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Review Article

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Systematic Approach of Autoclave Qualification: A Review

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ABSTRACT

Autoclave 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. Autoclave validation or qualification is mandatory to achieve a high level of sterility or quality of the product. Quality is the primordial intention of the industry and its product manufacturing. As part of the GMP, qualification is mandatory. Each company should identify what qualification work is required to prove the critical aspects of their particular operation control. Autoclaving is the most effective and efficient means of sterilization. Sterilization is a process to remove, kills, or deactivate all the microorganisms such as bacteria and spores. The purpose of this study is to give a systematic approach required for the qualification of the autoclave which contains USR, DQ, IQ, PQ, and OQ and also contains the factor that affects the sterilization and evaluation parameter test.

Keywords: Autoclave Qualification, Performance Evaluation Test

INTRODUCTION

As we know autoclave plays a curtail role in sterilization within the pharmaceutical and medical industries. For sterilizing materials, autoclaves are widely used. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes. Autoclaves vary in size, shape, and functionality. A very basic autoclave works similarly to a pressure cooker to kill bacteria, spores, and germs that are resistant to boiling water and powerful detergents.[1]

In 1879 the French microbiologist Charles Chamberland created a new version called the autoclave to be used in medical applications. In 1881 Robert Koch researched the disinfecting properties of steam and hot air. He illustrated how steam has a stronger ability to penetrate material than dry heat. Finally, 1933, the first pressure steam sterilizer that controlled performance by sensing the temperature in the chamber drain line was introduced (thermostatic trap).[2]

A steam sterilizer (Autoclave)

Heating water above the boiling point in an open vessel is difficult. This results from significant evaporation that takes place during boiling. It is possible to raise the boiling point of water if it is heated in a sealed vessel. An autoclave is a huge pressure cooker with a sealed lid that sterilizes objects by applying pressure to steam. Steam has a higher heat content and sterilizing power because high pressure makes it possible for steam to attain a high temperature.[3]

Steam instantly condenses to water upon contact with a cooler surface and produces a replacement in the form of folds, so by reducing the amount of steam, steam can permeate things with cooler temperatures. As a result, the condensation points experience negative pressure, which attracts more steam for additional condensation. This condensation continues so long as the temperature at the condensing surface is less than that of the steam present until temperature equilibrium is obtained; a saturated steam environment is formed.

Steam is one of the most efficient heat transporters since it can carry more heat when there is more moisture present. By triggering the coagulation of essential protein structures, including the nucleus and cytoplasmic membrane, moist heat kills microorganisms by rendering the cell non-viable. Temperature and time of heat exposure affect the rate of bacterial cells being thermally inactivated.[4]

Materials that can survive moisture and high temperatures are sterilized using steam. Since steam is simply water in a vaporized condition, it is non-toxic, generally accessible,

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and manageably simple. It's important to have a solid grasp of the fundamentals of steam sterilization in order to avoid mistakes that could result in nonsterile load items, poor equipment performance, harm to employees, decreased productivity, increased operation and maintenance expenses, and damaged load items. Numerous uses for steam sterilizers exist in the pharmaceutical and medical device sectors[5]. Steam sterilization cycles consist of three phases:

1. Pre-conditioning: Air is removed from the sterilizer chamber and the load is humidified using alternating vacuum and pressure pulses during this phase,
2. Exposure: The chamber temperature is raised and held at the programmed sterilizing temperature for the programmed exposure time (both are user selectable) during this phase. The exposure also may be controlled by accumulated F0 for liquids if a load probe and appropriate sterilizer controls are used.
3. Post-Condition: Dry goods load is cooled and dried or a liquids load is cooled during this phase. The chamber pressure is brought into the atmosphere.[6]

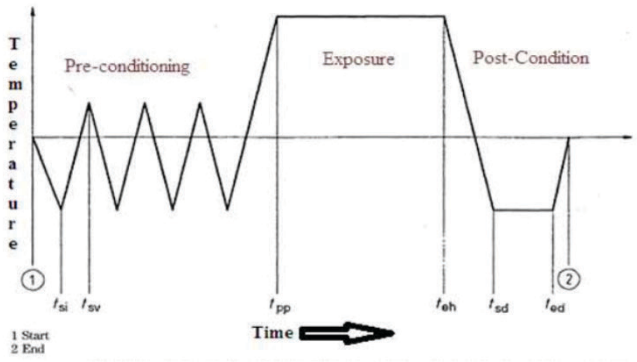

Figure 1: Steam sterilization cycle.

The most effective and efficient method of sterilization is the autoclave. Time and temperature have a linear relationship in all autoclaves. These two variables must be established through validation because they are crucial. A higher temperature guarantees 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. Surgical dressings, glassware, a variety of microbiologic media, liquids, and many other items can all be sterilized with an autoclave. No living organisms will survive a trip through an autoclave when the right circumstances and amount of time are used [7].

Some critical factors that are used to assure successful steam sterilization are as follows:

a) Time
b) Temperature
c) Moisture
d) Pressure
e) Direct steam contact
f) Air removal
g) Drying[5]

The process of microbial degradation is identified by "D" and "Z" values. A D-value is a 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 about thermal resistance are F-values and F0-values. An F0-value is the number of minutes to kill a specified number of microorganisms with a specified Z-value at a specific temperature. An F0– 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). [8]

QUALIFICATION APPROACH

User Requirement Specification (URS)

The user's requirements for output and product quality are specified in the user requirements specification. Equipment, facilities, utilities, and system specifications should be described in a URS and/or functional specification. The essential elements of quality need to be built in at this stage and any GMP risks moderate towards an acceptable level. Throughout the validation life cycle, the URS should serve as a point of reference.[9][10][11]

1. Design Qualification (DQ)

The documented verification that the proposed design of the facilities, systems, and equipment is suitable for the intended purpose. In this qualification, it needs to be demonstrated that the design complies with GMP. To achieve GMP objectives for equipment, design principles should be followed. It is important to look over the mechanical drawings and design features that the equipment manufacturer has provided.

Establishment based on background information and equipment application descriptions. Select the techniques and also types of equipment, utility, and applicable measuring devices with appropriate rationale or justification. Design Qualification (DQ) shall be provided by the vendor. The necessities of that user requirement specifications must be certified for the duration of this design qualification.[11]

Factory Acceptance Test

The Factory Acceptance Test (FAT) should be an important part of the manufacturing quality assurance program of the vendor. The FAT activities are conducted at the vendor facility to demonstrate that the equipment is constructed according to the design specifications and performance is in conformance with the functional requirements and user requirements.[12][13]

Site Acceptance Test

The site acceptance test (SAT) is a series of tests that are performed as part of commissioning after the unit has been installed in the final location. These documented tests establish the basic acceptability of the system as connected to the site utilities. During SAT, users should ensure that no damage occurred during shipment and installation, that the installation was performed properly, and that the unit is installed and sealed to maintain environmental conditions. [12][13]

FAT and SAT contain verification for the following concerning design qualification.

2. Installation Qualification (IQ)

The documented verification that the equipment and system as installed or modified, comply with the approved design and the manufacturer's recommendations. The establishment of purposes confirmation with all major features for the installation of process equipment as well as auxiliary systems are consistent with the manufacturer's authorized specifications and that the equipment supplier's recommendations have been adequately measured.[6]

The following main points should be included in the installation qualification:

- Checking of installation of equipment, piping, services, water system, and instrumentation.
- List of the operating guidelines, maintenance needs, and calibration specifications for the supplier.
- Verification of materials of construction.
- Sources of spares and maintenance.[9]

3. Operational qualification (OQ)

The documented verification that the facilities, systems, and equipment, as installed or modified, perform as intended throughout the anticipated operating ranges. Operational qualification should follow IQ. [9][13]

OQ should include the following:

- Tests developed using knowledge of the equipment, systems, and processes.
- Establishing lower and higher operating limitations. These are sometimes referred to as "worst-case" scenarios.

4. Performance qualification (PQ)

"It is a documented verification that the equipment and ancillary systems as compared together can perform effectively and reproducibly based on an approved method and specification." PQ is the process of gaining confidence that a process is effective and repeatable and that it is by the design requirements. Documented evidence that the equipment operates in your facilities exactly as intended is called performance qualification. This is done by confirming that the equipment is appropriate for the work at hand and the actual operating circumstances of the environment. Performance qualification examines the equipment's important parameters using appropriate test techniques. Test specifications are used to document these procedures. However, performance qualification is to be performed for all the process equipment and the critical equipment.[13][14]

Performances Evaluation Test

The different tests followed for qualification of autoclave are

1. Steam quality test

The objective is to determine that the pure steam supply to the steam sterilizer meets the specified acceptance criteria.

A. Physical test for pure steam

a. Non condensable gases test:

The non-condensable gases test is used to show that the amount of non-condensable gases in the steam won't prevent any part of the sterilizer load from reaching the condition. Gases released by the condensation of steam are known as non-condensable gases. The source of such gases is usually from the steam generator feed water and the impact of such gases that they modify the steam from being pure water vapor to a mixture of steam and gas is therefore an unwanted containment. The test method given should be viewed as a way to determine conformity with the specified acceptance criteria rather than as a way to precisely measure the level of non-condensable gases during regular sterilizer use.

Acceptance criteria:

The percentage of non-condensable gases present in pure steam should not exceed 3.5%.[15][16]

b. Test for Superheat

The superheat test is used to show that there is enough moisture suspended in the steam from the service supply to avoid it from overheating when it is expanded into the sterilizer chamber. A low-volume sample constantly drawn from the Centre of the steam service pipe is used in the test procedure outlined. Since condensate running along the inside surface of the pipe is not collected, the degree of superheat calculated by this method cannot be taken as an accurate representation of how dry the steam in the pipe is not collected. However, the steam delivery system to the sterilizer chamber incorporates components intended to separate free condensate, thus the level determined by this method is typical of steam conditions that are expected to exist inside the sterilizer chamber during the plateau period.

Acceptance criteria:

The superheat measured in pure steam at atmospheric pressure shall not exceed 25 °C. [15][16]

c. Dryness Value:

Steam sterilization requires a steady stream of saturated steam. Although lack of moisture in suspension cannot prevent the steam from overheating as it expands into the sterilizer chamber, too much moisture can result in damp loads. A measured value of 0 denotes 100% water and a value of 1 represents dry saturated steam, that is to say, steam as a vapor having no entrapped water. Therefore, steam with a dryness fraction of 0.95 will be a mixture of 95% dry saturated steam and 5% water. The test procedure described must be viewed as a means of proving the supply of appropriate steam quality rather than as a method for determining the actual amount of moisture in the steam.

Acceptance criteria: Dry saturated pure steam cannot have a dryness value below 0.95 or above 1.0. [15][16]

B. Chemical Analysis of Pure Steam Condensate:

Acceptance Criteria:

- pH of pure steam samples shall be between 5.0 to 7.0
- Pure steam condensate conductivity measurements must not exceed 1.3 s/cm.
- TOC analyzed of pure steam condensate shall not be more than 500 ppb.[6]

2. Vacuum leak test

Objective: To monitor for leakage in the sterilization chamber when the vacuum is being maintained during an empty sterilization chamber.

Principle: These tests are designed to show that the sterilizer chamber does not leak in the empty chamber. Leakage of air into the chamber is not acceptable for two reasons:

- Sterilization is prevented by air because it stops the sterilant (steam) from penetrating the load.
- There is a possibility that the load could get contaminated if air leaks into the chamber during the sterilizing and drying cycle because it is not being passed through the bacteria-retentive filter. When all the valves leading to the chamber have been closed and the vacuum source has been isolated, the test is conducted by determining the change in vacuum in the chamber.

Procedure: Make that the chamber temperature is constant at room temperature, that compressed air is on with high pressure, that the gaskets are properly lubricated, and that the switch on the panel board is working. Start the vacuum leak rate test cycle, check the steam sterilizer's pressure gauge to determine how much pressure is there, and then let the pressure fall naturally. The device will shut down all of the chamber's valves, halt the vacuum pump, and record the time and pressure (P1). Note the pressure once more after waiting 5 minutes (or 10 seconds) for it to stabilize (P2) Wait for another 1minuteste (± 1secondsnd) and then record the pressure once more (P3).Return to normal atmospheric pressure and go on for one more cycle, during which the vacuum leak rate shouldn't exceed the acceptable limits.[17]

Acceptance criteria: The recommended vacuum leak rate is NMT 0.013 bar/10 minutes.[6]

3. Bowie- Dick test

Objective: To facilitate the event and rapid steam penetration into all parts of the load, it is important to make sure that the vacuum pulses applied before the sterilization hold period are sufficient to remove any trapped air or non-condensable gases. This condition must be maintained throughout the sterilization holding period.

Principle: Sterilization is accomplished by quickly and evenly distributing steam across the entire load and maintaining these conditions for the specified holding period. To do this, it is crucial to remove air from the chamber and load and to supply steam that has a low concentration of non-condensable gases. The Bowie Dick test checks for the presence of non-condensable gases in the chamber to determine whether or not steam penetration is occurring, but it cannot determine whether or not the sterilizing condition in the load has been met.

Procedure: Just above the drain point, place the Bowie Dick test paper on the sterilizer's bottom shelf (100mm over the drain) The Bowie Dick test paper will be placed in the empty chamber to conduct an air removal study. It includes an indicator sheet and a pack of standard paper. 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 uniform color change. Given that Bowie Dick test paper is made to resemble a garment pack, used to evaluate the effectiveness of the steam sterilizer's air removal When performing the first three cycles of the air removal research, use brand-new indicator paper. The Bowie Dick test cycle must be used to conduct this test. The sterilization cycle must include a 17-minute sterilization time at 121°C to 123°C to meet the maximum exposure requirement.

Acceptance criteria:

After the cycle, the Bowie dick indication should display a consistent color change (from yellow to brown/black). Inadequate air evacuation from the sterilizing base chamber is indicated by no change, no change in consistency, or an area on the test sheet with air entrapment (bubbles).[18]

4. Heat distribution study (empty chamber)

Objective: To identify the cold point in the empty chamber and to confirm that the temperature is constant throughout

the chamber. During the sterilizing hold period in the Empty Chamber, the sterilizer can reach a temperature of 121° C.

Procedure: Several temperature sensors should be placed within the chamber through the sterilizer's validation port. To prevent steam leaks during sterilizer operations, plug the port with silicon sealant. Fix each probe in a distinct spot in the sterilizer so that sensors don't come into contact with the chamber's metallic surface. The data logger, which can scan and print the actual temperature and pressure at various locations, should be connected to the temperature sensors. Check the thermograph in the data logger to make that the sterilizing hold period's predetermined temperature and pressure were reached after the sterilization cycle has finished. Repeat the cycle after making the appropriate corrections if any deviations are noticed.

Acceptance criteria: Temperature distribution within the chamber must be between 121°C to 123°C at all locations during the sterilization period (dwell time) There should not be any slowest heating point (cold spot) in the autoclave chamber and equilibrium time should not be more than 30 seconds. [19][20]

5. Heat distribution study (loaded chamber)

Objective: To identify the cold point in the loaded chamber and to confirm that the temperature is constant throughout the chamber. During the sterilizing hold period in the loaded Chamber, the sterilizer can reach a temperature of 121° C.

Procedure: Several temperature sensors should be placed within the chamber through the sterilizer's validation port. To prevent steam leaks during sterilizer operations, plug the port with silicon sealant. Fix each probe in a distinct spot in the sterilizer so that sensors don't come into contact with the chamber's metallic surface. Each loading pattern must have its own loaded chamber heat distribution study completed. Each loading type must go through one cycle in the loaded chamber heat distribution investigation. 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 sterilizing hold period's predetermined temperature and pressure were reached after the sterilization cycle has finished. Repeat the cycle after making the appropriate corrections if any deviations are noticed.

Acceptance criteria: Temperature distribution within the chamber must be between 121°C to 123°C at all locations during the sterilization period (dwell time) There should not be any slowest heating point (cold spot) in the autoclave chamber and equilibrium time should not be more than 30 seconds. [19][20]

6. Heat penetration test

Objective: To ensure that the Steam sterilizer meets the temperature profile requirement and sterility assurance requirements during the sterilization for the various load patterns. The Steam Sterilizer under validation is subject to the test.

Principle: To identify the cold spot that is any location within the load where the temperature sensor is placed achieving minimum sterilization temperature throughout the sterilization hold period. The temperature spread is within the range of 121.1°C to 124.0°C during the 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 a lag period for attaining 121.1°C during heat penetration trials as the probes are placed deep into the load.

Procedure: Insert temperature sensors inside the chamber through the validation port of the sterilizer. Seal the port with silicon sealant to ensure that no steam leakage during the operations of a sterilizer. Fix all the probes at different locations in the sterilizer so that sensors do not touch the metallic surface of the chamber. In the autoclave chamber, load the object according to the loading pattern. For each loading pattern, a separate loaded chamber heat distribution study should be carried out. Set the HPHV Steam sterilizer and data logger for each run by connecting the pre-calibrated temperature sensors to the data logger and fixing the probes to the sterilizer chamber with the help of "the validation port" provided.

After completion of the sterilization cycle, stop the Data logger and open the non-sterile side door, take out the biological indicators from the load into a sampling bag with a proper identification number and send it to Microbiological Lab, with a duly filled analysis request form for testing of recovery study. Check the temperature profile from the Sterilizer chart recorder and equipment printout and attach it with the qualification report.

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

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

Acceptance criteria:

- The equilibration period shouldn't last longer than 30 seconds.
- The temperature should range from 121.3 °C to 124.0 °C during the sterilizing holding period of 30 minutes.
- During the sterilizing holding period, the temperatures should not vary from one another by more than 2°C.
- The F0 value for each sensor in the data logger should be more than 30 minutes.
- Throughout the sterilizing holding period, the chamber pressure should be measured between 1.00 bar and 1.30 bar.
- The biological indicators should indicate that sterilization is complete (i.e. no growth after defined incubation).
- Growth should be monitored for effective control.
- Upon visual inspection of the load following sterilization, there must be no signs of moisture or condensation.[21][22][23]

CONCLUSION

One of the core ideas of cGMP is qualification. where rubber stoppers, cleaning aids, garments, filters, utensils, vial filling machine parts, and other items are sterilized in an autoclave. Following this, the equipment had to be qualified, which included a comprehensive variety of evaluations, including the vacuum leak test, the Bowie-Dick test, the heat distribution study (with an empty and loaded chamber), and the heat penetration test. The acceptance criteria were found to be satisfied by all the factors and processes that are described. Autoclave is therefore regarded as qualified and appropriate for routine use.

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