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Equipment Validation of Moist Heat Sterilization

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Abstract:

The purpose of this paper is to provide an overview of the process validation and control validation protocols of a steam sterilizer for an injectable room in a pharmaceutical laboratory. The process validation is carried out by using a series of tests, including a Bowie Dick test, a vacuum leak test, and a swab test. This test is conducted to confirm that the steam is adequately penetrating into the deepest regions of the load being steered. The temperature variation between 121^0C and 124^0C during the steering hold time suggests that the load has no impact on the uniform heating procedure that was obtained in the empty chamber heat distribution investigation.

Keywords **—Validation, Sterilization, Moist heat sterilization, Equipment validation.**

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I. INTRODUCTION

Validation is a fundamental and integral part of the pharmaceutical industries to produce products of desired quality, accuracy, consistently and economically. It is required for compliance with national or international regulations. It is an essential requirement of a quality programme to maintain the consistency of quality products in all the stages. Process validation is the programme that is recorded and offers a high level of confidence that a particular process will consistently create a product that meets its predetermined standards and quality attributes. Process Validation is required under both the GMP standards for medical devices (21 CFR 820) and current good manufacturing practises (cGMP) for completed pharmaceuticals (21 CFR 211). As a result, it applies to the production of both drugs and medical devices. It is a methodical approach created to guarantee that a manufacturing process can reliably deliver high-quality goods. A validation team lead by the quality assurance director of pharmaceutical producers completes it. Generally, process validation is carried out before releasing a new product, A validation team lead by the quality assurance director of pharmaceutical producers performs it. If a change is made to an existing product, and on an ongoing basis to ensure the process is working as intended. A protocol should be established at the outset that details the parameters that will be tracked, the samples that will be collected, and the findings that will be accepted.

There are three stages in Process Validation

Stage 1- Process Design

The manufacturing procedure is established at this step so that it can replicate the delivery of a pharmaceutical product that satisfies certain requirements and quality characteristics. The validation team must fully understand how the process operates in order to do this. During this phase, the commercial process is established using the expertise amassed during development and scale-up activities.

- Product development protocols
- Functionality and requirements of production equipment
- Predicted efforts to variability
- Design of experiments (DOE) studies
- Risk analysis techniques
- Study achievement or demonstrations at laboratory or pilot scale
- Computer based or digital simulations

This stage also includes process management and creating strategies to reduce input variation and/or compensate for it throughout manufacture. Controls at significant processing locations frequently include equipment monitoring and material analysis.

Stage 2- Process Qualification

During this stage, the process design is confirmed as being capable of reproducible commercial manufacturing. Process design is examined as part of the process qualification to see if it is useful for high-quality manufacturing. First, the production facility should be built in accordance with modern good manufacturing practises standards (CGMP). After that, utilities and equipment should be qualified to ensure that they were constructed and installed in accordance with design requirements.

Finally, a method should be followed to complete process performance qualification and report on the results:

- Introduction, Objectives, and Scope
- Process Validation Studies based on Quality Risk Management (QRM)
- Responsibilities and requirements for production, quality control (QC), and quality assurance (QA) (eg. manufacturing records)
- A Validation Team (with members from Research and Development, Engineering, Production, QA, and QC)
- Product Details and Design Considerations
- List of Materials, Vendors, and Master Formula
- List of Equipment and Process Flow Chart
- Brief Manufacturing Process and evaluation of Critical Process Parameters (CPP)
- In-process Study and Specifications or Critical Quality Attributes (CQA)
- Limitations, Acceptance Criteria, and Experimentation Plan
- Yield Specifics (minimum of three consecutive batches)
- Analytical Method Validation
- Finished Product Specification
- Reviews of OOS test results, deviation monitoring, and change management (CC)
- Revalidation Criteria
- Review of Follow-up Action (if any)
- Summary and Conclusion

Stage 3- Continued Process Verification

Your The third step of process validation, which comes after process design and qualification, is putting in place procedures to continuously guarantee that the validated process continues to be in this state during ordinary production. Statistical process control (SPC), continuous monitoring and sampling of process parameters and quality characteristics, and planned maintenance of the facility, utilities, and equipment are all common components of ongoing process verification. Throughout the validation process, it is crucial to use excellent documentation methods. Continual confirmation that the process is under control is obtained throughout ordinary manufacturing.

III) TYPES OF PROCESS VALIDATION

Process validation is frequently divided into groups based on when it is carried out in relation to the production schedule. According to this definition, process validation may be divided into four different categories: prospective validation, retrospective validation, concurrent validation, and revalidation.

A) Prospective Validation

It is implemented whenever a product will be produced using a new recipe or at a new location. Prospective validation, often referred to as premarket validation, is typically done prior to starting ordinary manufacturing. As the beginning point for every product that will be issued under novel circumstances, it is also regarded as the fundamental sort of validation.

B) Retrospective Validation

For facilities, processes, and process controls that are currently in use but have not gone through a formally documented validation procedure, retrospective validation is used. In order to give the necessary documentation proof that the process is performing as it is supposed to, it is possible to validate these facilities, processes, and process controls using historical data. This kind of validation is therefore insufficient in situations where there have recently been modifications to the product composition, operational procedures, or equipment, and is only suitable for well-established processes.Since it is highly improbable that any existing product has not gone through the prospective validation procedure, this strategy is only briefly being employed today. It is only used to examine a procedure that has been validated.

C) Concurrent validation

On the basis of the description created during the actual execution of the process, concurrent validation is utilised to create recorded evidence that a facility and its processes do what they claim to. This tactic entails keeping an eye on crucial production processes and evaluating the final product to demonstrate that the manufacturing process is under control.

D) Revalidation

Revalidation entails using the first validation effort, in its entirety or in part, and involves a review of previous performance data. Maintaining the verified state of the facility, machinery, production processes, and computer systems requires this strategy. The following are possible causes for beginning the revalidation process:

- ✓ Transportation of a product between plants.
- ✓ Modifications to the product, facility, production method, cleaning procedure, or other factors that might have an impact on the quality of the final product
- ✓ The demand for routinely reviewing validation findings.
- ✓ Significant batch size growth or reduction.
- ✓ Recurring batches that don't adhere to the requirements for the product and the procedure.
- ✓ Revalidation methods have different goals depending on how much has changed and how it has affected the product.

IV) STERILE MANUFACTURING PROCESS

Sterile product manufacturing processes are very critical and precaution should be taken while carrying out the procedures. Following points should be considered:

1) Starting materials
2) Area of monitoring and control
3)Equipment Validation and control
4)Discipline in aseptic processing area
5)Washing and sterilization of primary containers
6)Solution preparation and filtration
7)Filling, sealing and inspection
8)Media fill

I) Starting Material

- Bulk row materials which are used should be monitored for bioburden periodically.
- Bioburden of bulk solution should not be more than 100 CFU per ml is maintained.

Environmental Acceptance criteria

Maximum number of Particles				
0.5 µm			5µm	
Class	Per cubic	Per cubic metre	Per cubic foot	Per cubic metre
100	100	3.5	-	-
10,000	10,000	350	65	2.3
100,000	1000,000	3,500	700	25

TABLE 1: AIR CLEANLINESS LEVEL : DEFINITION OF CLASSES

Source	Control
People	Total Body covering on critical areas Adequate personnel Flow Proper equipment location Limited number of personnel working in the critical environment.
Process	Cleaning and Sterilization of procedure and equipment Adequate operational procedures Proper process equipment designed for operation in critical areas Adequate vacuum cleaning devices for powder.
Materials	Adequate material control and selection Proper sterilization and filtration procedures Adequate handling of material procedures
Air	Adequate air filtration system Air cleanliness levels monitoring Air system validation procedure.

TABLE 2- CONTAMINATION SOURCES AND CONTROL

Operation	Class	Cleanliness level (Particles 0.5 µm and larger)
Warehousing	-	-
Preparation	100,000	Not more than 100,000
Filtration	100000	Not more than 100,000
Filling area	100,000 or better	Not more than 100,000
Filling line (point of use)	100	Not more than 100

TABLE 3 : GUIDELINES FOR CLEANLINESS LEVELS REQUIRED DURING MANUFACTURING OF A PARENTERAL DRUGS

Equipment Validation and Control Validation Protocol of HVAC system for an Injectable Room

Validation Protocol for HVAC system consist of :

- ***URS (User requirement specification)***

  Customer of the equipment has specific expectations about the equipment which he/she wants to use. Some of the general requirements are stated in the form of parameters like:

  - ✓ Size of the equipment
  - ✓ Speed of the equipment
  - ✓ Effectiveness of the equipment
  - ✓ Availability of spares, change parts, and prompt services with a reasonable cost
  - ✓ Ease of operation, cleaning, and maintenance
  - ✓ Low dust and sound generation
  - ✓ Lesser breakdowns
  - ✓ Materials of construction
  - ✓ Auto control system
  - ✓ Easy change over
  - ✓ Overall good construction and workmanship, etc.

  These requirements which are usually discussed with the equipment manufacturer or supplier and based on that discussion; the selection of the equipment is done. In case of any specific standard items, user have to accepts the standard specifications of the manufacturer.

- ***DQ (Design Qualification)***

If particular equipment is to be fabricated as per the user requirements, then it is very important to design detailed information of qualification document. It is advisable to work out the detailed specification of equipment by sitting with the manufacturer or supplier. Once this is ready, it should be agreeable to both the sides, that the purchaser and manufacturer. At this stage it may be recommended to identify the stages during the fabrication of the equipment, where visual,

instrumental, or even physicochemical testing is to be performed in the presence of the purchaser. Generally, the factory acceptance test (FAT) is performed at the manufacturer's premises before delivery of the equipment to the purchaser. DQ should provide the documented evidence that the design specifications were met.

- ***IQ (Installation Qualification)***

The following definition could be used to describe it: "Documented verification that all significant installation components comply with the manufacturer's recommendation, applicable codes, and approved design qualification." This statement simply means that the equipment in question can be installed once it has met the requirements for installation, or when it has successfully passed the IQ test. The installation should have been finished and satisfactorily installed, according to IQ's documentation. During IQ, it is important to verify the purchase specs, drawings, manuals, spare parts lists, and vendor information. Instruments for control and measurement ought to be calibrated.

- ***OQ (Operational Qualification)***

It might be described as "documented confirmation that the system or subsystem operates as expected within the whole stated operating range." Only once the OQ Test has been passed should the apparatus be used. Utility, system, or equipment and all of its components should perform in line with operational standards, according to recorded proof provided by OQ. Tests should be created to show that the device operates well under both its operating circumstances' upper and lower bounds (including worst case conditions). Operation controls, alarms, switches, displays, and other operational components should all undergo testing. All documentation for measurements made using a statistical technique should be provided.

- ***PQ (Performance Qualification)***

Many people perceive PQ to be the same as OQ. Some experts define OQ as the verification of system or subsystem performance without load, whereas PQ refers to the same thing with load. However, because these two ideas are often used interchangeably, it is tough to tell them apart clearly. Utility, system, or equipment and all of its components' ability to consistently work in accordance with the requirements while in regular use should be supported by documentation, according to PQ. To demonstrate consistency, test data should be gathered over an appropriate time span.

V) VALIDATION OF HVAC SYSTEM

In order to validate an HVAC system, a collection of documents is typically required, including functional specifications (the conceptual design), design drawings, plans, and specifications, validation master plans, contractor documents, testing, adjusting, and balancing (TAB), startup reports, commissioning reports (the actual execution of validation protocols), and validation (IQ, OQ, and PQ).

The following definition of IQ may be used: "Documented proof that all significant installation components comply with the manufacturer's recommendations, applicable codes, and approved design qualification." The purpose of IQ is to confirm and record the excellence, integrity, and installation of HVAC system components. Designing installation protocols involves using design manuals and publications. OQ may be summed up as "documented confirmation that the system or subsystem operates as intended over the whole specified operating range." Only once the OQ Test has been passed should the apparatus be used. When used regularly, utilities, systems, or equipment, including with all of its parts, should consistently function in compliance with the requirements, according to PQ's documentation. To demonstrate consistency, test data should be gathered over an appropriate time span. On the whole.

Methodology

The following technique was used to validate the HVAC system:

- ***Air flow or smoke pattern***

  For the characterization of this parameter, a titanium tetrachloride stick is taken and burnt and the burning stick is placed in front of the AHU. The distribution of smoke is observed. It have to be uniform.

- ***Air flow velocity and changes per hour***

  The HVAC area has been divided into four fake grids for this test. At each grid, the air speed is monitored, and the average air speed (V) is calculated after that. By dividing the area of the HEPA filter intake (A) in feet by the average air velocity (V), the total air volume (T) is calculated. T = A V is the equation that is produced. Once the room's volume has been determined, the total air change is divided by the room's volume to determine the air changes per hour.

- ***Filter leak test***

  The air velocity at each corner is measured using a velometer in front of the AHU system as part of the HEPA filter leak test. The air velocity must be lower than the HEPA filter's maximum allowable value. If it is discovered that the upper limit has been surpassed, a gas cut (silicon) is used to lessen the leakage.

- ***Particle count***

  The test is run with a particle counter. Prior to the procedure as well as while it is in progress, a particle count is taken. As per the requirements for the Grade A, B, C, and D areas, the particle count should fall within the acceptable range.

- ***Viable monitoring***

  Utilizing the swab test and nutrient agar medium for the microbial incubation allows for good daily monitoring. The numerous media plates are visible in every industrial location, including the HEPA filter's reverse air duct at the back of the cubicle. If the microbe count is twice found to be beyond the permitted limit, an effective remedial and preventative intervention is carried out.

- ***Pressure difference***

  It is estimated by using the manometer that is fixed to the nearby area's walls. The pressure differential is typically maintained between 5 and 20 mmHg.

- ***Recovery test***

  The Temperature and humidity recovery are monitored. The HVAC system is turned off to monitor the temperature and humidity for this. After raising the temperature to 400°C and the humidity to 75%, the temperature and humidity are once more recorded after turning on the HVAC system, and the time needed to stabilise the conditions is documented.

- ***Temperature and humidity uniformity test***

  A calibrated thermometer and a manometer are used to measure the uniformity of temperature and humidity, respectively. The stabilisation is ensured within the predetermined limit while the two parameters are tracked daily and reported in the format.

- ***Fresh air determination***

  The fresh air intake may be seen at the fresh air dumper's entrance. It calculates the overall air change. The percentage of fresh air inhaled by the HVAC system throughout each cycle in each of the different rooms is calculated by dividing the fresh air intake by the total air change in the room and multiplying the result by 100. clear-headed resolve.

Sterilization

Sterilization is a physical or chemical technique that eliminates all living forms and is particularly useful for eliminating viruses, spores, and microbes. It is specifically described as the total eradication of all microorganisms by the use of an appropriate chemical agent or heat, namely dry heat at 160 to 180 °C (320 to 360 °F) for three hours or wet steam under pressure at 120 to 250 °C for at least 15 to 20 minutes.

VI) Methods of sterilization

1) *Dry heat sterilization*

The most practical and preferred methods sterilisation is dry heat sterilisation, which uses h air to kill or degrade all life inside an industr oven's chamber. The earliest techniques sterilisation was dry heat, which also offers a hi degree of predictability and can be tailored to mee variety of various sterilising needs.

The intended target of the sterilisation is exposed dry air, which conducts energy to the target conduction (forced air). The forced air type desirable because it distributes the heat load to t item with higher homogeneity. Alternatively, oven might employ heated coils in place of fa (static air).

The operating conditions ask for the temperature be set at 160 °C (320 °F) for two hours, 170 °C (3 °F) for an hour, and up to 190 °C (375 °F) for six twelve minutes. These cycle periods are normal; the exact setting may vary based on the application. The amount of time needed to achieve sterilisation varies depending on the kind of oven and blower u employed. For instance, some ovens use hig velocity blowers with several, strong fans to se significant amounts of hot air onto the ite Consider that they have cycle times that are half those listed above for standard air circulati capabilities.

All bacterial spores, fungus, viruses, prions, a essentially all types of biological agents have th proteins denaturized as a result of the heat applied the item. Denaturation is the breakdown of nucl acids, which causes all living things to basically die from a lack of energy, whilst viruses are render permanently inactive owing to severe damage to the RNA or DNA that encodes them. It is crucial to remove any moisture from the air blown within the oven chamber since this moisture might obstruct the process of protein denaturation.

A distinct method entirely, moist heat sterilisation is employed for a different range of applications and sterilising goals.

Advantages of Dry-Heat Sterilization:

- The cost of buying dry heat ovens is often low.
- In general, operating and heating cycles are inexpensive.
- Thick things may be sterilised thoroughly since the heat can penetrate them deeply. In this manner, even items included in packaging can be sterilised.
- High temperatures may disinfect metallic things fast if they are heat-resistant.
- Due to the absence of moisture, dry heat is not detrimental to metallic objects.
- Since no poisonous chemicals are used in the process, no hazardous materials will be dumped into the environment.
- does not require human attention or involvement when operating. The oven has to be turned on and left alone for the cycle to finish.
- As it cools down fast, the object may be removed and utilised virtually immediately.

Disadvantages Compared to Other Processes

- Compared to the time needed for sterilisation by steam, flame, chemical sterilisation, or radiation, dry heat can take significantly longer.
- Sensitive materials or thin sheets may deform as a result of the heat.
- Plastics and rubber are not appropriate for dry heat due to the high temperatures' potential for permanent damage.
- Even for materials that can withstand it, excessive heat exposure can cause some compounds' chemical structures to alter unintentionally.

2) *Gaseous sterilization*

Equipment or devices are subjected to various gases in a sealed, heated or pressured chamber during the process of gaseous sterilisation. Due to the ability of gases to flow through a small aperture and produce more efficient outcomes, gaseous sterilisation is a more effective method. Additionally, heat treatment is frequently employed in conjunction with gases, which aids in the working of the gases. However, there is a problem with the process releasing certain poisonous gases, which the system needs to be cleaned out of on a regular basis. For various kinds

of gases, the method of action varies. The following is a list of some of the typical gases used in gaseous sterilisation:

- ***Ethylene oxide***

Due to its broad range of compatibility with various materials, For chemical treatments used to sterilise, pasteurise, or disinfect a variety of tools and surfaces, ethanol oxide (EO) gas is widely utilised. When an object is vulnerable to heat, radiation, or even chemicals, EO treatment frequently takes the place of these methods. This approach is widely utilised, accounting for around 50% of sterilisations of disposable medical equipment and over 70% of all sterilisations. It is thought that the sulfhydryl, amino, hydroxyl, and carboxyl groups of proteins as well as the imino groups of nucleic acids are alkylated by this gas, which is how it exerts its antimicrobial impact. In order to promote the action of the gas, EO treatments are often carried out for many hours at temperatures between 30 and 60 °C.The concentration of gas accessible for each object determines how effective the gas will be, and this is considerably aided by the gas's high degree of penetration through a variety of packing materials, including as rubber, plastics, fabric, and paper. Ethylene oxide is compatible with practically all materials and is effective against all known microorganisms, including bacteria (including spores), viruses, and fungus (including yeasts and moulds). The amount of gas in the steriliser continues to drop owing to absorption in this phase, and the treated items must go through a desorptionprocedure to eliminate the harmful residual wastes. In a dried condition, both organisms and those shielded from the gas by inclusion in crystalline or dry organic deposits are more resistant to ethylene oxide treatment.

- ***Formaldehyde***

Another significant highly reactive gas utilised for sterilising is formaldehyde. Heating formalin (37 percent w/v) to a temperature of 70–80 °C produces this gas. It has found use in the surface sterilisation of powders, the sterilisation of certain medical diagnostic, and electrical equipment, and the sterilisation of reusable surgical tools due to its broad-spectrum biocidal action. Although it doesn't penetrate as far as ethylene oxide, the alteration of proteins and nucleic acids is the same process used by formaldehyde. Its application is frequently restricted to paper and cotton materials due to its modest penetrating capability. Formaldehyde can typically be smelled at amounts lower than those allowed in the atmosphere, making leaks and other similar incidents detectable.

- ***Nitrogen dioxide (NO_2)***

Common bacteria, fungus, and even spores may be eliminated quickly and effectively with nitrogen dioxide. Due to NO2's low boiling point (20°C), a large vapour pressure is possible at room temperature. This characteristic of NO2 makes it possible to use the gas at normal pressure and temperature. As the exposed organism absorbs NO2, the biocidal activity of this gas destroys DNA by nitrating the phosphate backbone, which has fatal consequences for the organism. Due to the low amount of gas utilised and the high vapour pressure, this gas has the benefit of not condensing on the surface of the devices. As a result, there is no requirement for direct aeration following the procedure of sterilization.

- ***Ozone***

Ozone is a potent industrial gas that is frequently used to disinfect surfaces as well as to sanitise air and water. Ozone is a powerful oxidizer that may eliminate a variety of organisms, including prions, without the need of dangerous chemicals because it is often produced from oxygen of the highest quality for medical use. Similar to this, because of ozone's high reactivity, waste ozone may be removed by turning it into oxygen using a simple catalyst. Ozone may only be used in certain contexts since it must be manufactured on-site because it is an unstable and reactive gas. Due to its high level of danger, it should only be used at a concentration of 5 ppm, or 160 times less than ethylene oxide.

Advantages of Gaseous sterilization:

- Strong antibacterial and fungicide properties.
- best way to sterilise heat-sensitive biomedical materials (which are very expensive). Since

ethylene oxide sterilises at low temperatures, it is ensured that the elements won't be damaged or destroyed.
- good gas diffusion through the sheets and portions that are difficult for the sterilisation components to access.
- Sterilized components that would otherwise be destroyed can be reused, improving the cost-process relationship.
- Water can be used to neutralise ethylene oxide.

Disadvantages of Gaseous sterilization:
- It is a lengthy procedure since the ventilation time for the sterilised components must also be considered in addition to the sterilisation time. Depending on the sterilised material's kind (metal, glass, plastic, etc.) and whether it will be utilised intra- or extra-corporeal, the ventilation period may vary.
- Poor handling might have a negative impact on your health.

3) Moist heat sterilization

The most popular and reliable approach for sterilising is moist heat, which is achieved by the application of saturated steam under pressure. Moisture has a greater capacity to penetrate at a given temperature than dry heat, which speeds up the extinction of life. Steam sterilisation kills bacteria quickly, is sporicidal, nontoxic, and inexpensively. It permeates materials and warms them quickly. Heat- and moisture-resistant materials like aqueous preparation that are biohazardous are frequently sterilised using moist heat in an autoclave (culture media). The disinfection of surgical drapes and medical equipment also uses this technique. Gravity displacement steam sterilisers are the most prevalent kind in microbiology labs. Vacuum/gravity assisted autoclaves are another kind. For moist heat sterilization in an autoclave, pure steam is used in the chamber. Different load patterns to be pre-decided and validated by heat penetration studies. Temperature around 121^0 C for 15 minutes is required but actually 121^0 C for 30 minutes is used.

Advantages of Moist heat sterilization
- Non-toxic to users, workers, and the environment
- Simple to monitor and regulate a cycle
- Microbicidal quickly
- Of the mentioned sterilising techniques, the one least impacted by organic or inorganic soils
- Quick cycle time
- Penetrates device lumens and medical packaging

Disadvantages of Moist heat sterilization
- Harmful to heat-sensitive equipment
- Instruments used in microsurgery are harmed by frequent exposure.
- May cause instruments to get damp and corrode
- Possibility of burns

Pre-requisites for validation of Steam Sterilization
- IQ and PQ of steam sterilizer shall be completed.
- All the technical staff and operators shall be trained for validation
- procedure.
- All the measuring devices and sensors used for validation shall be calibrated.
- Steam shall be Qualified.
- Draft SOP for sterilization shall be ready.
- Biological indicators shall be qualified.

Qualification of Steam

The semi logarithmic model for saturated steam processes assumes that saturated steam is free from non-condensable gases and superheat. Non condensable, wet steam and other gases can adversely affect the load cycle. Thus, the extent of sterilization may be affected by steam quality. Hence, for sterilization, the steam quality should be evaluated as a part of qualification of steam and should be repeated at regular intervals.

A. D value & F0 value

D value: Log reduction is the amount of time needed to eliminate 90% of the current bacteria.

F0 value: The amount of lethality at the provided temperature for the specified amount of time in minutes is equal to that of 121^0 C.

Development of Sterilization Cycle

1) Bioburden Approach

Initial load = 134 CFU (Colony Forming Unit)
Log of 134 is 2.13
Additional 6 log reduction is required.
 Total log Reduction = 2.13 + 6 = 8.13
G. stearothermophillus spores D value is around 2 mins, Consider D value as 2.5 minutes,
Time required for sterilization = 8.13 x 2.5 = 20.32 minutes is the cycle time required.

2) Overkill Approach

The material is to be sterilized is having Bioload of 1 million CFU.
Log of 1000000 + 6 log
6 log + 6 log = 12 log reduction
 Cycle time = 12 + 2.5 = 30 minutes.

In both the approaches it is ensured that sterility assurance level(SAL) is better than $1x10^{-6}$

F_0 Value Calculation

By Biological indicators
$F_0 = D121(\log N_0 - \log N)$
D121 = D – value of reference spores at 121^0 C.
N_0 = Initial number of viable microorganisms
N = Final number of viable microorganisms
By Temperature profiling
$F_0 = \Delta t \sum 10\ T- 121.1/z$
T – current temperature in degree centigrade at time
Z- z value is considered as 10.

- ***OQ of Autoclave/ Pre-requirement of validation***

1) Vacuum test

To check for leakage in autoclaves, vacuum leak tests are conducted. The chamber integrity test is another name for this test. In VLT, a vacuum is initially applied within the chamber, followed by a wait for the vacuum to reach a certain point, a holding period during which the vacuum is maintained, and finally, vacuum breakage. Check the findings afterwards to see if the autoclave leakage was within the acceptable range. VLT acceptance standards: According to HTM 2010, VLT shouldn't exceed 1.3 mbar per minute. However, some autocalves' printouts only state the acceptance requirement of 0.013 bar without specifying whether the vacuum leakage limit is per minute or per ten minutes. The operator can become perplexed as a result.

Procedure: Make sure the compressed air is on and at a high pressure, that the gasket lubrication is enough, and that the switch on the panel board is functional. Also, make sure the chamber temperature is steady at ambient. Start the cycle for the vacuum leak rate test, watch the pressure in the steam sterilizer's pressure gauge, and then let the pressure fall. All of the chamber's valves will be closed by the machine, the vacuum pump will be turned off, and the time and pressure will be noted (P1). Wait for a stabilisation period of five minutes (about ten seconds), then record the pressure again (P2), and then wait for another ten minutes (about ten seconds) and record the pressure three times (P3). Return to normal atmospheric pressure and keep running until the following cycle, where the vacuum leak rate.

Acceptance criteria: The recommended vacuum leak rate is NMT 0.013 bar/ten minutes.

Frequency: At the time of Initial Qualification, run the test three times in a row.

2) Air removal test (Bowie-Dick test)

A Bowie-Dick test is a commonly used operational test that labs may do to make sure their autoclave satisfies the requirements for sterilisation and to identify the correct air removal from their pre-vacuum autoclave chamber. A Bowie-Dick test pack is a tiny, single-use container constructed from thermochromic paper, porous materials, and reticulated foam.
The thermochromic paper in the test pack must become entirely black to indicate that a Bowie-Dick test cycle was successful. This shows that the autoclaving is working properly and that all of the steam has permeated the load. The test was failed if the pack just partially or completely changes colour. Place the pack on the lowest shelf above the drain in the empty autoclave chamber before starting a Bowie-Dick test cycle.The demands of your institution will determine how frequently you

conduct a Bowie-Dick test cycle, since certain facilities, like hospitals or other healthcare facilities, require more regular testing than others. Please refer to the AAMI ST8 reference document for information on hospital steam sterilisers.

Procedure: Place the Bowie Dick test sheet on the lowest shelf of the steriliser, over the drain (100mm over the drain) To conduct an air removal study, the Bowie Dick test paper will be put in an empty chamber. It comes with a pack of standard paper and an indicator sheet. By pressing the enter key, begin the cycle. When the cycle is finished, open the door from the side facing the control area, remove the test paper from the autoclave, and inspect the indicator paper for a consistent colour change. Bowie Dick test paper is used to evaluate the effectiveness of air removal from the steam steriliser since it is designed to replicate the garment pack. The first three cycles of the air removal investigation must be carried out using brand-new indicator paper.

Set Parameters:17-minute cycle with a 121°C temperature

Place of keeping Bowie -Dick Indicator: Set the Bowie dick indication between 100 and 200 mm above the base of the sterilising chamber.

Acceptance criteria: After the cycle, the Bowie dick indication should display a consistent colour shift (Yellow to Brown/black). Inadequate air evacuation from the sterilising base chamber is indicated by no change, no change in consistency, or an area on the test sheet with air entrapment (bubbles).

Frequency: At the time of Initial Qualification, run the test three times in a row.

3) Heat distribution study

i. Empty chamber heat distribution study

This test's objective is to verify that the steriliser can function at 121^0C and 1.1–1.2 kg/cm2 of steam pressure for the duration of the sterilising hold time. The sterilising cycle's temperature spread between 121^0C and 124^0C will show how evenly the heat is distributed across the chamber. Any area where the temperature indicator is installed and does not maintain the sterilisation temperature hold at the sterilisation temperature minimum of 121^0C will be regarded as a cold spot.

Procedure: Place 16 temperature sensors within the chamber via the sterilizer's validation port. To prevent steam leaks during steriliser operations, plug the port with silicon sealant. Fix each probe in a distinct spot in the steriliser to prevent the sensors from coming into contact with the chamber's metallic surface. The data logger, which can scan and print the real temperature and pressure at various locations, should be connected to the temperature sensors. Check the thermograph in the data logger to make that the sterilising hold period's predetermined temperature and pressure were reached after the sterilisation cycle has finished. Repeat the cycle after making the appropriate corrections if any deviations are noticed.

Acceptance criteria: Throughout the sterilising period, the temperature distribution within the chamber must be between 121°C and 123°C in every region (dwell time). There shouldn't be any slowest heating sites (cold patches) in the autoclave chamber, and the equilibrium time shouldn't be more than 30 seconds.

ii. Loaded chamber heat distribution study (Heat penetration study)

In order to attain the target temperature of 121^0C for the whole sterilisation hold duration with steam pressure of 1.1 to 1.2 kg.cm^2, this test must demonstrate that steam is adequately penetrating into the deepest regions of the load being sterilised. The load arrangement or load size must be examined, and the cycle must be redone, if sterilisation temperature is not maintained throughout the cycle. Temperature variations between 121^0C and 124^0C during the sterilising hold time suggest that the load has no impact on the uniform heating procedure that was obtained in the empty chamber heat distribution investigation. As the probes are moving, there may be a delay before 121^0C is reached during heat penetration runs.

Procedure: Place 16 temperature sensors within the chamber via the sterilizer's validation port. To

prevent steam leaks during steriliser operations, plug the port with silicon sealant. Fix each probe in a distinct spot in the steriliser to prevent the sensors from coming into contact with the chamber's metallic surface. Place the object in the autoclave chamber according to the loading pattern. A separate heat distribution analysis for each loading pattern in the loaded chamber must be carried out. At least 15 biological indicators and 10 thermochemical indicators must be utilised for each cycle. Arrange the load as directed. In all three runs, the load must have an indication and a temperature sensor when it is positioned at the designated cold locations. At the drain point in each of the three cycles, a biological indicator, thermochemical indicator, and external temperature sensor must all be installed. Start the data logger and steam steriliser simultaneously, and then carry out the sterilisation by running the software designated for each kind of load in accordance with normal operating practise. Stop the data logger, open the steriliser when the sterilising cycle is over, remove the thermochemical and biological indicators from the load, and send them to the microbiological lab for analysis. The thermochemical indicator must be checked for conformity with the manufacturer's guideline for colour change after the biological indicator has been aseptically inoculated into sterile soybean casein digest media (SCDM) and incubated at 55 to 60°C and liquid load at 35 to 39°C for seven days (i.e Brown). Remove the temperature chart, data logger, andtemperature recorder from the device, along with the biological indicator report. Determine the Fo value, compare it to the acceptance criteria, and check for conformity with the requirements. Perform a vacuum leak rate test after removing the external temperature sensor from the chamber.

Acceptance criteria: Throughout the sterilising period, the temperature distribution within the chamber must be between 121°C and 123°C in every region (dwell time) For a minimum of 10 thermocouples, sterilisation temperature should be maintained for NLT 15 minutes. *G. bacillus Stearothermophillus*, a biological indicator, ought to exhibit full sterilisation (i.e no growth after incubation).

Documentation required for autoclave

- Calibration master plan (CMP)
- Validation master plan (VMP)
- SOP related to various calibrations
- Record & registers of calibration activity
- Room Description chart
- Protocol for IQ/OQ of equipment
- All the printouts of the cycles run shall be enclosed along with the report.
- Qualification report of biological indicators shall be enclosed along with the validation report.
- Pictures of loading pattern, Half load and full load shall be enclosed.

Conclusion

In cGMP, qualification is a basic idea. When using an autoclave to sterilise items like clothing, cleaning supplies, filters, utensils, vial filling machine components, rubber stoppers, etc. The equipment qualification procedure that covers the full test, including the vacuum leak test, the Bowie-Dick test, the heat distribution study (empty chamber, loaded chamber), and the heat penetration test, was then carried out. The acceptance criteria were determined to be satisfied by all of the procedures and parameters that are mentioned. As a result, autoclave is regarded as competent and suitable for regular usage.

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