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Review Article

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Validation of Aseptic Process in Sterile Pharmaceutical Facility in accordance with ICH Guideline

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Abstract

This review article focuses on the validation procedures and guidelines outlined by the International Council for Harmonization (ICH) for ensuring the efficacy and safety of aseptic processes in sterile pharmaceutical facilities. Aseptic processing is critical in pharmaceutical manufacturing to prevent contamination and ensure product sterility. The ICH guidelines provide a comprehensive framework for the validation of aseptic processes, encompassing facility design, equipment qualification, process validation, and ongoing monitoring. Aseptic validation is a systematic process that ensures sterile products are consistently manufactured under controlled conditions.
It begins with facility and equipment design, focusing on cleanroom layout, air handling systems (HVAC), and controlled environments that minimize contamination risk. Personnel qualification and training are critical to maintaining aseptic practices. The validation process typically includes: Installation Qualification (IQ), Operational Qualification (OQ), Performance Qualification (PQ), Media Fill / Process Simulation. Various techniques such as environmental monitoring, media fill studies, and microbiological testing are employed to assess the ongoing performance of aseptic processes. Adherence to ICH guidelines not only ensures regulatory compliance but also promotes the production of high-quality, safe, and efficacious sterile pharmaceutical products. This review consolidates key principles and best practices for validating aseptic processes, serving as a valuable resource for pharmaceutical professionals involved in sterile manufacturing and regulatory

Keywords: Validation; ICH guidelines; Sterile; Aseptic process; Process Validation; Qualification

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1. Introduction

The concept of validation was formally introduced in the United States in 1978 and has since evolved to address the growing need for consistency, quality, and public safety in pharmaceutical manufacturing. Today, validation serves as a systematic approach to demonstrate that a process consistently produces products meeting predefined specifications and quality attributes. Process Validation (PV) provides documented evidence that offers a high level of assurance that a specific process will reliably yield a quality product. (1)

A commercially sterile product is processed and packaged into sterile containers, then sealed hermetically with a sterile closure to prevent microbial recontamination — a method known as aseptic processing. This process involves manufacturing sterile medications in a controlled environment to minimize the risk of microbial contamination.

2. Aseptic Validation and key components

Aseptic validation is a systematic process carried out to ensure that aseptic manufacturing environments, processes, and operators consistently maintain sterility assurance. The primary objective is to demonstrate that sterile products are not contaminated during preparation, filling, or packaging. It is particularly critical for parenteral medications, as they bypass the body's natural defence mechanisms against infection. However, it is equally important for other aseptically prepared products such as ophthalmic preparations, nasal sprays, inhalation products, and sterile biologics (e.g., monoclonal antibodies, vaccines), which also require strict microbial control to ensure patient safety. (2)

2.1 Key components include:

- **Media Fill (Process Simulation):** Use of sterile growth media instead of product to simulate aseptic operations and detect potential sources of contamination.
- **Environmental Monitoring:** Continuous and routine monitoring of viable and non-viable particulates in cleanrooms and laminar airflow systems.

- **Equipment Qualification:** Validation of autoclaves, sterilizers, cleanroom HVAC systems, and isolators to ensure controlled conditions.
- **Operator Validation:** Gowning qualification and aseptic technique evaluation to confirm operator competency.
- **Sterilization Validation:** Confirmation that sterilization methods (e.g., steam, dry heat, filtration) achieve required sterility assurance levels.

3. Validation

Validation is a documented process that provides a high degree of assurance that a specific procedure, process, or system consistently produces results meeting predetermined quality criteria. In the pharmaceutical industry, validation is a key element of Good Manufacturing Practices (GMP) and ensures that products are safe, effective, and of consistent quality

3.1 Types of the Validation

- **Prospective**: Conducted before a new product is commercially distributed to ensure the process can consistently produce quality products.
- **Concurrent:** Performed during routine production of products intended for sale, with results evaluated in real time.
- **Retrospective:** Based on historical production data to confirm that a process has been operating within a state of control.
- **Revalidation:** Carried out periodically or after significant changes to confirm continued process control. (3)

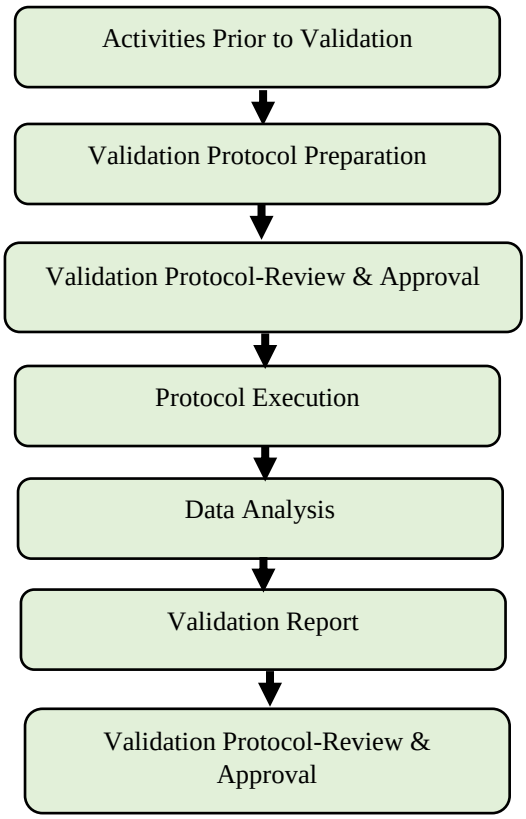

Figure 1. Validation process – Flow Diagram (4)

3.2 Validation of Equipment used in sterile pharmaceutical industry

Equipment should be chosen or built to ensure that the product specifications are regularly met. Below are the types of Equipment Qualification Types:

Design Qualification (DQ): This is formal confirmation that the facilities, processes, and equipment are designed with their intended use in mind. The parameters that are involved are as follows: kind, model number, construction material, and dimensions of various equipment pieces.

Installation Qualification (IQ): This checks the installations of machines, measurement tools and production spaces-that are employed during a process of manufacturing

Operational Qualification (OQ): This inspects systems, equipment, facilities to make sure they are functioning normally. It checks to see if the system functions as intended.

Performance Qualification (PQ): A set of test cases known as performance qualifications is used to confirm that a system operates as intended in settings that are modelled after real-world ones. The User Requirements Specification (or maybe the Functional Requirements Specification) defines the requirements for the performance qualifying tests.

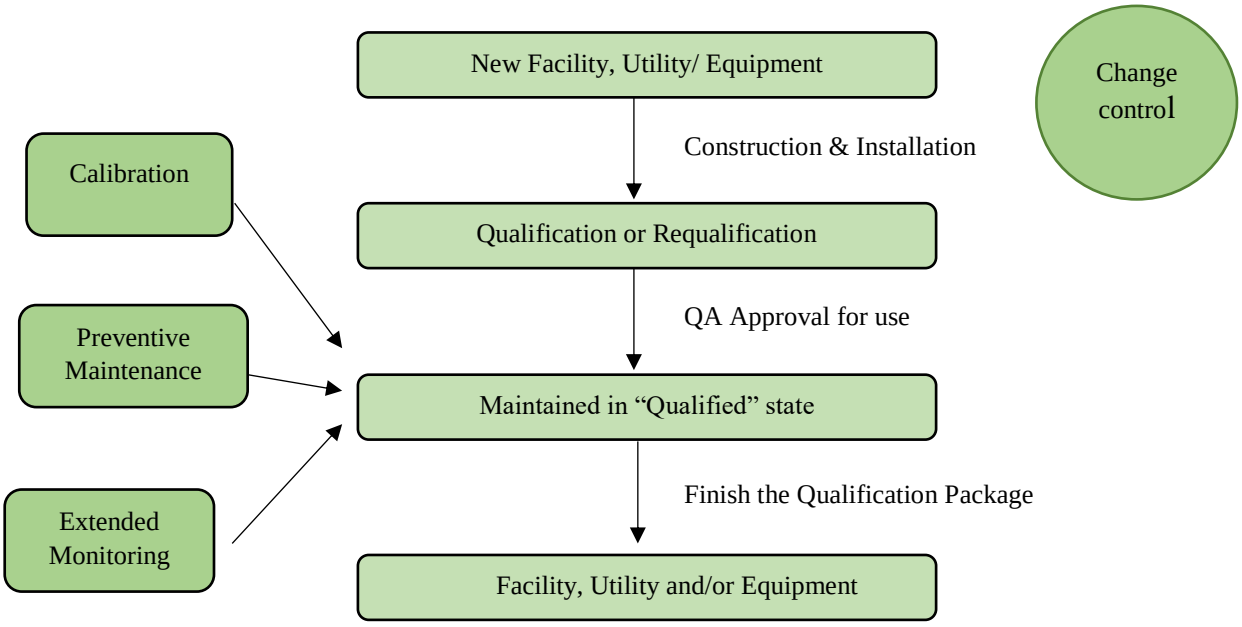

Figure 2. Qualification Lifecycle (2)

3.3 Validation of Sterilization Equipment

3.3.1 Validation of Autoclave:

a) By Using Biological Indicator

- Prepare 300 ml Soyabean Casien Digest Medium
- Transfer 100 ml of medium in 3 different 250 ml capacity and label it as A, B, C.
- Add one spore strip of Geobacillus stearothermophilus in flask B & plug all flasks
- Sterilized the entire flask in vertical autoclave at 121℃ for 60 min
- Remove the flask after sterilization and allow to cool.
- Transfer the flask to incubator at 55-60℃ for 7 days.
- Observe after 7 days
- **Acceptance criteria:** No growth in flask A B and C (5)

b) By Using Chemical Indicator:

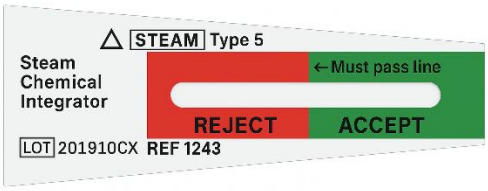

Figure 3. Steam Chemical Integrator (6)

Within a predetermined range of steam sterilization cycles, Steam Chemical Integrators are engineered to react to all important factors. Each container, pack, tray, pouch, or other containment device is supposed to have integrated indicator installed in it so that it can serve as a stand-alone monitor of crucial sterilization cycle parameters. (6)

3.4 Validation of Controlled Air System

3.4.1 Laminar Air Flow:

Laminar Air Flow (LAF) systems are designed to provide a continuous flow of HEPA-filtered air in a unidirectional pattern, thereby minimizing the risk of contamination in aseptic processing areas. These systems are critical in cleanrooms and sterile manufacturing zones, where maintaining controlled environmental conditions is essential for product quality and patient safety.

Validation of LAF Systems:

a) Particle count:

- This test methods specifies the measurements of air borne particle concentration with size distribution.
- Systems should be kept ON before starting measurements
- Ensure that the floor and area as well as equipments are well cleaned.
- Use calibrated particle counter and carry Out particles counting at corners and centres of Laminar Air flow unit.
- The limit is: >0.5 NMT 100 particle/ft^3 of air 5.0 g NIL particle/ft^3 of air
- Record the mean particle count in the format specified in the Report of Validation of Laminar Airflow (LAF). (7)

b) Air Flow Velocity Measurement:

- The purpose of this test is to calculate the air supply volume flow rate by averaging the air flow velocity, volume, and homogeneity.
- Clean the instrument with IPA and system should be kept ON before starting the measurements.

- Measure the air velocity near the HEPA filter at distance of 6 inch below the filter. Carry out measurement by using calibrated Anemometer. Check the velocity at corners and centre of the HEPA - Filter. The limit is 40-80 fpm. (7)

c) DOP Test:

The Dispersed Oil Particulate (DOP) Test, also known as the HEPA filter integrity test, is performed to verify the efficiency and leak-tightness of HEPA filters used in cleanrooms and laminar airflow units. It ensures that the filter can effectively remove particles from the air and maintain the required cleanliness classification.

- DOP is performed by presenting DOP aerosol at upstream in HEPA-filter & search for leaks by scanning the downstream of HEPA filter with calibrated photometer probe.
- Measure the upstream aerosol concentration using calibrated photometer Adjust the photometer to read 100 %. Hold the probes approximately6 inch from area to be tested and search the area for any leakage.
- DOP test passes with penetration less than 0.03%
- The report of DOP testing will be attached and received from the authorized agency. (7)

3.5 Heating, Ventilation and Air Conditioning (HVAC)

Heating, Ventilation, and Air Conditioning (HVAC) systems play a critical role in maintaining the required environmental conditions in cleanrooms and aseptic manufacturing areas. These systems control temperature, humidity, air cleanliness, and differential pressure to minimize the risk of contamination and ensure compliance with Good Manufacturing Practices (GMP).

3.5.1 Validation of HVAC System:

a) Pattern of Air Movement:

- Pick up the stick of titanium tetrachloride.
- Light the stick.
- Position flaming stick towards the Unit for Air Handling (AHU) that is operating.
- Used the room's smock distribution to observe the airflow.
- Draw a diagram showing how the air moves through each area. (8)

b) Air Flow Rate and Variation Hourly:

- Using the anemometer probe, scan the region of the HEPA filter six inches from the filter face.
- Assuming the trial, partition the surface area of the HEPA filter into four equal, imaginary grids.
- Note the velocity measurements made in 5.5 at the grid's center and the intersection of the dividing lines (the HEPA filter's center).
- Determine the average speed (V, expressed in feet per minute). (8)

c) Test for Filter Leaks

- Position the equipment i.e. velometer in front of the unit of AHU.
- Verify air speed in every corner of the AHU. The air speed has to be under the HEPA filter's upper limit.
- To stop air leaks, adjust the gas cut if the air velocity exceeds the upper limit. (8)

d) Particle Count

- Prior to an hour of test operation, on the air system. To inspect the particles in the room while it is not in use, take the appropriate particle counter and turn it on.
- Gather data from the particle counter and enter it into the format.
- When there is construction going on nearby, run the particle counter. After working in the region for more than an hour, the particles should be counted.
- Enter the data in the prescribed format. Run the particle counter in each room to ensure that grades A, B, C, and D are maintained.

e) Pressure Difference

- Fasten the manometer, which is fastened to the wall of the nearby area, to every room that is under test.
- In the tested zone, turn on the air system and wait for the local pressure to stabilize.
- Noted the variations in pressure throughout the entire space and between rooms.
- Use the format to record the data. (8)

f) Test of Uniform Humidity and Temperature

- Set the thermometer that has been calibrated in a new spot.
- Turn on the air conditioning and observe the time. Wait for the temperature in the area to stabilize within the given range.
- Take note of the area's temperature and record it in a format.
- Move the hygrometer that has been calibrated to a new spot.
- Turn on the air conditioning and observe the time. Wait for the humidity level in the area to stabilize within the designated range.
- Take note of the area's temperature and record it in a format. (8)

g) Check list item and qualification

1) Several item and qualification of the equipment is analysed during installation.

3.6 Illustrative examples of Validation Record for Sterilization of Equipment:

3.6.1 Validation of Autoclave:

a) By Using Biological Indicator:

Table 1. Illustrative Example: Validation Report of Vertical Autoclave

Validation Report of Vertical Autoclave	
Equipment Id No. ……...	Location……
Culture Name ……	
Validation Date: …….	Validation due on…….
Sterilization Parameter: ……..……	
Incubation Temp & Days: ……	

Growth Observations							
Date / Flask	02 Nov	03 Nov	04 Nov	05 Nov	06 Nov	07 Nov	08 Nov
A	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
B	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
C	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth

Done By…… Checked By…….

Table 2. Critical Parameters of Autoclave

Sr. No.	Type of Autoclave	Parameter	Set Temperature/ Pressure	Observed Temperature/Pressure	Limit
1.	Gravity-Displacement / Class N	Temperature	121.5 ºC	121.5 ºC	121 ºC to 124 ºC
2.		Pressure	15 lbs	15 lbs	15 lb to 18 lb

b) By Using Chemical Indicator

ACCEPTED	REJECTED
The colour bar indicates that the steam, temperature, and time requirements for sterilization have been satisfied if it enters or passes through the **ACCEPT** window.	It is deemed unsuccessful if the colour bar is on the line or in the **REJECT** region. It is essential to reprocess the pack and investigate the cause of the sterilization failure.

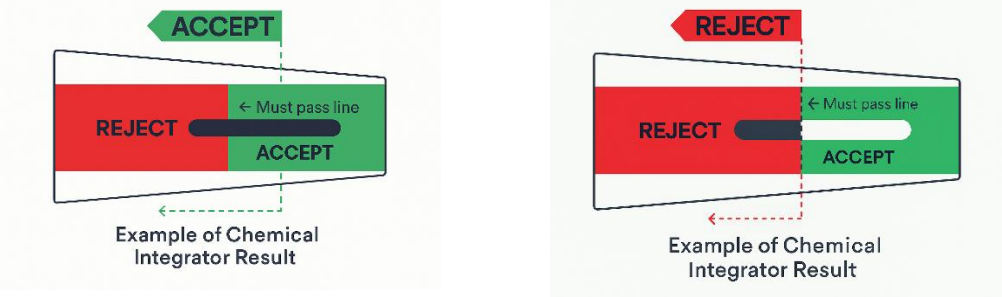

Figure 4. Interpretation of Results (Illustrative Example) (6)

3.6.2 Validation of the Sterilization Process in Autoclave

Validation of sterilization in autoclaves is generally demonstrated by monitoring multiple temperature probes (e.g., RTD sensors) placed at different locations within the chamber. Studies have shown that during a sterilization cycle, the recorded temperatures remain consistently around 121 °C, with minimal variation (121.1–121.9 °C), indicating uniform heat distribution. The coolest point within the chamber typically does not drop below 121.2 °C, confirming compliance with sterilization requirements

Table 3. Performance Qualification Tests for LAF

Sr. No.	Name/Title of Test	Rationale / Reference for Test	Rationale/ Reference for Acceptance Criteria
1	Differential Pressure test	This test's objective is to confirm that Dispensing LAF can sustain the desired pressure differential before and after the HEPA filter while staying below the Acceptance limit.	90-250 Pa (in house specification)
2	Air Velocity	The purpose of this test is to measure supply air velocity in the Booth	The obtained results must be between 0.36 m/s to 0.54 m/s
3	HEPA filter leakage test	The purpose of this test is to is to confirm that the filter system is appropriately installed and that no leaks have appeared while in use.	Should be less than 0.01%
4	Air Particle Count	The purpose of this test is to provide a quick reference of the overall cleanliness of the environment with respect to the concentration of non-viable particles	0.5 um – 35,20,000 5 um – 29,000
5.	Air Flow Direction Test (Smoke Test)	To verify the direction of the air flow, an air flow test ought to be conducted.	From +ve to -ve pressurized zone

Table 4. Qualification of HVAC

Item	Specification	Check method / Tester equipment	Conclusion	Checked by	Approved by
Air Conditioning Unit	Cooling capacity: Model: CLC P.025 Merk: Trane	Visual	OK	-	-
Laminar Air Flow	(2400x5400) +(1200x9600) + (2400x4800) 304 thickness 2 mm 6 pcs filter efficiency 99.99 % 6 no. Centrifugal fan 1 set Air Curtin kaca thickness 6 mm	Visual	OK	-	-
Booster Fan Unit	Capacity: 21,00 m^3/H Static Pressure: 3'' WG Merk: Kruger	Visual	OK	-	-
Body / Box A.H.U	No Leakage	Visual and Soap Foam	OK	-	-
Ducting Installation	No Leakage	Visual and Soap Foam	OK	-	-
Electric Installation (P&ID)	Available	Insulation test	OK	-	-
Grounding	0.5-5 ohm	Insulation test	OK	-	-
Insulation test		Insulation test	OK	-	-
HEPA Filter unit (Dimension)	Size: 610 x 610 x 292 Cap: 99.97 % - 0.3 u	Visual	OK	-	-
Pre Filter unit Afi 75%	Size: 610 x 610 x 292 Cap: 99.97 % - 0.3 u	Visual	OK	-	-
Bag Filter nbs 90%	-	Visual	OK	-	-
Filter position	Confirm	Visual	OK	-	-
Certificate of components	Available	-	-	-	-
Safety aspects	Confirm	Visual	-	-	-

Table 5. Critical Parameter for HVAC Validation

Sr. No.	Test/Critical parameter	Acceptance criteria
1.	DOP test	NMT 0.01%
2.	Air velocity	90 ± 20% FPM
3.	Air changes	Not Less Than 25 Air differentiate
4.	Pressure differential	For similar class NLT 6Pa and for distinct class NLT 15 Pa
5.	Temp and Humidity	Temp. :23+2°C, Humidity: NMT 55%
6.	Non-viable count	In accordance with the ISO specification
7.	Viable count	In accordance with the IHS specification
8.	Air flow pattern	Uniform all the way up to the operational level
9.	Decontamination time	NMT for eight minutes

4. Conclusion

Aseptic process validation remains a cornerstone of sterile pharmaceutical manufacturing, ensuring that drug products are consistently safe, effective, and free from contamination. By following the International Council for Harmonization (ICH) guidelines along with Good Manufacturing Practices (GMP), manufacturers can establish a robust framework for facility design, equipment qualification, process validation, and ongoing environmental monitoring.

This review highlights the importance of integrating risk-based approaches, personnel training, media fill simulations, and regular revalidation to maintain a state of control throughout the product lifecycle. Aseptic validation should be viewed not as a one-time activity but as a continuous commitment to quality, supported by periodic review of critical parameters and process improvements.

Ultimately, adherence to global regulatory expectations and scientific best practices enhances patient safety, minimizes the risk of contamination, and safeguards public health.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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