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Research Paper

GUIDANCE FOR PERIODIC QUALIFICATION OF STEAM STERILIZER USED FOR THE STERILE PHARMACEUTICAL INDUSTRY

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Abstract

Steam sterilization used to sterilize items that can withstand moisture and high temperature. An autoclave or steam sterilizer is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. Steam Sterilizer or autoclave Validation / periodic Qualification is mandatory for all machines used for biological sterilization, in the biomedical and pharmaceutical industries. The purpose of the periodic requalification is to verify the performance of the equipment with respect to time. Autoclaving is the most effective and most efficient means of sterilization. All autoclaves operate on a time/temperature relationship. These two variables are extremely important and need to establish through validation. Periodic requalification required to challenge the pre-established time/ temperature relationship during the equipment life cycle. Periodic qualification divided into two parts. First part is physical verification and critical operational control checks. Second part is performance evaluation. First step is to assure the equipment is in state of control. Second step deals with the output. Heat penetration study performed with pre-established worst load items. Study mapped with external temperature sensors for mapping the thermal effect on the load articles during sterilization. Biological indicators also used to challenge the microorganism challenge efficiency.

Key words: Steam sterilizer, periodic qualification.

INTRODUCTION

The invention of the autoclave sterilizer is attributed to Charles Chamberland, in 1879. Around that time, researchers started to understand the advantages of sterile surgery, and doctors needed a more reliable sterilization method than open flaming. The autoclave's benefits were soon evident, and it became an essential part of every clinic and hospital.

An autoclave or steam sterilizer is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes. Autoclaves vary in size, shape and functionality. A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents.

Steam sterilization used to sterilize items that can withstand moisture and high temperature. Steam is water in vapor state; therefore, it is nontoxic, generally readily available and relatively easy to control. A good understanding of basic steam sterilization principles and cycles is necessary to avoid mistake that can lead to non-sterile load items, poor performance of the equipment, personnel injury, lower productivity, higher operation and maintenance costs, and damage to load items. Steam sterilizers are used for numerous applications in the pharmaceutical and medical device industries [1].

Steam sterilization cycles typically consist of three phases:

1. Pre-conditioning: during this phase, air is removed from the sterilizer chamber and the load is humidified by means of alternating vacuum and pressure pulses.
2. Exposure: during this phase, the chamber temperature is raised to and held at the programmed sterilizing temperature for the programmed exposure time (both are user selectable). The exposure also may be controlled by accumulated F_0 for liquids if a load probe and appropriate sterilizer controls are used.
3. Post-Condition: during this phase, dry goods load are cooled and dried or a liquids load is cooled. The chamber pressure is brought to atmosphere.

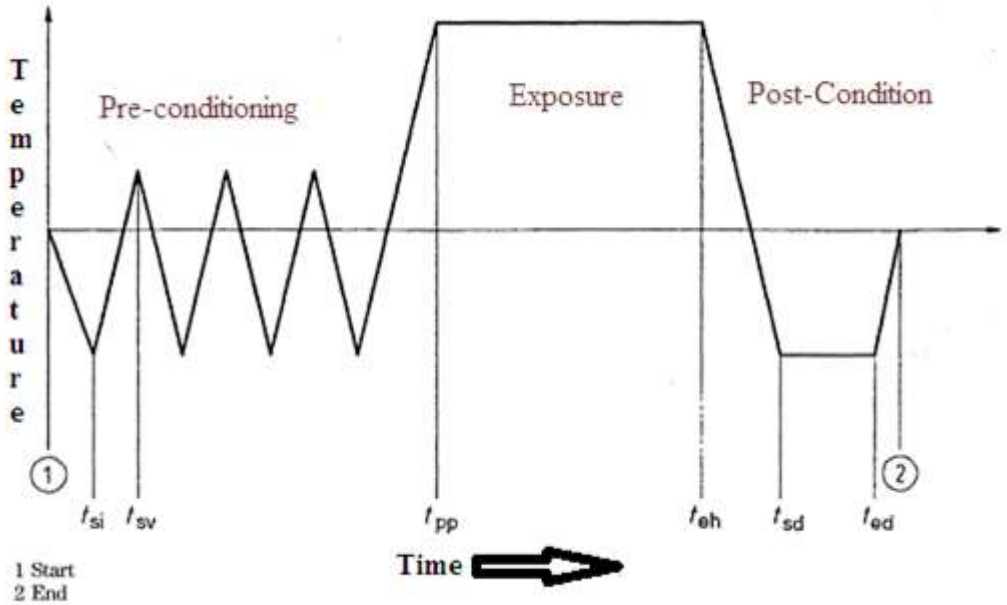

Diagram of a specimen sterilization cycle given as an example only

Steam Sterilizer or autoclave Validation / Qualification is mandatory for all machines used for biological sterilization, in the biomedical and pharmaceutical industries within the FDA, WHO & EU controlled areas. Sterilization can be accomplished by either physical or chemical means.

The principal physical means is autoclaving; other physical methods include boiling and dry heat. Chemicals used for sterilization include the gases ethylene oxide and formaldehyde, and liquids such as glutaraldehyde.
Of all these sterilants, autoclaving is the fastest, most reliable and hence; most commonly used within the FDA, WHO and EU zones of influence. It must always be remembered that it is also one of the easiest processes to get wrong. This is why regulators will nearly always scrutinize and ask about validation methods used in autoclave qualification / Qualification activities.

Autoclave or steam sterilizer validation / Qualification must follow the routine validation / Qualification document string of;

Sr. no.	Qualification Step Name	Activity to be perform
Step 1	User Requirement Specification (URS)	Identification of user specific requirement
Step 2	Design Qualification (DQ)	Identification and documentation of mechanical specification, electrical specification, software specification and operational specification.
Step 3	Installation Qualification (IQ)	Identification and documentation of critical components of the steam sterilizer
Step 4	Operational Qualification (OQ)	Vacuum supply water temperature monitoring , Vacuum Rate Test , Steam Quality testing , Air removal test (Bowie Dick test) , Cycle Tests , Empty chamber heat distribution, Air detector Test, Power failure recovery test, Autoclave cycle test
Step 5	Performance Qualification (PQ)	Cycle development for heat penetration study of user identified sterilizable load. Heat penetration study of sterilizable load along with biological indicator.
Step 6	Requalification (RQ) or Periodic Qualification	This article has been focus on that activity.

Autoclaving is the most effective and most efficient means of sterilization. All autoclaves operate on a time/temperature relationship. These two variables are extremely important and need to establish through validation. Higher temperatures ensure more rapid killing. Some standard temperature/pressures employed are 115ºC/10p.s.i., 121ºC/15 p.s.i., and 132ºC/27 p.s.i. Longer times are needed for larger loads, large volumes of liquid, and more dense materials. Autoclaving is ideal for sterilizing biohazards waste, surgical dressings, glassware, many types of microbiologic media, liquids, and many other things. When proper conditions and time are employed, no living organisms will survive a trip through an autoclave.
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_0-values. A F_0-value is the number of minutes to kill a specified number of microorganisms with a specified Z-value at a

specific temperature. A F_0– 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° (250°F).
It is not unusual to find people thinking 121° C is the temperature for sterilization. In the early days of steam sterilization a standard temperature was used in order that studies could be accurately compared, the temperature chosen was a nice round figure of 250° F (121.1° C). The F_0-value can be determined as per the following
$F_0 = 10 (T – 121.1)/10$
Where T = sterilization temperature (° C) and F_0 = equivalent sterilization time (minutes)
Suppose a given bioburdens of 1215 CFU having a D-value of 1.6 min/log at 121.1°C. If required a target SAL of 10^{-6}.
Then: Log (1215) = 3.08
Loge reduction = 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.

Validation or qualification of steam sterilizer is a kind of approach, which establishes a time temperature relation for the sterilizable loads. As mentioned above, performance qualification is the validation step where effective sterilization cycles establish.
After successful completion of performance qualification a risk assessment must be perform to identify the difficult to sterilize items and worst load for sterilization. Food and Drug Administration states the following guidelines on identification of difficult to sterilize items and hence the difficult loads: [2]

- Validation studies should be conducted to demonstrate the efficacy of the sterilization cycle. Requalification studies should also be performed on a periodic basis.
- It is important to remove air from the autoclave chamber as part of a steam sterilization cycle. The insulating properties of air interfere with the ability of steam to transfer its energy to the load, achieving lower lethality than associated with saturated steam.
- Potentially difficult to reach locations within the sterilizer load should be evaluated. For example, filter installations in piping can cause a substantial pressure differential across the filter, resulting in a significant temperature drop on the downstream side.
- When determining which articles are difficult to sterilize, special attention should be given to the sterilization of filters, filling manifolds, and pumps. Some other examples include certain locations of tightly wrapped or densely packed supplies, securely fastened load articles, lengthy tubing, the sterile filter apparatus, hydrophobic filters, and stopper load.
- The sterilizer validation program should continue to focus on the load areas identified as most difficult to penetrate or heat. The suitability of the sterilizer should be established by qualification, maintenance, change control, and periodic verification of the cycle, including biological challenges.
- Routine evaluation of sterilizer performance-indicating attributes, such as equilibrium (come up) time is important in assuring that the unit continues to operate as per the validated conditions.
- In adherence to the above reference guideline the worst case-loads were identified based on several important factors:
- The requalification loads should cover the sterilizer programs used for routine production (drug product manufacturing) loads sterilization.

- The loads shall present a challenge to the sterilizer program for air removal (Tanks, Filters, Pumps, Lengthy tubing, Manifolds, Garments)
- The loads shall present a challenge to the sterilizer program for qualifying the wrapped items (Garments, Gloves)
- The difficult to sterilize load can be demonstrated with the comparison on the equilibration time that assures effective air removal and the lethality that assures difficulty in steam exposure.

So, based on the assessment sterilizable load for heat penetration study identify for periodic requalification. Details study for the periodic qualification focus in the article.

MATERIALS AND METHODS FOR PERIODIC QUALIFICATION OF STERILIZER

A) Physical verification of equipment, major key functionality of equipment, safety features and alarms check of the equipment

A.1 The machine shall be undergone through couple of basic physical verification for wear & tear along with ancillary requirements.

- **Acceptance criteria**

  - Equipment should not have any physically damage.
  - Utilities lines, gauges shall be properly identified with labels /color code and flow marks to identify the proper process pathway.
  - All inbuilt instruments must be in calibrated state during requalification activity.
  - All the major components shall be securely anchored, and protected from shock for safe operation.

A.2 Door functioning, vacuum pump operation, jacket functioning (if any) verification need to be perform.

- **Acceptance criteria**

  - Sterilizer has two doors, sterile side and non-sterile side door. Both the door cannot be open at a time.

  - Vacuum pump must operate at deep vacuum. Pump should have the capability to hold the maximum vacuum for definite time (as per design).

  - Jacket heats up system shall verify during periodic qualification and system shall function as per design.

A.3 Alarms are the inbuilt control of equipment to run the equipment with predefines parameters. Like high temperature alarm, low temperature alarm, steam supply failure alarm, utility failure alarm, vacuum pump function error alarm, timer failure alarm etc.

- **Acceptance criteria**

  - All the design alarms shall simulate during requalification and must meet the design specification. Checking of the alarms during periodic qualification ensure the better control on equipment performance.

B) Performance evaluation

B.1 Procedure for performance evaluation

B.1.1 Pre-calibration of data logger and temperature sensors / validator system to be used:

- **Objective**

  - Data logger and temperature sensors to be used for temperature mapping should be pre-calibrated to ensure that the temperature measurement system is accurate and precise.

  - This test applies to the calibration of Data logger and T-type thermocouple sensor before qualification activity.

- **Methodology and sampling plan**

  - Data logger with T type thermocouple sensor to be used for qualification studies shall be calibrated before qualification activity to ensure that the temperature measurement system is accurate and precise

  - Calibration status of pressure devices of the respective steam sterilizer should be reviewed and recorded before commencement of requalification. Calibration temperature shall cover the entire range of temperature (e.g. 90°C,130°C and 121.1°C) which is subjected for the qualification of Steam sterilizer [3].

  - Check the calibration reports for its compliance with respect to the acceptance criteria and traceability of calibration standards.

  - Record the observations in respective observation sheet.

- **Acceptance criteria**

- All measuring and controlling devices including data logger and temperature sensors should be in valid state of calibration, and if the due date potentially occurs during the testing period, then the instrument shall be calibrated prior to beginning of qualification.

- The measurement loop including the data logger and the temperature sensors should be accurate with respect to secondary reference standard. Reference Standards used for calibration shall be traceable to national or international standards.

B.1.2 Vacuum leak test (with probe and without probe):

- **Objective**

- This test is performed to ensure that the sterilizer complies to leak test requirements indicating that the integrity of the chamber is maintained during application of vacuum after the sterilization cycle hold.

- This test is applicable for the sterilizer where vacuum cycle is applied after sterilization phase.

- **Methodology and sampling plan**

- Perform the cycle for "Vacuum leak test" in sterilizer.

- Stabilize the temperature of the sterilizer chamber so that there is no change in temperature by more than 10°C during the period in which the chamber pressure is monitored.

- When the temperature has stabilized, start the vacuum leak test cycle.

- Monitor the process and note down the observations.

- Perform the vacuum leak test before and after the validation execution to ensure the chamber integrity with and without probes.

- The test shall be executed in normal condition and with required number of thermocouples attached to ensure chamber integrity during validation.

- **Acceptance criteria:**

- When the sterilizer is tested as described above, the rate of pressure rise shall be within limits specified by the manufacturer and in any case, shall be not more than 0.013bar during the hold period of 10 minutes [4].

B.1.3 Air removals test (Bowie - Dick test):

- **Objective**

- Objective of this test is to ensure that the vacuum pulses applied before the sterilization hold period are sufficient to remove the entrapped air or non-condensable gases so as to facilitate rapid and even steam penetration into all parts of the load and maintaining these conditions for the specified temperature holding time.

- If air is present in the chamber, it will collect within the Bowie-Dick test pack as a bubble. The indicator in the region of the bubble will be of different color as compared to the color on the remaining part of the test paper, because of a lower temperature, lower moisture level or both.

- **Methodology and sampling plan**

- A ready "Bowie and Dick test kit" or "Bowie and Dick test sheet" should be used.

- Check and ensure the validity of test kit / sheet to be use with respect to its expiry date.

- Place the standard test pack above the nominal geometric center of the horizontal plane of the usable space supported at a distance of between 100 mm and 200 mm above the chamber base.
- Perform a "Bowie and Dick" test cycle in sterilizer. Perform the test as described in design specification of the equipment.

- After completion of the cycle, retrieve and examine the test sheet for the compliance against the acceptance criteria.

- Document the test details and attach the test sheet to validation report.

- **Acceptance criteria:**

- When the sterilizer is tested as described above, the indicator shall show uniform color change throughout the indicator as per the test certificate.

- It is important to compare the color of the indicator at the corners of the paper with that at the center so that any difference can be clearly seen. If there is any discernible difference the test should be recorded as failed, and the paper marked accordingly. A large area of unchanged indicator points to a gross failure [5].

- Reference Image:

B.1.4 Steam quality tests

- **Objective**

- The Steam Quality Test is performed to ensure the quality of steam entering the sterilizer during sterilization cycle is within acceptable limit with respect to Non-Condensable Gas, Super Heat, Dryness Fraction, Microbial and chemical quality [6].

- **Methodology**

- Check the dry and saturated pure steam for non-condensable gases present, for Super heat and for Dryness Factor.

- Collect the sample of pure steam condensate and subject the sample for MLT and BET test as per any pharmacopoeia. .

- Collect the sample of pure steam condensate and check for pH, TOC & Conductivity as per WFI specification (any pharmacopoeia).

- Record the observations in validation report.

- **Acceptance criteria:**

- The Fraction of non-condensable gases in dry saturated pure steam should be less than 3.5% v/v [6].

- Super Heat measured in the expansion tube of dry saturated pure steam should not exceed 25°C [6].

- Dryness value of dry saturated pure steam should not be less than 0.95 and should not be more than 1.0 [6].

- Microbial quality of pure steam condensate should comply as per WFI Specification [6].

- Chemical quality of pure steam condensate should comply as per WFI Specification [6].

B.1.5 Heat penetration studies with loaded chamber:

- **Objective:**

- The objective of the study is to ensure that the Steam sterilizer meets the temperature profile requirements, sterility assurance requirements during the sterilization for the various load patterns.

- The test is applicable for the Steam Sterilizer under validation.

- **The approach for this qualification test is overkill with an objective to ensure that,**

- The steam is sufficiently penetrating the load subjected for sterilization to achieve desired temperature of 121.1°C during the complete sterilization hold period of 30 minutes with steam pressure of 1.0 Bar to 1.3 Bar [7].

- The penetration temperature sensors/thermocouple are positioned within the components using

    1. Locations that are deemed to be most difficult to steam penetrate.
    2. When the load consists of multiples of the same item, the probed items should be distributed uniformly throughout the items.
    3. For loads where there are different types of items ("mixed load"), representative items of each type should be studied based on loading pattern rationale.

- The temperature spread is within the range of 121.1°C to 124.0°C during sterilization hold period of 30 minutes. Temperature variation criteria are applied from the time the “last” validation probe reaches the minimum temperature specified in the sterilization specification. There could be the possibility of lag period for attaining 121.1°C during heat penetration trials as the probes are placed deep into the load [8].

- For porous loads only, the equilibration time (time difference between the sensor in active chamber discharge or drain reaches sterilization temperature and sterilization hold start) to reach the minimum temperature specified in the sterilization specification should not be more than 30 seconds as chamber size is more than 800 liters [9].

- To identify the cold spot that is any location within the load where temperature sensor is placed achieving minimum sterilization temperature throughout the sterilization hold period.

- **Methodology and sampling plan**

- Before initiating the periodic qualification, ensure that the load patterns should have finalized and approved along with the rationale document for the location of sensors and the biological indicators in initial qualification.

- Instrument: Calibrated multi-channel data logger with RTD/ T type thermocouple sensors or Validator system with T-type thermocouple sensors shall be used. One calibrated pressure monitoring probe shall be used.

- Ensure that steam quality test is performed at the inlet of steam line of sterilizer. The steam quality test shall be carried out during empty chamber operation.

- A minimum one run of heat penetration shall be performed during periodic requalification for each identified load configuration to confirm uniform heat penetration inside the loads.

- Number of temperature sensors required to use for sterilizer chamber qualification shall be followed as mentioned in attachment.

- In the heat penetration run along with the penetration probes the sterilizer chamber shall be mapped with distribution probes in close proximity to the inbuilt sensors.

- Arrange and load the inputs (load pattern) to be studied as per the diagrams/photographs mentioned in the initial qualification or performance qualification.

- Verify the load for its correctness with preapproved standard load pattern diagram/ photographs.

- Placement of identified temperature sensors and biological indicators inside the sterilization load and temperature sensors inside chamber as per the preapproved protocol by considering the following,

    1. The items difficult to sterilize shall be identified from the development studies. The locations which are slowest to heat shall also be identified. During heat penetration studies, the items and locations shall be mapped with sensor and Biological Indicator.

    2. In case of same type of articles, place biological indicator in one article and place in temperature mapping sensor in article next to biological indicator article.

    3. For heterogeneous loads consisting of different of items probes shall be placed in representative items which are difficult to sterilize, including the slowest-to-heat items.

4. For loads consisting of only one item type, such as stoppers, probes will be placed throughout the load in a random or geometric pattern, as well as in identified cold and hot zones within the chamber.

5. Rationale for probe locations and number shall be provided in the validation documentation by referring the Rational document.

- Sterilization load orientation shall be well defined to facilitate air removal, condensate drainage and steam penetration (examples empty buckets should be sterilized upside down).

- In case of loads with wrapping, semi permeable portion of wrapping shall be bottom position and non-permeable portion shall be on the top position.

- While loading in case of loads with wrapping, ensure that the semipermeable portion of wrapping used should be always facing downwards in autoclave chamber and non-permeable portion shall be on top position or as per development of individual load.

- For wrapped loads, make small incision on the non-permeable portion of the pack and pass the sensor through the same to reach the identified location where steam penetration could be difficult. After placing the sensor, close the incision with silicon sealant / autoclavable tape to prevent the ingress of steam during the sterilization.

- Place pre-enumerated biological indicators of Geobacillus stearothermophilus containing minimum10^6 spores per strip, with proper identification inside the load article near each temperature sensor kept checking heat penetration, or at the locations where sensor cannot be placed, but the location is defined as difficult for steam penetration (if any).

    1. Place the biological indicator and temperature sensor inside silicon tubing and seal the portion with silicon sealant.
    2. Place biological indicator and temperature sensor inside the filter at the downstream portion and ensure the sensor tip touches the filter medium.

- Biological indicators used for biological qualification of porous/hard goods loads are typically obtained from commercial sources and may be mounted on paper, stainless steel, or other substrates [10].

- Biological indicator challenge systems are placed in slowest-to-heat locations in items that are considered most difficult to sterilize. These may be, for instance within cartridge filters at the center of a length of tubing where there is the potential for air to be trapped, or on rubber stoppers where it may be difficult for steam to penetrate.

- To evaluate the correlation between F_{PHY} and F_{BIO}, biological indicators should be placed near thermometric probes. When positioning the probes and

indicators, care should be taken to not artificially increase or decrease air removal or steam penetration to a particular area. It may be necessary to place duplicate items in the load, where one item contains a thermal probe and the other a biological indicator to obtain representative results

- Collect the Biological indicators separately in a closed container/sampling bag for each cycle individually to avoid mix-up or collect Biological indicator for each cycle once after successful completion of previous validation cycle and ensure the submission of the exposed biological indicator in a closed container/sampling cover with proper identification number.

- Set the temperature logging interval in the data logger for not more than 10 seconds in case of chamber volume is more than 800 liters and set the temperature logging interval in the data logger for not more than 5 seconds in case of chamber volume is less than 800 liters. Compare the timer/clock of the sterilizer with that of the Data logger.

- Operate the Steam Sterilizer as per the equipment "Design specification".

- Set the HPHV Steam sterilizer and data logger for the each run by connecting the pre-calibrated temperature sensors to the data logger and fix the probes to the sterilizer chamber with the help of "validation port" provided.

- Select the sterilization cycle in User Interface "Avanti", verify the sterilization cycle parameters with the sterilizer / approved protocol and start the process.

- After completion of sterilization cycle, stop the Data logger and open non-sterile side door, take out the Biological indicators from the load in to a sampling bag with proper identification number and send it to Microbiological Lab, with duly filled analysis request form for testing of recovery study.

- Check the temperature profile from Sterilizer chart recorder and equipment printout and attach with the qualification report

- After completion of sterilization cycle, stop the data logger and check the temperature profile from the Data logger for its compliance and attach the reports to the qualification document/report.

- In-case of garments load, estimate the garment dryness after sterilization. For this weigh one standard garment pack before sterilization cycle and record initial weight as 'A'. Once after completion of sterilization cycle, collect the weighed garment pack in a closed bin and weigh the test pack and record the final weight as 'B', calculate the load dryness as per below formula.

- Calculate the dryness by using the following formula

  Weight of garment before sterilization (A)

  Weight of garment after sterilization (B)

Weight of moisture present in garment W= (B-A).

$$Dryness\ \% = \frac{W}{A} X\ 100$$

Note: before loading garments into the sterilizer ensure that the garments are fully dry to avoid false pass results & to evaluate accurately.

- In case of rubber plugs loads, after completion of sterilization cycle, aseptically collect representative sample from the load in clean and dry sampling bottle and send it to Lab with duly filled analysis request form, to check for the moisture content by loss on drying method or by Karl Fischer method.

- Calculate equilibration time, by considering the time difference when minimum specified sterilization temperature (121.1ºC) is attained in drain sensor of data logger and the last sensor of data logger (penetration sensor) attaining minimum sterilization temperature.

- Check the F0 value for each temperature sensor of data logger.

- Calculation of F0 value is based on the given formula. Check the results against the acceptance criteria for compliance.

  Formula: $F0 = dt \sum 10^{(Ta- Tb)/Z}$ [10]

  Where, Tb = 121.1°C,

  Z = 10°C

  Ta = Actual temperature

  DT = Time interval between two successive temperature measurements.

- After getting the reports of biological indicator, check it for compliance against acceptance criteria and attach with the qualification report.

- After getting the reports of moisture content, check it for compliance against acceptance criteria & attach it to the qualification report.

- Temperature sensors used should be post calibrated after completion of activity to ensure that the temperature measurement system is accurate and precise

- Check the calibration reports for its compliance with respect to the acceptance criteria and attach the Calibration reports along with qualification report.

- Record all the observations in respective observation sheet later given in this protocol.

Precautions:

1. The rubber plugs used for the qualification and re-qualification purpose should not to be used for routine production.

2. Other materials like filling assembly, tanks, glassware, garments etc. used for the qualification purpose shall be used for routine production only cleaning & re-sterilized.

3. Semipermeable pouch shall be changed for each cycle once after completion of each cycle to simulate actual production process conditions.

4. When placing a sensor in free space within chamber, ensure the sensor tip should be facing upwards, to avoid temperature variation due to traces of moisture condensation (if any) along with the sensor tip.

5. In case of failure to meet acceptance criteria of any test as mentioned in the protocol an QMS tools need to be used and a thorough investigation is to be initiated to find out the root cause and the complete study or a part of the study may be repeated after the corrective action based on a suitable assessment of the situation. However, if the failure is due to some obvious reason like breakdowns, power failure during the runs or sensor fault or instrument failure then the affected run may be aborted and repeat run shall be initiated but the aborted run result along with description of the incident must be reported in the qualification report.

6. Use sterile tape with indicator to make the intact the biological indicator in the predefined positions.

7. Don't overwrap the biological indicator portion of strip while placement.

8. Avoid using of the Kapton tape during the qualification i.e Kapton tape should be exclusively used for High Temperature (Dry Heat Sterilization) qualification purpose instead use the steam sterilizable tape with indicator for confirmation

- **Acceptance criteria:**

- **External temperature mapping probes**

  - The temperature measured, throughout the sterilization holding time, should be within 121.1 to 124.0°C.

  - The duration of sterilization peak dwell time (exposure) shall be not less than 30 minutes.
  - In case of some special articles like glove port gloves, sterilizable paper load or mops the temperature measured may be higher than that specified above due to heat of hydration, in that case the maximum temperature attained will be recorded but the criteria shall be temperature should be above 121.1^0C for the sterilization hold time. [9]

- The temperature measured, throughout the sterilization holding time, should not differ from each other by more than 2°C at a single point of time.

  Note: The temperature variation criteria are not applicable for liquid media load, where the load containers are filled with different liquid volumes.

- Chamber pressure observed during sterilization temperature hold shall be maintained within the range of 1.0 to 1.3 bar (2.0-2.3 bar absolute). [8]

- The equilibration time shall not be more than 15 seconds' case of sterilizer chamber volume less than 800 liters, wherein if the chamber volume is greater than 800 liters the equilibration time shall not be more than 30 seconds. [7]

- The F0 - value of all the temperature sensor of data logger at the end of the exposure phase should be equal to or more than set sterilization hold time.

- Minimum F0 at end of the exposure phase must equal or exceed the calculated required SAL of 10^{-6} for overkill cycles.

- The measurement loop including the data logger and the temperature sensors should be accurate with respect to secondary reference standard.

- Sensor location shall be ensured before start of cycle and the same shall be verified once after completion of cycle for any dislocation of sensors. All temperature sensors shall remain in same location after completion of cycle.

- Reference/ Standard used for calibration shall be traceable to national / international standards.

- Biological indicators

  - Exposed biological indicator should show complete sterilization (i.e. no growth after defined incubation).

  - Positive controls should demonstrate the growth of the biological indicator in culture in the presence of the carrier. Samples of each manufacturer's batch must be cultured as positive controls.

  - Negative controls should demonstrate that the media is free from contaminants which could grow at the specified incubation temperature. These are done by incubating inoculated samples of media at the temperature used for recovery of the biological indicator. Negative controls are valid if there is no growth.

- Load acceptance

  - Load dryness (for garment load) shall not be more than 1.0% from its initial weight. Also, LOD for Rubber Stoppers shall not more than 1.0%.

- After completion of sterilization, there shall not be any visual traces of moisture/condensate in the load.

- Sterilized items wrapping should be intact (The load shall be checked for damage to the packaging material and overwraps post sterilization).

- **Process equipment printouts Equipment print and recorder printout),**

  - The sterilization temperature as measured by all process sensors will be within 122-124ºC during the exposure phase.

  - The duration of sterilization peak dwell time (exposure) shall be not less than 30 minutes.

  - Chamber pressure observed during sterilization temperature hold shall be maintained within the range of 1.0 to 1.3 bar (2.0-2.3 bar absolute).

  - The F0 - value of all the temperature sensor of data logger at the end of the exposure phase should be equal to or more than set sterilization hold time. (Except liquid or nutrient media load).

  - There should not be any critical process alarms.

B.1.6 CALCULATION OF STERILITY ASSURANCE LEVEL (SAL) IN RQ

- Heat Penetration Studies are performed to calculate the accumulated lethality, F0, in the load the objective is to determine the amount of lethality delivered in the process.

- This is based on the assumption that the lethal effect obtained at different temperatures is additive

- The calculated minimum F0 value should be more than biological F0 value for the biological indicator exposed for the bio-challenge studies i.e. Therefore, the minimum calculated process lethality, F0 value required for more than 12-log reduction of the Geobacillus Stearothermophilus indicator, which is exposed during the sterilization cycle should not be less than the biological F0 value for the biological indicator exposed during the bio-challenge studies for an overkill cycle [11].

- The F0-value allows inclusion of lethal input during heat-up and cool down phases above 100°C.

  Formula: $F_0 = DT \sum 10^{(Ta- Tb)/Z}$

  Where, Tb = 121.1°C,

  Z = 10°C

DT = Time interval between two successive temperature measurements.

Ta = the observed temperature at that particular time (As per the actual temperatures recorded)

Z value is the number of degrees of temperature required for a one-log cycle or a 90% change in the D-value of Geobacillus stearothermophilus spores. Values for moist heat processing of bacterial spores vary from approximately 8-12°C, but a z-value of 10°C is widely assumed for moist heat.

- The bio-challenge qualification test is performed to calculate of the biological F0 value. It is to be ensured that the process accumulated lethality; F0 value is more than the biological F0 value at all temperature-mapping locations for the sterilization cycle.

  Biological F0 value for specific biological indicator spore shall be calculated as follows,

  Biological F0 = SLR X D^{121} value

  Where,

  D-value of the BI system at the reference temperature (121°C) and SLR is actual SLR is the actual logarithmic reduction (log N0 – log NF) of the biological indicator population achieved during the cycle.

  Biological Indicator Population = N0 = 10^6

  Biological Indicator D-Value D121°C = 1.0 minute

  z = 10°C

  And, to achieve the necessary PNSU, NF = 10^{-6}

  Using the above values, the design requirements for delivered lethality, FBIO, can be calculated as follows.

  F_{BIOL} = D at 121°C × (log N0 − log NF)

- Therefore, the minimum calculated process lethality, F0 value required for more than 12-log reduction of the Geobacillus stearothermophilus indicator, which is exposed during the sterilization cycle should not be less than the biological F0 value for the particular biological indicator exposed during the bio-challenge studies for an overkill cycle

B.1.7 Selection criteria for placement of temperature sensors and biological indicators:

- Suppose volume of Steam Sterilizer is approximately 2000 liters ≅ 2.000 m^3**.**
- No of temperature sensor selected: 16 nos.
- For thermal mapping studies, minimum number of temperature sensors to be used for qualification study shall be 12 for sterilizers up to $1M^3$ usable chamber capacity and for each additional cubic meter of sterilizer chamber use minimum of 4 sensors.
- For heat penetration studies in porous/hard goods and mixed loads the number of sensors used shall be guided by the placement in representative item types those are difficult to sterilize including the reference points like the drain and close to the chamber inbuilt sensors.
- Minimum no of temperature sensor to be used for heat distribution study in loaded chamber is: 03 (Including one at drain point), and rest to be used for heat penetration study in loaded chamber.
- If the load is homogeneous like the rubber stopper loads then probes shall be placed throughout the load in a randomly generated or geometric pattern, as well as in any cold or hot zones within the chamber that may have been identified.
- Temperature sensor locations for each qualification load and the rationale for selecting each location predefined in pre-approved loading pattern.
- Place the Biological indicator (Geobacillus stearothermophilus) with details, in the middle of sterilization load near temperature sensor kept checking heat penetration, and also at the locations where probe cannot be placed but the location is identified as difficult for steam penetration.
- Placement and collection of numbers of the biological indicators before and after the study to be recorded in respective load pattern diagram of the cycle (As per Attachment to the qualification).

B.1.8 Temperature sensors used (post calibration):

- **Objective**

- The objective of this test is to ensure that the T type thermocouple used for qualification of sterilizer is in calibrated state during the entire qualification period.
- This test applies to the calibration of temperature sensors after qualification activity.

- **Methodology and sampling plan**

  - T type thermocouple used for qualification studies shall be calibrated after completion of activity to ensure that the temperature measurement system is accurate and precise.

  - Calibration temperature shall cover the entire range of temperature (including one at 121.1°C) which is subjected for the qualification of Steam sterilizer [3].

  - Check the calibration reports for its compliance with respect to the acceptance criteria and traceability of calibration standards.

  - Record the observations in respective observation sheet later given in this protocol, and attach the calibration reports along with qualification report.

- **Acceptance criteria:**

  - The measurement loop including the data logger and the temperature sensors should be accurate with respect to secondary reference standard. Reference Standards used for calibration shall be traceable to national or international standards.

DISCUSSION

The autoclave used for sterilization of the different loads used for drug product manufacturing in pharmaceutical industry employs sterilizer programs that include the following key sequential stages:

- Using a negative/dynamic pulsing and positive pulsing in the cycle to enhance removal of air from the autoclave and its load, thereby allowing subsequent replacement with dry saturated steam. This stage is of major significance to sterilization of porous loads.
- Elevation of the temperature of the load by injection of steam under pressure to a specified sterilization temperature.
- Maintenance of the specified sterilization temperature for a period of time sufficient to achieve a satisfactory assurance of sterility.
- Allowing the load to cool and dry at the end of the sterilization period.
- Equalizing chamber pressure to atmosphere by introduction of filtered (through sterilizing grade) air.

So by design the cycles are able enough to sterilize difficult items however to demonstrate the effectiveness of the sterilization cycles the periodic requalification are carried out with worst case-loads using both thermocouples and biological indicators.

Hence, periodic requalification is important to assure the performance of the steam sterilizer/ autoclave with respect to time. The full article focus on the tolls to check the performance of the steam sterilizer equipment.

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