

## Review

# Validation of aseptic processes for pharmaceuticals

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### VALIDATION OF ASEPTIC PROCESSES

Sterile Products may be broadly classified into two main categories, according to the manner in which they are produced: those which are sterilized after the product has been filled and sealed in the final container(s) (“terminally sterilized” products) and those where the sterilization stage (or stages) takes place is it before or after the bulk product filled in to final container. In this latter instance, all subsequent processing (typically, the filling and sealing operations) must be conducted aseptically in order to prevent recontamination of the sterilized product. The two most common pharmaceutical applications of aseptic processing methods are (a) the filling of liquid products following sterilization by filtration and (b) the filling of previously sterilized bulk powder products. An aseptic processing operation should be tested using a microbiological growth medium (media fill) during lyophilized injection formulation, filling, loading, lyophilisation, stoppering, and unloading activities.

Key words:

#### PURPOSE

The process simulation tests (media fill) provide a way to evaluate changes made to an aseptic processing operation which may affect the sterility of the final product. It will be useful in identifying potential weakness in an aseptic processing operation which might contribute to the microbiological contamination of the product.

The purpose of a process simulation test is to:

- Demonstrate the capability of the aseptic process to produce sterile drug products.
- Quality or certify processing personnel.

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- Comply with current good manufacturing practice requirements.

#### PROCESS SIMULATION (MEDIA FILL)

The “Media Fill”, or “Broth Fill”, technique, is one in which a liquid microbiological nutrient growth medium is prepared and filled in a simulation of a normal manufacturing operation. The nutrient medium processed and handled in a manner which simulates the “normal” manufacturing process as closely as possible with the same exposure to possible contamination (from operators, environment, equipment, and surfaces) as may occur during routine manufacture. The sealed containers of medium thus produced are then incubated under prescribed conditions and examined for evidence

of microbial growth, and thus can obtain indication of the level of contaminated units produced.

An approach must be used to validate adequately and control aseptic processes. A process simulation test is only a point-in-time representation of the capabilities of an aseptic processing system, including environment, equipment, procedures and personnel. It does not ensure that drug products produced on the same line at other times will have the same level of microbiological quality. However, through control and validation of the related processes, such as environmental monitoring, qualification of personnel and sterilization cycles, it is possible to maintain the level of asepsis demonstrated during the processes independently, such as sterilization/depyrogenation of the product, container, closure and all product contact surface.

Following activities shall be performed for the process simulation studies (media fill) for aseptically filled lyophilized products and verification of performance attributes.

- Frequency of process simulation
- Process simulation run
- Forced simulation study
- Introduction of interruption during the process

### FREQUENCY OF PROCESS SIMULATION

#### Process simulation for a new facility or filling line or process

Three consecutive successful process simulations are required to demonstrate acceptable limit of performance for new facility or filling line and process.

Since the process being introduced use a range of different container sizes, for example the filling of several vials having different sizes in a filling machine where all other aspects are the same, and the process simulation shall be carried out at least 3 consecutive runs irrespective of vial sizes.

#### Ongoing process simulation (validation maintenance)

Process simulation on the extremes of the container sizes from the smallest volume to the largest

volume will be considered for one process simulation trial in a frequency of alternative every 6 months. The smallest and largest volume containers are considered as the worst cases due to the difficulty to handle (fastest speed, more interventions) and maximum exposure of the open container (slowest filled speed) respectively. These extremes cover all other intermediate size container within this range.

Based upon frequencies a requalification schedule shall be drawn up by team. The process simulation must be performed within  $\pm 1$  month of the scheduled limit.

### PROCESS SIMULATION RUN

Each process simulation run is designed to cover all normal processing operations and including some of the possible interventions that could realistically be encountered during routine filling operations. The following aspects must be considered.

The process simulation should cover all normal processing steps right from the compounding operation to the exterior vial washing and visual inspection. The following table provides the comparison of normal manufacturing steps to be followed for process simulation test.

SNO	Manufacturing operation A / steps	Normal production	Process sim- ulation
1	Dispensing	✓	✓
2	Formulation (mixing and dissolution)	✓	✓
3	Sterile filtration-1&Bulk solution collection	✓	✓
4	Sterile filtration-2 (in the filling CRABS)	✓	✓
5	Vial filling	✓	✓
6	Half-stoppering	✓	✓
7	Tray loading	✓	✓
8	Lyo loading	✓	✓
9	lyophilization		
9a	freezing	✓	X
9b	Primary drying	✓	X
9c	Secondary drying	✓	✓
10	Vacuum break	By Nitro- gen	By filter compress air

11	Rubber stoppering (inside Lyophilizer)	✓	✓
12	Unloading of vials	✓	✓
13	Aluminum cap over sealing	✓	✓
14	External vial washing & visual inspection	✓	✓
15	Vial coding	✓	✓
16	Vial labeling	✓	X
17	Vial incubation for 14 days (22.5 ± 2.5°C) For 7 days followed by 32.5 ± 2.5°C for 7 days	X	✓
18	Vial visual inspection (contamination)	X	✓

S.NO. B	Manufacturing support process	Normal production	Process simulation
1	Product and vent filters sterilization	✓	✓
2	Product and vent filters integrity testing (pre & post use)	✓	✓
3	Filling machine parts sterilization	✓	✓
4	Interior vials washing & depyrogenation	✓	✓
5	Rubber stopper & over seals sterilization	✓	✓
6	Solution preparation vessels cleaning & sterilization	✓	✓
7	Mobile (collection) vessels cleaning & sterilization	✓	✓
8	Lyophilizer cleaning & sterilization	✓	✓

S.NO. C	In-process controls	Normal production	Process simulation
1	Bulk solution sampling for chemical testing	✓	X
2	Bulk solution sampling for bioburden	✓	✓
3	Pre -fill growth promotion test (for holding tank)	X	✓
4	Post incubation media growth promotion test	X	✓
5	Continuous environmental monitoring (viable & non viable)	✓	✓
6	Fill volume check	✓	✓

7	Personnel monitoring	✓	✓
8	Surface monitoring	✓	✓
9	Empty depyrogenated vials from turntable before filling for sterility	X	✓
10	Sterilized rubber stopper from hopper before & after filling for sterility test	X	✓
11	Sterilized aluminum seal from hopper before & after filling for sterility test	X	✓
12	Compressed air sampling from manufacturing filtration & filling area during operation for sterility	X	✓
13	Compressed air sampling from lyophilization area during operation for bioburden	X	✓

## MEDIA SELECTION

The media selection for process simulation test should demonstrate the capability to promote recovery of wide spectrum of microorganisms. The medium growth promotion properties should be evaluated using pharmacopoeial methods. The media can also be demonstrated for the growth promotion properties using environmental isolates.

**Soybean casein digest medium (SCDM)(PIC, 2007)** 1% should be used for the process simulation test. selection of the low nutrient medium is due to following factors.

- Low nutrient medium supports even the growth of injured organism.
- Comparative study protocol & study report showing better growth in 1% medium.
- Ease in cleaning post media fill.

## PROCESS SIMULATION PROCEDURE

### Compounding/manufacturing

A quantity of suitable growth medium, soybean casein digest medium (SCDM) is prepared using water for injections and mixed for a nominal 30 minutes maintaining the temperature at 50 - 60°C.

The media shall be heated up to  $80^{\circ}\text{C} \pm 5^{\circ}\text{C}$ .

#### First filtration

The liquid growth medium should be sterilized by passing through sterilizing grade filters in a manner similar to the production process being simulated. After sterilization, the growth medium should be passed through the equipment train as through it were an actual production batch and all routine procedures used in the manufacturing of the batch are performed, such as in-process sampling filter integrity testing.

After first filtration of the media, adequate quantity should be sampled for growth promotion test of the media.

#### Second filtration

The liquid growth medium should be sterilized by passing through sterilizing grade filters in a manner similar to the production process being simulated. After sterilization, the process repeated as did in the first filtration the growth medium should be passed through the equipment train as through it were an actual production batch and all routine procedures used in the manufacturing of the batch are performed, such as in-process sampling filter integrity testing.

### FILLING

The filling machine should be operated at the pre-determined fill rate the container size being utilized on a particular line filling the smallest unit at the fastest speed (handling difficult) and the largest unit at the slowest operating speed (maximum exposure).

#### Filling duration

The overall length of the processing run should be similar to or greater than that normally encountered in routine manufacturing. The process simulation tests should be of sufficient duration to include a representative number of common interventions,

which might occur during an actual product filling operation. Where they are part of normal operation, gown changes, shift changes, breaks etc. should be simulated. If all normally expected interventions are not covered with the normal operating line speed for the desired media vials, this may be achieved by running the filling machine at slower speed.

#### Filling volume

The container need not be filled to its normal fill volume between 50% to 80% of the volume of entire internal surface area, and to allow the visual inspection to easily detect any microbial growth / contamination.

The following nominal fill volume with respect to the container size should be used for process simulation studies.

S.NO.	Vial capacity (ml)	Fill volume/vial (ml)
1	5	3.5
2	10	7
3	50	30
4	100	50

### HALF STOPPERING

The filling process followed by the stoppering process under the cRAB. (Closed restricted access barrier system) The stoppering should be partially stoppered to simulate the lyophilization process. The slotted rubber stopper should be used for stoppering process.

### TRAY LOADING

After the stoppering operation the vials should be transported to the tray loading system for loading in the lyophilizer without sealing under the cRAB. The trays should be completely filled and then transported in to lyophilizer.

### LYOPHILIZATION

The lyophilization process should follow the

simulated load /unload with shortened hold time. Partially stoppered vials are loaded in to the lyophilizer. A partial vacuum of 400m bar should be drawn on the chamber and this level should hold for a period of NLT 6 hr. at ambient temperature. Then the chamber should vent with filtered compressed air and stoppers should close completely within the chamber of lyophilizer before opening the door. The lyophilized stoppered vials should be removed from chamber for sealing.

### **SEALING, EXTERNAL WASHING OPTICAL INSPECTION**

The lyophilized stoppered vials should be removed aseptically from lyophilizer chamber to sealing station for sealing, then transported to external decontamination machine for washing of the external surface of the vials. All the filled vials should be optically inspected with trained operator.

### **ENVIRONMENTAL MONITORING/ PERSONNEL MONITORING**

Environmental monitoring including non-viable air bone particulate, settle plates, active air monitoring, swabs/contacts and personnel (at the end of processing).

### **FORCED SIMULATION STUDY/ INTERVENTIONS**

Following interventions/ forced situations shall be created during process simulation for checking the robustness of the aseptic process.

- Maximum number of personnel: continue media fill with maximum number of personnel that may be present at any point of year in a production shift (all operators, maintenance persons, supervisors, cleaners etc.)
- Change of operators.
- Repairs by maintenance personnel.
- Power failure – put off the power for 2 - 3 min

(maximum time required for generator to supply the electricity).

- Putting off Laminar Air Flow (LAF) – put off LAF for 3 - 5 min with continuing the filling activity.
- Manual filling operation.
- Perform following operation manually during the media fill operation. Mark all such vial incubate the same separately.
- Pick up the vial on the turn table.
- Remove the vial manually under the filling needle.
- Stopper the vial manually.

### **NORMAL INTERVENTION SIMULATION**

The process simulation should essentially include the activities that occur and/or being followed during normal vial filling operation such as

- The normal line set-up activities.
- Microbiological monitoring.
- Component addition.
- In process sampling activities.
- Lunch breaks.
- Anticipated interventions, such as in-process weight adjustments and/or checks, container/closure re-supply.
- Correction and /or simulation for correction of various non-planned interventions, such as container breakage, fluid leakage, container/closure jams, container tip-over etc.

### **POST FILLING INSPECTION**

Performed visual inspection of vials. The standard of inspection and criteria used for rejection should be the same as used in routine manufacture.

Unit that would be excluded during routine production, especially related to the integrity of the container closure, such as capping rejection, cracked or chipped vials should not be considered for incubation. as they are not representative of the microbial quality of the product. The reason for discarding all such units should be documented.

All filled units discarded due to the cosmetic defects, such as deform container etc. should be collected separately and should be incubated and assessed as part of the process simulation test.

### INCUBATION

The units should be transferred to a temperature controlled incubator as quickly as practically possible after the completion of the filling process. If there is a delay of more than 24 hrs between the filling and incubation steps, the incubation time should be counted from the start of incubation.

All media filled units should be incubated in inverted position in suitable trays/boxes. All media filled units should be incubated in a suitable and qualified incubator for a minimum of 14 days. Incubation temperature should be appropriate for the growth requirements of the microorganisms that are anticipated in the aseptic area.

The units should be incubated for 7 days at  $22.5 \pm 2.5^\circ\text{C}$  followed by further 7 days at  $32.5 \pm 2.5^\circ\text{C}$ .

### STASIS TEST (POST INCUBATION GROWTH PROMOTION TEST)

The media filled vials after the incubation should demonstrate the capability to promote recovery of wide spectrum of micro-organisms. The medium growth promotion properties should be evaluated using pharmacopoeial method, and the inclusion of environmental organisms may be beneficial. In general at least 5 vials for each test organism should be considered for stasis test.

### POST INCUBATION INSPECTION

Inspect 100% vials at an interval of 1, 2, 3, 7, 9, & at the end of the 14 days incubation period and recorded. In all cases the inspection must be performed by trained inspectors and must be supervised by a microbiologist.

### ACCEPTANCE CRITERIA

*Pre-filtration bioburden:* for information only.

*Growth promotion test: (GPT)* The media should be able to promote growth as per the requirement.

*Filtered bulk media:* The filtered bulk shall undergo the following in-process test and should meet the below specified criteria.

S.NO.	IN -PRO-CESS TEST	ACCEPTANCE CRITERIA
1	Sterility	No visible growth on completion of 14 days of incubation after filtration
2	GPT	Promotes the growth promotion even after incubation of 14 days

### INTERPRETATION OF TEST RESULTS AND PROCESS SIMULATION FAILURES

Any contaminated unit should be considered as objectionable & the underlying cause should be thoroughly investigated. The causative microorganism should be identified up to the species level.

The following table is useful for assessing the level of control in the aseptic manufacturing process & recommendation the subsequent steps/ actions needed.

S. NO.	Batch size (units)	Number of contaminated units	Interpretation
1	5000	1	Cause for revalidation, following an investigation
2a	5000 to 10,000	1	Investigation, including consideration for revalidation
2b	5000 to 10,000	2	Cause for revalidation, following an investigation
3a	10,000	1	Investigation
3b	10,000	2	Cause for revalidation, following an investigation

In the event of a process simulation failure the failure investigation shall immediately be initiated. However it must be ensured that, at least the following points are essentially considered as part of the process simulation test failure investigation.

- Allocation of sufficient & justifiable time for

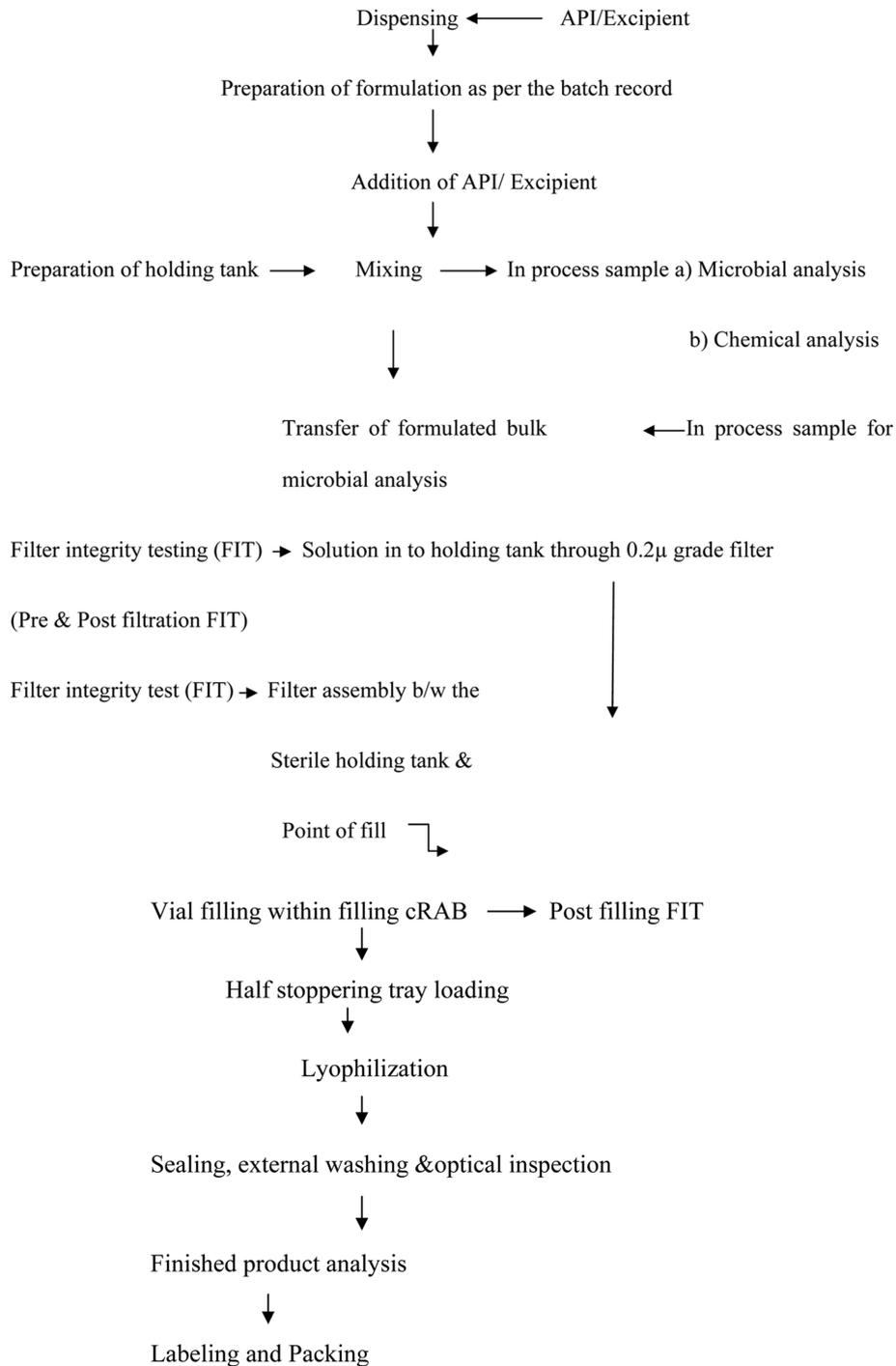


Fig. 1. The routine manufacturing of finished product.

- complete investigation.
- Identification of the causative organisms up to the species level.
- Comparison of the contaminant with the available

data base of the organisms recently identified from sterility tests, bioburden, and environmental monitoring program.

- Literature reference detailing possible sources of the organism to identify point of entry during the process.
- Review of processing records and/or video tapes for any deviations/non-compliance from the established procedures.
- Review of any deviations, down times and repairs before or during filling.
- Review of filter integrity test result.
- Review of all sterilization records associated with product components and equipments.
- Review of training records for all individuals (production, maintenance, microbiologist, cleaning etc.)
- Review of validation records to evaluate any changes in process.

Based upon the outcome of the investigation a comprehensive failure investigation report should be prepared, which should essentially describe the following points.

- A summary of occurrence.
- Investigation of all systems including those, which may be indirectly linked to the failure.
- The conclusion as to the cause & supporting documentation.

- Potential effect on the previous batches produced.
- Corrective & preventive action taken.
- Outcome of additional process simulation test if, required.

## CONCLUSION

Based on the above discussions, it can be concluded that, the purpose of an aseptic process is highly essential to prevent any type of contamination in pharmaceuticals especially in parenterals. For ensuring the sterility of pharmaceuticals, sterilization and aseptic filling including lyophilization techniques must be validated before proceeding to routine manufacture of those pharmaceuticals. Sterility of the pharmaceuticals depends upon how precisely and accurately process simulations can characterize a system of control intended to exclude maximum contamination.

## REFERENCES

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