Review article

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Critical review on qualification of sterilization equipment in aseptic processing

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Abstract

Aseptic filling is an aseptic process that requires the close coordination and complex interaction between personnel, sterilized product, the fill/finish equipment system, clean room and support facilities and sterilized filling components. Aseptic manufacturing means drug substance and excipients were sterilized appropriately and all materials, equipment and container closure systems were used only after sterilization. Regulatory GMP for the aseptic manufacture of human and veterinary products mandate that an incidence involving product sterility failure or media fill contamination is fully investigated and also manufacturer establishes an environmental monitoring program that is properly validated to ensure that environmental contaminates are detected. The stability of the aseptic filled drugs will be affected by steam (autoclave), dry heat (depyrogenation tunnel) and rubber stopper. Hence there is a need to utilize an aseptic process to fill certain biological, pharmaceuticals and biotechnology drugs.

Keywords: Keywords: Qualification, depyrogenation tunnel, autoclave, rubber stopper washer.

INTRODUCTION

Definition of Sterilization

Sterilization can be defined as any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses) from surface, equipment, foods, medications or biological culture medium.[1] A sterility assurance level (SAL) of 10^{-6} means that there is less than or equal to one chance in million that particular item is contaminated or unsterile following sterilization process.[2,3] These events raised awareness across first regulatory agencies, but soon after among companies that something was seriously amiss in the production of sterile product. But other in United Kingdom speaks of something new "validation". "Validation is the attaining and documentation of sufficient to give reasonable assurance, given the state of science, which is the process under consideration, does, and/or will do, what it purport to do". This definition has changed minimally over the year and prevailing USFDA definition in use today is quite similar. "Validation is establishing documented evidence which provided a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specification and quality characteristics" [4]

Need and importance

1. The proper sterilization of medical devices, surgical instruments, supplies and equipment utilized in direct patient care and surgery is a critical aspect of the modern health care delivery system and directly impacts patient safety
2. Sterilization is very important in the medical industry. Without sterilization, infections would fly around and thousands of lives would be lost. Sterilization helps to prevent the development and spread of infection.
3. In the pharmaceutical industry it used for surgical dressings, sheets, surgical and diagnostic equipment, containers, closures, aqueous injections, ophthalmic preparations and irrigation fluids etc.
4. It is generally accepted that sterility assurance level (SAL) of 10^{-6} is appropriate for items intended to

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come into contact with compromised tissue, which has lost the integrity of natural body barriers.[5]

Sterilization methods

Dry heat sterilization

Heat is most reliable and rapid method of sterilization. The killing of dry heat is due to protein denaturation, oxidative damage and toxic effect of elevated level of electrolytes. Microorganism is more resistant to dry heat as compared to moist heat. So, this process requires higher temperature and longer exposure time. This method of sterilization can be applied only to thermo stable products but it can be used for moisture-sensitive materials for which dry heat (350-360^{0}C) sterilization.eg: Hot air oven.

Moist heat sterilization

Sterilization by moist heat means killing of microorganism with hot water or steam. The lethal effect of moist heat is due to denaturation and coagulation of protein. Moist heat sterilization involves the use of steam in the range of 121-124^{0}C.eg: Autoclave

Chemical sterilization

The chemically reactive gases such as formaldehyde (HCHO) and ethylene oxide $(CH_2)_2O$ possess biocidal activity. Ethylene oxide is colourless, odourless, and flammable gas.

Radiation sterilization

Many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light) particulate radiation (e.g. accelerated electrons).

Filtration

Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles.[2,3]

Responsibility of department

Production

Ensures implementation of BPR and protocol, sample withdrawal and sending to QC for testing as per specification. Ensures all the equipment used during process validation was documented in respective equipment usage log.

Validation

Prepare the protocol and review

Quality Assurance

Review and approval of protocol report and raw data generated were in accordance with protocol and expected result achieved.

Quality control

Testing of sample

Basic Qualification approach

User requirement specification (URS)

User or customer of equipment has certain expectation about the equipment which wants to use. These expectations are generally in the form of his requirements. It is called as user requirement specifications.

Design qualification (DQ)

Design qualification may verify that design of equipment, system/facility is according to requirement of user and current good manufacturing practices.

Installation qualification (IQ)

Installation qualification is conducted to prove that equipment/system has been installed as per user and manufacturer recommendation and verifying that all required utilities have provided safe operation of equipment/system.

- Utilities specification
- Drawing specification(electrical, mechanical)
- Construction material in product contact
- Operating and maintenance manual

Operational qualification (OQ)

The operational qualification process is intended to demonstrate that the components are operating properly and ready for performance or load testing. Operational qualification shall be done “without load”

Performance qualification (PQ)

Performance qualification is documented evidence to prove that equipment/system is performing under specified condition. It involve in taking trial under "loaded condition"[6].

Qualification of equipment

Steam Sterilizer (Autoclave)

Steam sterilizer sterile the article using saturated steam and equipped specifically for application in the pharmaceutical field. Sterilizer is an effective equipment to ensure the sterilization of the garment, cleaning aids filter, utensil, vial filling m/c parts, rubber, etc. The set of main activities for treating the processed materials in order to sterilize them is termed as sterilization process/cycle [Figure 1 and.2].

Figure 1: Autoclave

The following main activities can be distinguished in the sterilization process

- Autoclave preparation
- Load sterilization
- Load cooling
- Final phase

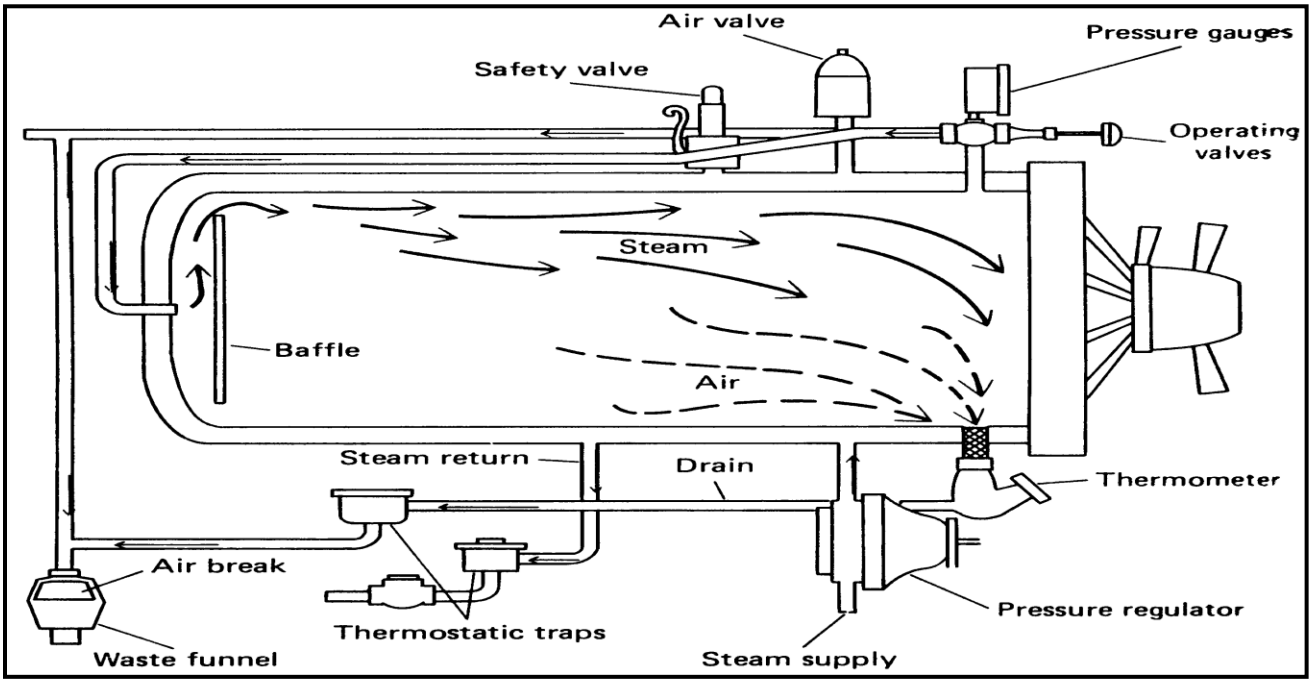

Figure 2: Steam Flow

Performance of autoclave

It involve in taking trial under "loaded condition". The calibration of instruments, apparatus carried out at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision. Operating range, approval standard operating procedure is used for verification. Performance qualification is integrate, procedure, personal, system and material verify pharmaceutical grade utility, and environment, equipment, and system produce required output[7, 8].

Different tests are follows

Vacuum leak test

Objective

To prepare master chart of sterilization cycle for reference during normal production cycle and demonstrate that autoclave is leak proof and there no leakage from the chamber.

Procedure

- Ensure that the chamber temperature is stable at ambient and compressed air is on at pressure of 6 bar
- Ensure gasket lubrication is proper and switch provided on panel board
- Start the vacuum leak rate test cycle
- Observe the pressure in the pressure gauge of steam sterilizer
- Cycle allow the pressure to drop up to 450-470 mmHg
- Machine will close all the valves connected to the chamber and stop the vacuum pump and note the time and pressure (P_1)
- Wait for stabilization period of 5 minute (±10 second) and note down the pressure again (P_2)
- Wait for another 10 minute (± 10 second) and note down the pressure third time (P_3)
- Return to atmospheric pressure and continue to run for next cycle of Bowie Dick test.

Acceptance criteria

The vacuum leak test should not more than 1.0 mmHg

Air removal test (Bowie Dick test)

Objective

If commercially available Bowie-Dick test pack is used, make sure that all tests are conducted with test pack of the same manufacturer's specification. If you are at this point and have not identified the source of the problem, inform and request assistance in resolving the problem by contacting the sterilizer manufacturer, the maintenance or service provider, the test pack manufacturer and facility engineering department.

Procedure

- Load the tray in the autoclave chamber
- Place the bowie dick test pack on the bottom shelf of the sterilizer just above drain point (100mm over the drain)
- Air removal study shall be performed in empty chamber by placing the Bowie Dick test kit. This shall be reusable or disposable Bowie Dick test kit manufactured by standard manufacture. it consist of standard paper pack and indicator sheet
- Start the cycle by pressing enter key
- After the cycle is over open the door from control area side and take the sterilized test pack from the autoclave and check the indicator paper/strip for uniform colour change
- As Bowie Dick test kit is designed to simulate the garment pack, it used to test the efficiency of the air removal from the steam sterilizer
- Three cycle of air removal study shall be performed (initially) by using fresh indicator kit pack and only one cycle during re-validation
- This test shall be performed by using Bowie Dick test cycle. To fulfil the maximum exposure requirement, the sterilization cycle shall have 10 minutes at 121^0C to 123^0C sterilization period.

Acceptance criteria

The indicator paper/sheet should be uniformly changed in colour (brown) over the entire pattern of indicator sheet when compared with control.

Heat distribution study (empty chamber)

Objective

The chamber provides a uniform sterilizing environment and verifies the temperature stability.

Procedure

- Connect the thermocouple with SIM to the kaye validator and distribute these thermocouple throughout chamber
- Pass the probes into the chamber through validator port by using Kaye 'feed thru' and seal the port properly to avoid any steam leakage
- Place the external temperature sensor, one each, geometrically and equally distributed in the sterilizer chamber

- Fix 10 pre-calibrated thermocouple (type T) with in chamber in different location for mapping the distribution temperature with in empty chamber
- As in-built control temperature sensor is fixed, locate the external temperature sensor at the pre-determined location
- Theoretically the drain point is considered to the coldest point because steam condensate and air is removed through this point
- It shall ensure that temperature probes/sensor do not touch any metal surface or walls of chamber. In case sensor touches the metal surface, it may show steam sterilizer body part temperature, and not the chamber temperature. And set the data logger
- Start the data logger and steam sterilizer simultaneously. Stop the data logger when the entire sterilization cycle of the sterilizer is over
- Check both the profile against the acceptance criteria for compliance
- Change the location of the remaining external temperature sensor to different location to cover the maximum area
- Attach the temperature profiles of data logger and in-built temperature recorder of all run in report.

Acceptance criteria

Temperature distribution within the chamber must be between 121^0C to 123^0C at all location 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 second.

Heat distribution study (loaded chamber)

Objective

It identifies the cold spot in the loaded steam sterilizer

Procedure

- Connect the thermocouple with SIM to the kaye validator and distribute these thermocouple throughout chamber
- Pass the probes into the chamber through validator port by using Kaye 'feed thru' and seal the port properly to avoid any steam leakage
- Fix minimum 10 pre-calibrated thermocouple (type T) with in chamber in different location for mapping the distribution temperature with in loaded chamber
- Load the article as per loading pattern in the autoclave chamber
- Loaded chamber heat distribution study shall be performed separately for all loading patterns. One cycle shall be performed for each loading type for loaded chamber heat distribution study
- Place the external temperature sensor in the chamber as far as possible, near to the location as in the empty chamber heat distribution study trials at the outside of article
- It shall be ensure that temperature probes/sensor do not touch the object surface or walls of chamber. The probes shall remain suspended in the chamber
- One external temperature sensor shall be placed at identified cold point as per empty chamber heat distribution
- One external temperature sensor shall be placed near inbuilt drain point temperature sensor and set the data logger
- Start the data logger and steam sterilizer simultaneously. Perform the cycle as per set parameter and stop the data logger when the entire sterilization cycle of the sterilizer is over then check both the profile against the acceptance criteria for compliance

Acceptance criteria

Temperature distribution within the chamber must be between 121^0C to 123^0C at all location 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 second.

Heat penetration study

Objective

In order to verify sterilizing temperature has been reached in each load subjected to moist heat sterilization, it is necessary to conduct heat penetration studies. This study is conducted to ensure that the coolest unit within a pre-defined loading pattern (including minimum and maximum loads) will consistently be exposed to sufficient heat lethality (minimum "F_0").

Procedure

- Pass the probes into the chamber through validator port by using Kaye 'feed thru' and seal the port properly to avoid any steam leakage
- Fix minimum 10 pre-calibrated thermocouple (type T) with in chamber in different location for mapping the penetration temperature with in loaded chamber
- Place the external temperature sensor one each inside the object. Location of different external temperature sensor shall be changed in each cycle
- Three cycle of heat penetration study should be performed for all loading pattern (initially or any new loading pattern introduced) and in case of revalidation one cycle shall be performed. Separate set of cycle shall be performed for each loading pattern as applicable
- Arrange the load as per specified diagram. At least 15 biological indicators and 10 thermo chemical indicators shall be used for each cycle. If number of object is less than 10 then number of biological indicator shall be equal to number of object
- Place thermo chemical indicator 3m type (mark with detail) biological indicator (*Geobacillus stearothermophillus* ATCC 7953) strip/ampoule (only ampoule used in water for injection load) having spore population of minimum 1.0×10^6 placed in the middle of sterilizing material along with external temperature sensor. Load placed at the identified cold points must have indicator and temperature sensor in all three runs. One biological indicator and thermo chemical indicator along with external temperature sensor shall be placed at drain point in all three cycle (at the time of initial validation or when any new load pattern introduces)
- Place one control probe on the identified cold spot of heat distribution study
- Perform the sterilization by operating the program specified for each load type as per standard operating procedure
- Start the data logger and steam sterilizer simultaneously
- After the sterilization cycle is completed, stop data logger and open the sterilizer
- Take out challenge biological indicator and thermo chemical indicator from load and send to microbiological lab for testing
- Challenge biological indicator shall aseptically inoculate into sterile soyabean casein digest media (SCDM) and incubated at 55 - 60^oC and liquid load at 35 - 39^0C for 7 days
- Check the thermo chemical indicator for the compliance as per manufacturer recommendation for colour change (i.e. Brown)
- Take out temperature chart/data logger and inbuilt temperature recorder, report of biological indicator. Check against acceptance criteria for compliance
- Determine the F_o value and compare against acceptance criteria. Take out external temperature sensor from the chamber and perform vacuum leak rate test.

Acceptance criteria

Temperature distribution within the chamber must be between 121^0C to 123^0C at all location during the sterilization period (dwell time)

Sterilization temperature should be maintained for NLT 15 minute for minimum 10 thermo couple during hold time. Biological indicator (Geobacillus stearothermophillus) should show complete sterilization (i.e. no growth after incubation)[9,10,11,12].

Depyrogenation tunnel (Sterilization tunnel)

Depyrogenation can be defined as the elimination of all pyrogenic substances, including bacterial endotoxin and is generally achieved by removal or inactivation. The sterilization tunnels have been designed to continuously sterilize dehydrogenated glass vial containers in a class 100 and environment. The tunnel is comprised of the In feed chamber, Sterilizing chamber and Cooling chamber. In feed chamber (where the vials enter) creates thermal barrier between the vial washing room and the sterilizing chamber to protect the vials from contamination and to pre heat the vials. The vial is heated up to 85-90^{o}C. Sterilizing chamber is responsible for sterilization and depyrogenation. The sterilizing chamber is fully insulated and can be heated up to temperature 350-390ºC for (3-4 min). Heaters generate heat, the sterilizing time of the vials depends upon the temperature and air velocity. If the temperature is high and air velocity is available as required then less time will be required for sterilization. After passing through the conveyor, the air taken up by fan with continuous speed adjustment to required f_H value.

Process for flow of sterilization tunnel

Washed glass vials

↓

Drying zone

↓

Sterilizing zone

↓

Cooling zone

↓

Filling table

The cooling chamber, depending upon the tunnel size, comprises of one or two cooling coils, which assist in the cooling of the vials to ambient temperature. Regular chilled water may be used, vials stay in the cooling chamber for 15-20 minutes. [13,14,15]

Different tests are as follows

Air velocity

Objective

To determine that factors that affect cross-sectional air velocity distribution in tunnel-ventilated system and is capable of delivering air velocities, as per the requirement to maintain continuous laminarity of HEPA filter installed in tunnel.

Figure 3: Anemometer

Procedure

- This test shall be performed by trained person and training record should be attached in report. Performed at least 30 minute.
- Measure the velocity above the conveyor for the different zone of tunnel sterilizer and measure the air velocity 6 inches below filter.
- Take the velocity of air at five locations (on centre and four corners) of each zones of sterilizer tunnel. Calculate average velocity for each filter
- If velocity is not within the limit, inform the manufacturer of the sterilizing tunnel for corrective action.

Acceptance criteria

Air velocity should be maintained within 90 fpm ± 20% of mean unit velocity for even distribution of temperature.

HEPA filter integrity test

Objective

To verify the integrity of HEPA filter installed in the sterilization and depyrogenation tunnel. HEPA filter installation has been done properly and qualifies the filter integrity test.

Figure 4: Aerosols Photometer

Equipment

Aerosol generator

Aerosol photometer

Procedure

- Place the aerosol generator to introduce an aerosol challenge upstream of the HEPA filter in zone wise manner in concentration of 80-120mg/m^3 of air by opening appropriate number of nozzles
- Measure upstream concentration of aerosol by using zone wise upstream (in feed zone, hot zone 1, hot zone 2 and cooling zone)
- Adjust the photometer gain/span control for full scale deflection on 100% range
- Scan the downstream side of the HEPA filter, its perimeter, the seal between the filter frame and grid structure including its joints using overlapping strokes with the photometer probes
- The photometer probes should move at transverse rate not more than 10ft/minute with sample flow rate of 1cft/min ±10%
- If any leak is more than the specified limit, the above test should be repeated after taking the recommended corrective action

Acceptance criteria

Photometer reading downstream of the HEPA filtration unit caused by the leakage should be less than 0.01% of the upstream challenge concentration of the aerosol 100%.

Air flow visualization study

Objective

To determine the air flow pattern of HEPA filter installed in sterilization and depyrogenation.

Procedure

- Take the glass rod with cotton or sponge tied to it
- Take a smoke pencil/titanium tetrachloride swab and bring it near to infeed of tunnel sterilizer.
- Check the air flow direction at the downstream of the filter face
- Observe and record the same with video camera

Acceptance criteria

The direction of flow should be from;

In- feed area to vial washing zone

Away from all the opening i.e. from the sterilization tunnel to washing area

From sterilization zone to in feed/drying zone

From sterilization zone to cooling zone

Vial filling room to the cooling chamber of sterilization tunnel

Non-viable borne particulate count

Objective

To establish that at different location within the tunnel, count size of particle per cubic meter is within the limit.[Figure 5].

Figure 5: Particle Counter (Climet)

Procedure

- The particle count test should be performed by qualified or trained person
- Start blower of the sterilizing tunnel
- Calculate the number of location by the following formula
- Number of sampling location $N_L = \sqrt{A}$
- Whereas; the minimum number of sampling locations
- Switch on particle counter and place the iso-kinetic suction probes at specified location under

the filter of conveyor belt of tunnel and observe the reading, record in reports

- Take the particle counts for all zones of sterilizing tunnel

Acceptance criteria

The particle counts taken under the HEPA filter in the different zones of sterilizing tunnel should meet the requirement of ISO 5/class A.

Grade/class	Maximum permitted no. of particle/m^3	
	0.5µm	5µm
A	3500	20

Heat distribution study (empty tunnel)

Objective

The sterilizing and depyrogenation tunnel when operated with empty chamber is capable of producing the temperature profile as per temperature set point set in PLC of the equipment. The temperature distribution is uniform throughout the sterilization period.

Procedure

- Place 10 temperature indicating probes across the width of the conveyor of an empty tunnel, in such a way that probes junction do not touch solid surfaces as to determine the air temperature profile
- Use zig to hold the probes in place as the probes travels through the tunnel
- Attach the connecting cable of probes to data logger, which can scan the date, time and temperature of probes at every 10 seconds
- Set the temperature/cycle condition as per set parameter
- Record the set parameter for the sterilization cycle operated during test
- Operate the tunnel as per the standard operating procedure and start the data logger to record the actual temperature within the sterilization zone of tunnel by passing the zig plate holding the external temperature sensor through the sterilization zone
- When the probes cross the sterilization zone stop the conveyor belt of sterilizing tunnel, switch off the data logger and pull out the probes
- When the validation is completed, collect the batch report of sterilizer
- Record the observation from the data logger in to the computer and take printout for analysis of the data collection. Also record temperature observation at different location
- Report the time for each probes for which the vial are exposed to temperature of 300^oC or above

Acceptance criteria

The temperature at each temperature probes should be ≥ 300^0C during the cycle.

Heat distribution study (loaded chamber)

Objective

The sterilizing and depyrogenation tunnel when operated with empty chamber is capable of producing the temperature profile as per temperature set point set in PLC of the equipment. The temperature distribution is uniform throughout the sterilization period

Procedure

- Place 10 temperature indicating probes across the width of the conveyor of tunnel, in such way that probes junction do not touch solid surfaces but remain in hanging condition inside the vials
- Use zig plate to hold vial with the probes (sensor) in place as it help smooth travel through the tunnel
- Attach the connecting cable of probes (sensor) to data logger, which can scan the date, time and temperature of probes at every 10 seconds
- Set the temperature/cycle condition as per set parameter
- Record the set parameter for the sterilization cycle operated during test
- Operate the tunnel as per the standard operating procedure and start the data logger to record the actual temperature within the sterilization zone of

tunnel through probes (sensor). Allow the zig plate having vials containing the external temperature probes (sensor) to travel along with other washed vials through the sterilization zone. Operate vial washing machine continuously to fill the tunnel chamber back to back

- When the vials having probes cross the sterilization zone stop the conveyor belt of sterilizing tunnel, switch off the data logger and pull out the probes along with zig
- Take one validation run for loaded chamber heat distribution studies for each type of load.

Acceptance criteria

The temperature at each temperature probes should be ≥ 300^0C during the cycle.

Heat penetration studies

Objective

To ensure that heat is sufficiently penetrating into the inner most portion of the vial subjected for sterilization and depyrogenation to achieve desired temperature during the sterilization and depyrogenation cycle. The recovery of endotoxin concentration after exposing to depyrogenation tunnel should show more than 3 log reduction.

Procedure

- Get the 9 spiked vial with approx. 10,000 EU/vial of bacterial endotoxin from microbiology
- Place minimum 10 number of probes, one probe each inside the endotoxin spiked 8 vials and 3 without spiked vials at the junction of the bottom of the container and side wall. The containers inner surface should be in contact with the probe because for sterilization and depyrogenation of the inner walls of the container as well as inner space. Tie the probes firmly with the vial and place these vial inside the washed vial load
- Use zig to hold the spiked vials containing probes in place, as vial travel through the tunnel
- Set the temperature/cycle condition as per set parameter
- Record the set parameter for the sterilization cycle operated during test
- Operate the tunnel and pass the endotoxin spiked vials along with the washed vials as per standard operating procedure and start the data logger to record the actual temperature inside within the sterilization zone
- When the vial attached with temperature indicating probes cross the sterilization zone, stop the conveyor belt of sterilizing tunnel, switch off the data logger and pull out the probes. Wrap the exposed endotoxin indicator vials with aluminum foil and label properly
- Send the exposed vials to microbiology lab for testing of residual endotoxin in the vials after sterilization as per standard procedure
- Record the result and take validation run for each set of vial normally used in routine production with complete load and re-validation one run on rotation for different type of vial size
- Record the temperature observation at different location.

Acceptance criteria

All temperature measured in the chamber is ≥ 300^0C. The recovery of endotoxin concentration after in sterilization and depyrogenation should at least 3 log reductions [1,5,7,8].

Rubber Stopper Washer

Rubber stopper are washed to make free from particulate contamination in this machine prior to sterilization. The cycle is controlled by microprocessor through different solenoid valves, washing is done in two vessels M1 and M2 which can be programmed separately. The process cycle can be run separately or at same time[16].

Different test are follows

Removal of soluble and insoluble matter

Objective

To ensure that the vial washing machine meets the acceptance criteria for washing efficiency when challenged with sodium chloride spiked vial.

Procedure

- Prepare 0.5% w/v slurry of sodium chloride and talc/lycopodium slurry solution by adding 5g of sodium chloride and talc/ lycopodium in 1000 ml of purified water.
- Transfer 5000 numbers i.e. 1 bag each rubber stopper in two stainless steel (SS) container
- Contaminate the rubber stopper of each container (i.e.5000) with the above 0.5% slurry.
- Allow to air dry the rubber stopper over-night.
- Transfer the rubber stopper of each stainless steel (SS) container (5000) in to washing bucket (vessel m1 and m2) of rubber stopper washing machine.
- Sample 10 rubber stoppers (contaminated, before washing) from each bucket in separate clean conical flask containing 100 ml of water for injection (WFI). This will be used for positive control.
- Run the rubber stopper washing cycle as per standard operating procedure keeping all the process parameter identical to routine washing cycle.
- After completion of washing cycle take out 3×10 washed rubber stopper each from both the washing bucket into separate clean conical flask containing 100 ml of water for injection (WFI) in each flask. These will be used as test sample after washing. Take out sample for soluble and insoluble matter
- Send all the sample to quality control lab/microbiology lab for soluble matter (sodium chloride) and insoluble particulate matter (by liquid particle counter) analysis
- Inspect the washed rubber stopper visually with magnifying glass for presence of any particulate matter

Acceptance criteria

All challenged vials shall show negative test for presence of chloride.

Endotoxin challenge test

Objective

It ensure that the rubber stopper washing machine meets acceptance criteria when challenged with endotoxin

Procedure

- Send 10 washed rubber stopper of different color to microbiology lab for inoculation spiking of endotoxin one day prior to test
- Get 7 numbers of colored rubber stopper spiked with known amount of endotoxin i.e. 1000 (I.U)/rubber stopper in properly closed Petri dish from microbiology lab, number them from 1 to 7
- Charge 5000 rubber stopper and 7 spiked rubber stoppers in each process in each washing vessel in such way that both washing bucket the spiked rubber stopper are kept at different layer respectively
- Run the washer as per standard operating procedure with all the process parameter kept identical to keep in normal production run
- After the cycle is over take out the spiked rubber stopper and send them to microbiology lab for determination of residual endotoxin

Acceptance criteria

There should minimum of 3 log reduction of endotoxin content in all spiked vials.

Steam Sterilizer (Autoclave)

All the tests were carried out as per procedure and parameter were found meeting the acceptance criteria. Hence steam sterilizer (autoclave) is successfully validated [1,4,9,10,13,14].

Depyrogenation Tunnel

All the test was carried out as per procedure and parameter were found meeting the acceptance criteria. Hence sterilization tunnel is successfully validated [1,5-8,10,12].

Rubber Stopper Washer

All the test was carried out as per procedure and parameter were found meeting it's the acceptance criteria. Hence rubber stopper washer is successfully validated. [16]

CONCLUSION

It is based on result or observation it was concluded that result of the entire equipment performance test was found meeting the acceptance limit and all load pattern. Hence steam sterilizer (autoclave) and sterilization tunnel is successfully validated. The vial washing machine has been successfully qualified for removal of soluble and insoluble matter, residual water test of washed vial and endotoxin study test. All washed vial are required for quality and hence the vial washing machine stand for validation. The rubber stopper washer has been successfully qualified for removal of soluble matter, insoluble matter and endotoxin study from washed rubber stopper when operated as per standard operating procedure and comply acceptance criteria. Hence rubber stopper washer too, successfully validated.

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