP.

Changes in any of the following items can invalidate an autoclave validation: pressure gauge, temperature gauge, control panel programming, temperature-control valve, steam trap, and steam source. Changes introduced by preventive or unplanned maintenance procedures must be evaluated and documented in regard to a decision for revalidation.

**Table IV: Sterility assurance levels (SALs) attained for each run.**

Run	$F_0$ Values Representing the Calculated Lower Limit of the 95% Confidence Interval	SAL
1	24.14	$10^{-10.09}$
2	23.97	$10^{-9.98}$
3	22.80	$10^{-9.2}$
4	25.47	$10^{-10.98}$



**Figure 1:** Representation of the autoclave chamber (overhead view). B is the number of the bioindicator ampul, and T is the thermocouple number. Heat-distribution thermocouples are marked with a number and letter indicating their position in the chamber: u = upper position, m = middle position, b = bottom position, r = under the rail, d = in the chamber drain.  $T_8$  was placed in the piping heading to the pressure gauge. The dotted rectangles represent the two rails of the autoclave chamber. The circles represent the 50 bottles, some of them containing thermocouples and bioindicator ampuls.

During routine use, personnel should ensure that the process specifications established during qualification testing are followed and remain valid. The critical parameters governing the process must be monitored routinely to confirm that previously determined conditions are achieved.

### Estimation of probability of nonsterility of a load during validation of the steam sterilization process

Qualification-validation activities were performed for a steam sterilizer, model 2020, from Amsco AHSC-South Africa (Pty) Ltd. The following example demonstrates the validation of a saturated steam-vented cycle used with a load containing 50 250-mL bottles filled with 200-mL trypticase soy broth (TSB). TSB is a heat-sensitive medium that is prone to alteration during the sterilization process. Therefore, the parameters for sterilization had to be carefully chosen. The microbiology labora-

tory referred to in this article uses the sterilized broth for microbiological testing of nonsterile products and stab cultures.

**Materials and methods.** As recommended by pharmacopoeias (2,9,10), the reference sterilization conditions for liquid media (121 °C for an exposure of 15 min) were tested. A preliminary experiment was performed to determine the lag-time characteristic for this loading configuration. A simulation load comprising 50 bottles filled with 200 mL of tap water was used first.

To monitor the temperatures attained in the chamber under loaded conditions, 9 thermocouples were placed in various locations within the chamber, and another 10 were placed in the middle of the liquid contained in the bottles. The placement of the thermocouples in the chamber and the loading configuration are presented in Figure 1, which shows the overhead view of the autoclave chamber.

Timing the lag time began when the thermocouple placed in the drain next to the sterilizer temperature probe reached the sterilizing temperature. Timing stopped when the last thermocouple placed in the load reached the sterilizing temperature.

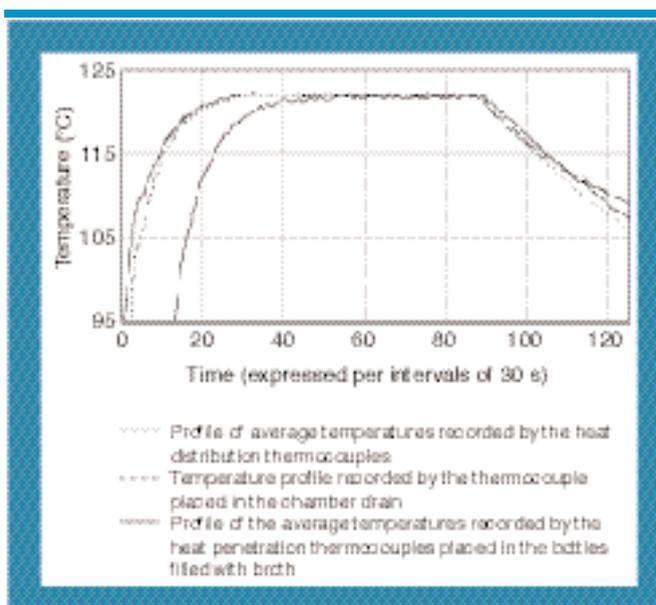
The heat penetration studies were conducted concomitant with the BI studies. An ampul containing a population of  $\sim 10^6$  spores of *Bacillus stearothermophilus* was placed next to each thermocouple inserted into the load. In the loading diagram (see Figure 1) the positioning of the thermocouples and BIs is indicated by the letters T and B. For the ampuls, the number marked on each of the 50 bottles was assigned. After insertion into the thermocouples, the sterilized ampuls were incubated with the positive controls (nonsterilized ampuls) for seven days at 55–60 °C and were checked daily for growth. As a means to verify the sterilization efficiency, 5 of the 50 bottles containing TSB were incubated after sterilization for five days at 32 °C. The broth was checked daily for growth.

To check the effectiveness of the culture medium after its sterilization, the TSB was tested in accordance with the USP Growth Promotion Test (11). The broth was tested for growth in the presence of *Bacillus subtilis*, ATCC 6633, spores and incubated at 37 °C for 48 h.

Another means for verifying the integrity of the physical properties of the broths and buffers is to measure their pH values before and after sterilization. For TSB a pH of  $7.3 \pm 0.2$  should be obtained after its preparation.

**Results.** For the simulation load, a lag time of  $\sim 13$  min was measured. The TSB has a slightly higher viscosity than tap water. A lag time of 15 min was considered sufficient to be added to the required exposure time of 15 min.

Temperature profiles recorded by using thermocouples inserted into a simulation load have indicated that during the exposure phase the temperature of the load is slightly below the set temperature of 121 °C. To achieve the prescribed sterilization temperature of 121 °C inside the load, the set temperature of the PLC was raised to 122 °C. Therefore the experiments for the validation loads were performed with the new set parameters of 122 °C and 30 min. As an example, Figure 2 shows the middle portion of the temperature profiles achieved within the autoclave chamber, in the chamber drain, and inside the load during the second run.



**Figure 2:** Representation of the middle portion of the temperature profiles in the chamber recorded during the second run.

The data acquired by heat distribution thermocouples during four runs are summarized in Table I. The data acquired by heat penetration thermocouples introduced into the bottles filled with broth during four runs are summarized in Table II.

When observing the mean temperature values per run calculated for heat distribution (see Table I), one will note that position 4 is the hottest spot in the chamber, and positions 6 and 7, situated under the rail, can be considered as the coldest spots within the chamber. Analysis of the data with the *t* test indicated no statistically significant differences in mean temperature values at a 95% level of confidence between the drain location and any of the arbitrarily chosen points in the chamber, with the exception of thermocouple T<sub>3</sub>, which was situated on the top corner of the chamber farther from the steam inlet. This fact is not critical because loads will not be placed in the top corner.

The heat-penetration temperature data (see Table II) indicate positions 11 and 17 as the hottest spots and positions 14 and 19 as the coldest spots in the chamber. The temperature data recorded at various points in the load proved not to be statistically different from each other at a 95% level of confidence.

Temperature readings taken every 30 s were used for the computation of the *F*<sub>0</sub> value (8). The value was calculated with

$$F_0 = \Delta t \sum_{j=1}^n F_{z_j} = \Delta t \sum_{j=1}^n \left( 10^{\frac{\sigma_{z_j} - 121.1}{z}} \right) \quad [1]$$

in which  $\Delta t = (t_n - t_0)$ . The sterilizing effects for temperatures >100 °C attained during the total set time of the cycle, including the entire exposure phase and portions of the heating and cooling phases, were considered in the calculations. The time at which the autoclave temperature probe reached the sterilizing temperature was considered as *t*<sub>0</sub>, and therefore the timing the cycle started. The lethality values (see Table III) were analyzed to determine the 95% confidence interval for the mean *F*<sub>0</sub> value per individual run.

The SAL achieved was determined for each of the four runs. The calculated lower limit of the 95% confidence interval was introduced in Equation 4 of Part I of this article indicated by *EP* (9) for the calculation of the *F*<sub>0</sub> value. In rearranging that equation, one can define a probabilistic term *P* as the absolute value of *N*<sub>*t*</sub> expressed as

$$P = N_t = -\text{antilog} \left( \log N_0 - \frac{F_0}{D_{121}} \right) \quad [2]$$

To calculate the probabilities of nonsterility of the product, the equations obtained were solved for *N*<sub>*t*</sub>.

As indicated in the certificate of conformity provided by the manufacturer for the *B. stearothermophilus* spores, *D*<sub>121</sub> was considered 1.5 min and *N*<sub>0</sub> as 10<sup>6</sup> spores per vial. The results of the *Nt* calculated at the lower limit of the 95% confidence interval are shown in Table IV. It can be concluded that, when reproducible sterilization conditions are applied to the studied load, a 95% confidence level exists that the probability of a single organism's survival will be 10<sup>-9.2</sup> or better.

## Discussion

The previously described approach is based on a worst-case assumption that a population of 10<sup>6</sup> spores per item exists and must be sterilized and that the microorganisms in the bioburden have the same heat resistance as the spores of *B. stearothermophilus*. However, achieving an SAL of 10<sup>-12</sup> is not necessary for this situation because the raw materials used for the preparation of the broth are pure and possess a low bioburden.

An adequate SAL also was indicated by the results of the experiment using BIs. After sterilization, which was followed by seven days of incubation at 55–60 °C, all the spores contained in the ampuls were killed. The positive controls showed growth after the incubation period.

The visual examination of the broth after five days of incubation indicated no spore growth. A bottle containing unsterilized TSB was incubated as a positive control, and the medium turned turbid after the incubation period. This confirmed the conclusion that the sterilization process was efficient.

The effectiveness of the culture medium after its sterilization was also confirmed by the growth promotion test. After sterilization, the broth, spiked with *B. subtilis* and incubated for 48 h at 37 °C, showed a positive spore growth. The fact that the medium turned turbid after the prescribed incubation period confirmed that its stability and chemical composition were not altered through the sterilization process and that the medium still supports growth when spiked with a suitable microorganism.

Four pH measurements of the sterilized broth were performed. All pH values ranged between 7.15 and 7.17, which confirmed the preservation of the initial physical and chemical properties of the broth after sterilization. Also, a visual test performed by three independent observers confirmed no color changes or precipitation phenomena in the broth after sterilization.

It can be concluded that, for the studied load, the study's objectives for the PQ phase have been fulfilled. Monitoring the physical parameters attained during the sterilization phase revealed an even distribution of temperatures in the chamber dur-

ing the run, even penetration at all points within the load, and reproducible conditions across three of the four runs. The second run was significantly different from the first, but an SAL of  $10^{-9.98}$  was attained. This ensured compliance with the BP, which states that an SAL equal to  $10^{-6}$  or higher must be achieved (10).

The conditions used to sterilize the load are halfway between the overkill approach and the bioburden approach, which were described in Part I of this article. They provide an optimum balance between an acceptable degree of sterilization and an acceptable stability of the broth after sterilization. The authors concluded that for this step of qualification the autoclave worked properly within its functional parameters in four consecutive runs and provided an SAL of  $10^{-9.2}$  or higher.

### Conclusion

Although the validation approach follows several standard steps, exact methods for validation cannot be offered because various autoclave types exist and each unit has specific operating parameters. Practical examples and requirements for validation are offered in this article for readers who may be inexperienced at qualifying steam sterilizers. Some of the principles presented here can be applied to other sterilization procedures.

As long as the strategy for validation is based on a logical, systematic approach, and meaningful tests are performed to prove the suitability of the autoclave for its intended use, the validation can only be successful. A key factor for a compre-

hensive validation is the meticulous documentation of all activities performed during the process. Documentation provides a comprehensive review of validation results that is essential for acceptance by the quality control unit and also serves to satisfy the scrutiny of regulatory bodies.

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