Review Article
Available online at www.ijpras.com

ISSN 2277-3657

Volume 3, Issue 4 (2014),10-15

International Journal of Pharmaceutical Research & Allied Sciences

Sterility Assurance Level and Aseptic Manufacturing Process in Pharmaceuticals

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Subject: Pharmaceutics

Abstract

Contamination of sterile areas is a concerned issue in pharmaceutical companies, it is very hard to overcome these issues if you want to get aseptic environment. Bioburden increases in a manner that microorganism travels along with dust particle and these particle destroys the aseptic environment. There are different sources through which contamination can occur in aseptic environment but the main source is the airborne particles. The predictable bacteriological value of the product can be assessed by process repetition by the containers used for filling with bacteriological medium. On the other hand, these tests are not much sensitive to indicate the level of contamination particles below 1000 and these tests are also limited in number. This paper includes the discussion about the procedure to control the contamination and the means of contamination through airborne particle of aseptic room. The discussion includes the counting of CFU by plate exposure method and fill trial test to check the level of contaminating microorganism in aseptic environment. The confidence must be found in the methods to calculate the contaminants if they are to be adopted. The methods used currently in our pharmaceuticals are lack of accuracy and they are suggested to be improved to enhance their accuracy. The company's financial ability, reputation and license to manufacture is seriously affected by contamination in aseptic area. To guarantee the proper and validated monitoring of environmental to detect contaminants, regulatory GMP codes for the manufacturing of human as well as veterinary products aseptically are provided that show the frequency of sterility failure. Autoclave, driers, Ethylene Oxide and irradiation, either with Cobalt 60 Gamma or with E Beam affects the stability of the drug filled in aseptically. Therefore the product that are either biological or pharmaceutical or it may be biotechnological product all are filled in aseptic environment.

Keywords: *Sterility Assurance, Aseptic Manufacturing, Aseptic Environment*

Introduction

The most serious process in manufacturing of pharmaceutical product is the aseptic filling of drugs. This is due to the possible safety of the end users and its complex technique to run the process. Once the drug is filled, plugged with stopper and capped in sterile area then only indirect factors can affect the sterility. Steam autoclave, Ethylene Oxide, drying ovens, and irradiation affects the stability of drugs filled aseptically which is not the case with terminal sterilization of drugs. Therefore aseptic process is used to fill many biological, biotechnology pharmaceutical drugs. The number of bacteria living on a surface that has not been sterilized is known as Bioburden. To measure the entire viable microorganism on a device before its sterilization prior to use is the main purpose of bioburden. This step is important to reduce the bioburden in the filling area. Different methods are used to sterilize the final product but its Depyrogenation is not possible so the older methods of sterilization should be reviewed. For our product to be safe for the growth of microorganism we mostly use large number o preservatives which can be hazardous to health. To reduce the contamination occurring due to the older process of producing aseptic condition many anti-fungal reagents are also used in the parenteral products. The Parenteral Drug Association (PDA) published its Aseptic Validation Technical Report in1981 to improve uniformity in aseptic processing. The Food & Drug Administration (FDA) follows with its Aseptic Processing Guidelines. The International Society of Pharmaceutical Engineering (ISPE) as component of their Guidelines Series published its Sterile Facilities 2013. The Concept paper of FDA has been published recently: Aseptic Guidelines in2013. The close management and complex communication between personnel, the finish equipment system, sterilized product, clean

room and support facilities, and sterilized filling components is required by aseptic process.

Aseptic manufacturing:-

Filtration of the solution using membrane filter of pore size (0.2 µm or less) in sterile area is done to achieve sterility. Separate sterilization of products their containers and their closure are done appropriately. Several continuous and important steps are present for these complex working processes, each step contributes its part to achieve the plan of manufacturing an aseptic product. All the physical or mechanical treatment of the sterilized drug its components and its assembly carefully controlled. Aseptic manufacturing is preferred, where the sterilization of final product in its container is not possible due to its instability against heat. Manufacturing the product aseptically means that the drug, its excipients and other materials and equipments were sterilized with proper care before using. To avoid contamination all the steps were carried out in clean areas. Hence high standards have to be made about the clean room, the equipments that were being used, the available personnel, and the supply systems (air system, WFI, sterile gases; for example compressed air, nitrogen etc.)

Aseptic Processing in Pharmaceutical Companies:-

The most important demand of pharmaceutical manufacturing is the aseptic processing. The specific attention to operator training and performance, validation of process, documentation of process involved in production, plant and maintenance of equipments is required. Regulators will attempt to make sure that the safety of the customer is never compromised. Due to the risks of manufacturing by aseptic processing and adverse reactions on the customer the increased level of regulatory inspection is attracted. The company's financial ability, status and license to manufacture are seriously affected by contamination in aseptic area/product. To ensure the proper and validated environmental monitoring program to detect contaminants , to guarantee the proper and validated monitoring of environmental to detect contaminants, regulatory GMP codes for the manufacturing of human as well as veterinary products aseptically are provided that show the frequency of sterility failure.

Sterile Product:-

Sterile products are free from microorganism and are used therapeutically. Mainly, these include parenteral, ophthalmic and irrigating preparations. From these products, parenteral products are injected via the skin or mucous membranes into body compartments are unique among all other dosage forms. Because they have bypassed the highly efficient first line of defense of the (i.e. the skin and mucous membranes) they must be free of microbial contamination and from components that are toxic to body and have a great level of purity. The possible contamination of physical and chemical microbial in origin can be prevented by selecting such method that should eliminate all kind of contamination. Various routes are available for the administration of parenteral products: Intraspinal, intramuscular, subcutaneous, Intravenous, and intradermal. No absorption is necessary when drug is injected through intravascular route in which availability occurs immediately. For the administration of drug through other routes blood vessel or other tissues may be punctured before administration.

Medium /Vehicles

Water is used as a vehicle for all sterile products as it is a naturally occurring in body fluids. The quality of vehicle that is required for use is explained in the monograph in USP. Requirements may be even more strict for some products, however one of the most important test performed for the selection is the gravimetric evaluation its dissociation of substances present in water and the total solid content level of water. The most easily performed test for the measurement of electrolyte is the conductivity of water and it is less time consuming.USP monographs also consist of other tests to check the quality of WFI. On comparing the total solid contents of WFI and sterile water for injection it is noted that higher values are permitted for sterile water for injection. Pyrogen free water is used for parenteral solutions.

Source of Pyrogen:-

Pyrogens are metabolic products of micro-organism. Pyrogens are produced by man bacteria, molds and viruses. The potent pyrogenic substances are produced by gram-negative bacteria and are known as endotoxins. Pyrogens can enter a product by different ways that may be able to introduce microorganisms or the crops of their growth. The most important source of Pyrogen is water, solutes and containers. The containers of solute which are opened invite such contamination.

Pyrogen Elimination:-

The Depyrogenation of containers can be obtained by heating usually at 210°C for 3-4 hours and proper cleaning of containers. Studies revealed that the autoclavation at normal cycle do not remove Pyrogens while heating at 650°C for 60 sec removes all Pyrogens. Following process may be used for the removal of Pyrogens

Overview Aseptic Processing
To examine detailed issues relating to the manufacture of products prepared aseptically:
Manufacturing environment
• Clean areas
• Personnel
Preparation and filtration of solutions
Pre-filtration bioburden
Filter integrity/validation
Equipment/container preparation and sterilization
Filling Process
Aseptic process Validation
Specific issues relating to Isolators, BFS and Bulk

Particulate matter: -
The critical environment monitored should not be more than 30 cm during filling.

Differential pressures: -
Between the rooms the Positive pressure is maintained which should be not less than10-15 Pascal's.

Pattern of airflow and Air changes: -
Air flow and its pattern should be such that the entire particle should be carried away by air from filling area. And for area B, C, D it should be 20-30 changes/hr

Clean up time/recovery:-
The particle count for grade A can be maintained at rest by cleaning foe 20 minutes after completion of operation. And in operation state it is maintained whenever material container is opened.

Relative humidity and Temperature of product:-
The temperature should not be high which can cause the generation of particle.

Velocity of Airflow:-
Laminar airflow air speed is approx 0.45m/s± 20% at place where work is being carried out.

Aseptic Filling:-
All the filling material can be sterilized prior to filling in aseptic environment. Sterile products are aseptically filled in container and are aseptic in their container. For further increase in stability of drug the solution can be lyophilized. To ensure the mangment of operating challenges the container must be more unique. How do we complete the aseptic filling process? All the personnel, drugs, machine parts must be aseptic and proper sanitation is provided. This all can be achieved by good aseptic practices and simplified processes.

Machine of vial/ampoules line:-
Following Machines are used in vial/ampoules line:-
Double Rotary vial/ampoules washer
Sterilization tunnel
Autoclave
Vial/ampoules filling machine
Vial/ampoules cap sealing machine
Visual inspection machine
Vial/ampoules sticker labeling machine
Vial/ampoules packaging machine

Double rotary machine for washing of vial/ampoules:-
0.4 μm hydrophobic, size of filter for air
10 μm filter size
Removal of particulate matter by using washing and air blow stations.
The capacity of vial/ampoules washer is from 5 – 32 ml. glass.
Output dependent on size and is up to 120 vial/ampoules per min.
The parts of machine are made up off SS because it does not reach the product.

Sterilization Tunnel
The design of the sterilization tunnel is such that it can provide continuous sterilization in Class 100.
Following are the 3 different chambers present in the tunnel:

i. feed chamber in tunnel
ii. sterilizing chamber in tunnel
iii. cooling chamber in tunnel

Feed Chamber
The barrier from transferring heat between washing and sterilization area is prevented so that pre heating and contamination can be prevented is provided by in feed chamber. Air flows over the vial/ampoules at 0.5m form HEPA filters which are vertical and unidirectional. The pressure of feed chamber is 28Pa which is less than sterilization chamber. This decrease in pressure causes the air to flow between sterilizing chamber and feed chamber.

Sterilizing chamber
The chamber used for sterilization can be heated up to 350ºC for 3-4 min and it is completely insulated. The time required for the sterilization of vial/ampoules depends on the circulation of air and the temperature given, temperature is provided by heaters which provide heat. Less time is required for sterilization if high temperature and air circulation is provided. The temperature of 250ºC for 15 minutes is provide for Depyrogenation as a standard cycle. The pressure of sterilization chamber is 29 Pascal's which

1 Pascal's is less than the cooling chamber. This drop in pressure causes the flow from one chamber to other i.e. cooling chamber to sterilization chambers.

Cooling Chamber
Different no. of coils may be used for cooling of vial/ampoules to ambient temperature in the tunnel depending on its size, vial/ampoules should stay in the chamber for 15-20 minutes and chilled water may also be used for cooling.

Autoclave
The process used is described by the following steps.
HPHV sterilizing cycle:

1. It undergoes following.
2. Vacuum
3. Pre Heating (85º - 95ºC)
4. Initial vacuum hold 1 minute
5. 121ºC for 30 minutes for sterile hold
6. Heating
7. 450 mm/µg for 20 min final vacuum holding.
8. Vacuum Release

Drying cycle:
The most ideal machine for sterilization of parts of machine, cloths, rubber stopper and gloves is autoclave. The important feature of it is the removal of air phase by vacuum and steam. Quick exhausting is done to achieve quick drying. All the parameters are controlled precisely by using PLC based control. Display of all the parameters are shown by machine interface in a line. GMP describes that the temperature though out is distributed uniformly. Following are the steps followed to achieve uniform distribution of temperature.
Steam should be supplied to the chamber and the jacket by single supply apparatus.
Dimple jacket chamber.
Baffles present in the chamber are used for distribution of steam.
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Vial/ampoules filling machine:
For filling the empty vial/ampoules are raised bellow the filling nozzle by applying pressure.
For sensing the pressure of vial/ampoules in the line a sensor is arranged.
For counting the vial/ampoules and bottles one more sensor is arranged and all this procedure is carried out under Laminar Air Flow hood.

Steps for Sterile powder filling:-
vial/ampoules washing
Washed vial/ampoules unloading on to tunnel entry-class A LAF
Tunnel preheating zone
Tunnel heating zone for Depyrogenation
Tunnel cooling zone
Vial/ampoules enters class 100 area under LAF on to A turn table
Vial/ampoules is purged with nitrogen gas
Vial/ampoules is filled with sterile powder
Vial/ampoules is bunged or plugged
Vial/ampoules goes on to a turn table
Vial/ampoules goes on conveyer to capping head-vial/ampoules are capped with flip off seals-class 100 LAF
Vial/ampoules come out
Vial/ampoules are inspected- optical inspection for weights and particulates and vial/ampoules non conformities
Vial/ampoules are quarantined 100-A area.

Visual/ampoule inspection machine:
After sealing the optical inspection of the vial/ampoules are carried out.
The operator working in this area should check the vial/ampoules for any metal piece, black particles or white particles occurring as contamination during the filling before finally releasing the batch. This procedure is carried out to prevent damage to the batch. For optical checking 5-6 vial/ampoules are held for 1 minute to check any particle.
The concentration should be given on inspection during checking.
Vial/ampoules are inspected in a machine fitted with lights. First for checking the efficiency of operator vial/ampoules containing particle are selected and passed in front of the operator for inspection.
For the visual inspection of vial/ampoules the light of 700 Lux is provided in inspection area.
The visual inspection machine consists of 2 mirrors.
1st mirror is white in color and is used for the detection of any black particle in the product.
2nd mirror is used for the detection of white particles and any crack in the container.
All the persons which are doing visual inspection should be changed in every 2 hours.

Requirements of Aseptic Filling

Vial/ampoules:-
Vial/ampoules are the primary packing materials (container) for the products which are used in aseptic filling of dry injectable powder or liquid injection.

Vial/ampoules washing:-
For the entry into washing area separate rooms should be used and vial/ampoules are supplied to machine under Class 100. DM & WFI is used for washing the vial/ampoules and the person operating the machine should be properly dressed, wear mask and gloves. Sterilizing grade membrane is used for

the sterilization of WFI and filters of size 10 and 5 microns are used for the filtration of DM. the area in which all this procedure is being carried out should lie in Class 1000 and air supplied should be of sterilization grade. The area in which vial/ampoules are unloaded should follow class 100.

Sterilization of vial/ampoules:-
Sterilization process is used under LAF for the Depyrogenation and sterilization of vial/ampoules. Following are the zones of sterilization tunnel:

a. Pre heating Zone in tunnel
b. Heating Zone in tunnel
c. Cooling Zone in tunnel
d. Stabilizing Zone in tunnel

A. Pre heating Zone
This type of zone consist of temperature of 50 – 55ºC.
All the contents of moisture are removed from vial/ampoules in this region.
B. Heating Zone
This Zone consists of temperature that is around 340 – 360ºC.
At this temperature all the microorganisms and endotoxins produced by them are destroyed.
C. Cooling Zone
Cooling zone consists of temperature around 80ºC.
The temperature of the vial/ampoules is reduced by the cool air under laminar air flow.
The air flow speed is more in class 100 i.e. flow speed at least 1500 CFM and the air enters from class 100 into cooling tunnel but the opposite is prevented. Vial/ampoules in this area should not be touch physically otherwise they will not be used for manufacturing.
Temperature in this area is maintained at 25-30ºC.

- Time taken by vial/ampoules to pass sterilization tunnel is 7.40 seconds.
- For Depyrogenation temperature of 300°C for 3 min is required.

Vial/ampoules Enters Class 100 Area under LAF

Nitrogen gas is passed through vial/ampoules so that oxygen is displaced. Nitrogen is supplied through filter of sterilizing grade. Microbial content in nitrogen at any point and from time to time is carried out. Sterility Assurance Limits (SAL) should be tight. The area used in this falls under class 100 and the background class is 1000. According to the latest GMP guidelines class 10000 is never allowed.

Sterile Powder- entry
Dry powder injectable are already sterile powder which is used intravenous or sub-coetaneous and coetaneous after reconstitution with medium like WFI.

- It refill in Aseptic filling room and transfer through pass box present in vial sterilization room.
- Sterile powder is packed in sterile steel containers.
- The aseptic filling area should be maintained at 20-22C temperature, Relative Humidity should be 35%-40% and Class 100 maintained during whole Filling Set.

Vials should be proper presterilised, Soiliconised, & dried to remove moisture or water. Vials should be handled with proper care.

Vials are inspected:-
The inspection of weight, vials and optical inspection of particulates non conformities-class 10000 area

Vial/ampoules are quarantined:-
Tight control is made on the vial/ampoules under quarantine and segregates the vial/ampoules under rejection and don't allow them to mix. Vial/ampoules should be properly labeled with date, batch number, batch size, no. of vial/ampoules in each container, visual inspection date and signature of QA incharge and supervisor. The rejected material should be in Lock and Key and no one is allowed to remove it without permission. Then afterwards the QA incharge will reject them under its own supervision and show them in statement as a settlement.

Conclusion:-

Aseptic production is used in cases, where the different drugs material is instable against heat; hence steam or heat sterilization in the final container closure system is not possible. Aseptic manufacturing is the very demanding now days in pharmaceutical manufacturing processes. It requires accurate attention to operator training and behavior, process validation, production process documentation, plant and equipment maintenance and change control management.

"Cite this article"

Yasir Mehmood "Sterility Assurance Level and Aseptic Manufacturing Process in Pharmaceuticals" Int. J. of Pharm. Res. & All. Sci.2014;3(4):10-15

References

1. *Mosby's Dental Dictionary*, 2nd edition. © 2008 Elsevier, Inc.
2. Pace Analytical Life Sciences has methods in place to perform bioburden testing in accordance with ISO 11737-1.2014
3. Validation of Aseptic Filling for solution Drug Products, PDA Technical Monogram, Number 2, pub. 1981.
4. Guideline on Sterile Drug Products Produced by Aseptic Processes,FDA, pub. 1987.
5. ISPE Baseline Pharmaceutical Engineering Guide, Volume 3, Sterile Manufacturing Facilities, Jan 1999.
6. FDA Concept Paper on Aseptic Processing:www.fda.gov/cder/dmpq/aseptic-cp.pdf.
7. Technical Report No. 36, ―Current Practices in the Validation of Aseptic Processing," Parenteral Drug Association, Inc., 2002.
8. FDA, Guidance for Industry for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products (Rockville, MD, Nov. 1994).
9. W. Whyte, "A Cleanroom Contamination Control System," Eur. J. Parenter. Sci. 7 (2), 55–61 (2002)
10. ISO 14644-1: Cleanrooms and Associated Controlled Environments,Classification of Air Cleanliness.
11. J. Agalloco and J. Akers, "Aseptic Processing: A Vision of the Future,"Pharm. Technol. 29 (Suppl. Aseptic Processing), 16–23 (2005).
12. Ljungqvist, B., and Reinmuller, B., Cleanroom Design: Minimizing Contamination through Proper Design; Interpharm Press, 1997.
13. J.E. Akers, "Environmental Monitoring and Control: Proposed Standards, Current Practices and Future Directions," J. Pharm Sci. Techn. 51(1), 36- 47, 1996.
14. ASTM, "E 2500-07 Standard Guide for Specification, Design, and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment," (ASTM, West Conshohocken, PA, 2007).
15. FDA, Draft Guidance for Industry—Process Validation: General Principles and Practices (Rockville, MD, Nov. 2008).
16. W. Whyte, "A Cleanroom Contamination Control System," Eur. J. Parenter. Sci. 7 (2), 55–61 (2002)
17. J.E. Akers and Y. Oshima, "PAsepT, Aseptic Vial/ampoules Filling Processing Based on Principles of PAT," in Presentation at the ISPE Annual Conference (San Antonio, TX, 2004).
18. Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice (2004).
19. B. Ljungvist, B. Reinmüller, and R. Nydahl, "Microbiological Assessment in Clean Rooms for Aseptic Processing," J. R3 Nordic 23(3), 7-10,1995.
20. J. Agalloco, "The Validation Life Cycle," J. Parenter. Sci. Technol. 47(3), 142–147 (1993).
21. K. Chapman, "The PAR Approach to Process Validation," Pharm.Technol. 8 (12), 24–36 (1984).
22. FDA, "Pharmaceutical CGMPs for the 21st Century—A Risk-Based Approach," Final Report (Rockville, MD, Sept. 2004).
23. J. Agalloco, "Compliance Risk Management: Using a Top Down Validation Approach," Pharm. Technol. 32 (7), 70–78 (2008