VALIDATION OF ASEPTIC PROCESS

MEDIA FILL
VALIDATION OF ASEPTIC PROCESSES

Validation Aseptic of a test is the simulation of aseptic operations in which the product is replaced by a culture medium (nutrient for microorganisms). The goal is to provide guidance for the correct test, giving recommendations for the validation of aseptic processes. Provide guidance for GMP inspectors and for the purpose of training and preparation for inspections of company facilities.
Definition: Media FILL

• To ensure the sterility of products purporting to be sterile, sterilization, aseptic filling and closing operations must be adequately validated.

• The goal of even the most effective sterilization processes can be defeated if the sterilized elements of a product (the drug formulation, the container, and the closure) are brought together under conditions that contaminate any of those elements.
Definition: Media FILL

- An aseptic processing operation should be validated using a microbiological growth medium in place of the product. This process simulation, also known as a media fill, normally includes exposing the microbiological growth medium to product contact surfaces of equipment, container closure systems, critical environments, and process manipulations to closely simulate the same exposure that the product itself will undergo.

- The sealed containers filled with the medium are then incubated to detect microbial contamination. Results are then interpreted to assess the potential for a unit of drug product to become contaminated during actual operations (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing).
Definition: Media FILL

- Environmental monitoring data from the process simulation can also provide useful information for the processing line evaluation.

The purpose of Media-Fill Test, as the association says U.S. pharmaceutical PDA is:

- Demonstrate the ability of the process to handle Products in sterile.

- Qualify or certify the operators of aseptic area

- Comply with the requirements of good manufacturing practices.
SCOPE

• This test applies to all manufacturers involved in aseptic processing of finished dosage forms (human and veterinary) as well as manufacturers of sterile labelled bulk drug substances (active pharmaceutical ingredients).

• Liquid products of small and large volumes, Sterile powder, Aseptic manipulation, Creams and ointments, Oftálmilcos; antibiotics, lyophilized, suspensions, ointments and powders.
Frequency and Numbers of Runs

• When a processing line is initially qualified, individual media fills should be repeated enough times to ensure that results are consistent and meaningful. This approach is important because a single run can be inconclusive, while multiple runs with divergent results signal a process that is not in control.

• Recommend that at least three consecutive separate successful runs be performed during initial line qualification. Subsequently, routine semi-annual qualification conducted for each processing line will evaluate the state of control of the aseptic process.
Frequency and Numbers of Runs

• Activities and interventions representative of each shift, and shift changeover, should be incorporated into the design of the semi-annual qualification program. For example, the evaluation of a production shift should address its unique time-related and operational features.

• All personnel who are authorized to enter the aseptic processing room during manufacturing, including technicians and maintenance personnel, should participate in a media fill at least once a year. Participation should be consistent with the nature of each operator’s duties during routine production.
RE-VALIDATION INCLUDES

• Regular performance of process simulation studies;
• Monitoring of environment, disinfection procedures, equipment cleaning and sterilisation (including containers and closures);
• Routine maintenance and re-qualification of equipment, e.g. autoclaves, ovens, HVAC (heating, ventilation and air conditioning) systems, water systems, etc;
• Regular integrity testing of product filters, containers, closures and vent filters;
• Re-validation after changes.

It is the sum total of all validation data that provides the necessary level of assurance for aseptically produced products.
PROCESS SIMULATION

• Process simulation studies (media fills) are simulating the whole process in order to evaluate the sterility confidence of the process.

• Process simulation studies include formulation (compounding), filtration and filling with suitable media. Simulations are made to ensure that the regular process for commercial batches repeatedly and reliably produces the finished product of the required quality. However, each process simulation trial is unique and so it is not possible to extrapolate these results directly to actual production contamination rates.
The media fill should simulate the regular product fill situation in terms of equipment, processes, personnel involved and time taken for filling as well as for holding.

Where filling takes place over extended periods, i.e. longer than 24 hours, the process simulation test should extend over the whole of the standard filling period. In order to prevent excessively high numbers of units being filled it is usually acceptable to just run the machine for a reasonable time, if the validity of the simulation is not diminished by this procedure.
It should be considered that inert gases will prevent the growth of aerobic microorganisms. Therefore for process simulations sterile filtered air should be used instead of inert gases, also for breaking a vacuum.

Where anaerobes are detected in the environmental monitoring or sterility testing, the use of an inert gas should be considered for a process simulation, as inert gas is supporting the growth of anaerobes.
Preparation of liquid media

• Where a liquid nutrient medium is used it should be prepared in a similar manner to the product. The medium should be dissolved in Water for Injection in a standard manufacturing vessel.

• If heat is required to dissolve it then only minimal heat should be used. The pH of the medium should be measured and, if necessary, adjusted to bring it into the required range. The medium should be aseptically filtered into an aseptic holding vessel using the normal rodution filter and processing procedure. In justified cases it may be also acceptable to sterilise the media. All aseptic holding vessels should be covered by a process simulation test on a regular basis unless a validated, pressure hold or vacuum hold test is routinely performed.
Liquids Products

• The liquid growth medium for the simulation test is prepared as above and kept in a sterile holding vessel for the maximum permitted holding time before starting the simulation test. If the bulk solution is stored under refrigerated.

• Conditions during the holding time then this should also be performed for the medium. Vials and closures should be prepared as in regular production.
Sterile Products in Plastic Containers

• Ear and eye drops are typically marketed in plastic containers. Containers, inserts, closures and where applicable overseas are washed and sterilised as in regular production. Instead of sterilisation with heat, irradiation or ethylene oxide are used.

• Whilst clear plastic containers are frequently used for process simulation trials, the plastic is usually slightly opaque and thus hinders identification of contaminated units that show only a slight haze. In such case examination under natural or room lighting would not suffice.

• Where opaque containers are used for process simulation trials the whole contents should be removed for examination.
Ampoule Products

- Open or closed ampoule types may be used. They should be sterilised by dry heat and afterwards used in the simulation test as per the regular production run.

- Ampoules should be prepared as in regular production.
Injectable Powder Products

There are two possibilities for simulation of this process:
1º: Either by filling a sterilised liquid growth medium into the sterile container or
2º: Adding a powder (inert or growth medium) before or after a sterile diluent (WFI or growth medium).

Inert materials commonly used include: polyethylene glycol 8000 and carboxymethyl cellulose. These materials are usually sterilised by irradiation.
Suspension Products

• This procedure is comparable to the filling of liquid products, except for the process step of maintaining suspension of the ingredients. The stirring or recirculation should be part of the simulation.

• If aseptic additions are made to the bulk solution these should be simulated by the use of inert sterile liquids/powders.
Freeze Dried (Lyophilesed) Products

• Crystallisation of the medium should be prevented because it may reduce the likelihood of recovery of organisms.

Two simulation methods are commonly used:
• In the first one a dilute medium is subject to a cycle that removes water until a determined medium strength is obtained, but is not subject to freezing.
• The second method uses full strength medium and requires only a partial vacuum be drawn whilst the chamber should be kept at ambient temperature. There is a risk that the medium may boil over and contaminate the chamber unless conditions are tightly controlled. The absence of boiling under the defined cycle conditions should be confirmed.
Semi-Sólids Products (e.g. sterile ointments)

• For this simulation test the liquid growth medium is thickened to the appropriate viscosity, used as in the routine production procedure suitable thickening agents are agar and carboxymethyl cellulose.

• Other agents would need to be validated with regard to lack of their bacteriostatic and fungistatic properties.

• Metal and plastic ointment tubes prevent the examination of the medium in-situ. Usually the whole content of the tube should be examined and this is usually achieved by squeezing the contents into a plate (petri dish), and after whirling it is examined for turbidity and fungal colonies under defined light conditions or by performing a sterility test.

• If properly validated, an alternative method for detection of contamination of semi-solid products could be the use of media which changes colour in the presence of contamination.
Biological and Biotechnology Products

The manufacture of these products varies, such that there is not one single process. It may be more practical to validate the various segments of the process individually. The frequency of the revalidation should relate to the one of regular, commercial production.
TEST PERFORMANCE:
PROCESS SIMULATION TEST CONDITIONS

• The process simulation test should follow as closely as possible the routine aseptic manufacturing process and include all critical subsequent manufacturing steps.

• All equipment should remain the same wherever practicable as for the routine process. Appropriate combinations of container size and opening as well as speed of the processing line should be used (preferably at the extremes).

• The process simulation test should represent a “worst case” situation and include all manipulations and interventions likely to be represented during a shift.
TEST PERFORMANCE: PROCESS SIMULATION TEST CONDITIONS

• Worst case conditions are often thought to be the largest container with the widest mouth as it is exposed longer to the environment. However, there are exceptions to this and one of them is small ampoules run at the highest speed as the ampoules may be unstable and cause frequent jams thus necessitating frequent operator intervention.

• The fill volume of the containers should be sufficient to enable contact of all the container-closure seal surfaces when the container is inverted and also sufficient to allow the detection of microbial growth.
Simulation tests should be performed on different days and hours during the week and not only at the beginning of a work day.

If the same process is conducted in a separate clean room, this should also be validated.

In order to find the possible source of contamination it may be a good advise to video tape the aseptic fill and also number the individual vials or segregate vials in chronological order during incubation.
Selection of Growth Medium:

• The criteria for the selection of growth medium include: low selectivity, clarity, medium concentration and filterability.

• Low Selectivity: The medium selected should be capable of supporting a wide range of microorganisms, which might reasonably be encountered and be based also on the in house flora (e.g. isolates from monitoring etc.).

• Media used in the evaluation must pass a growth promotion test. The control organisms used should include those relevant strains of test microorganisms identified by relevant Pharmacopoeias as being suitable for use in the growth promotion test.
TEST PERFORMANCE:
PROCESS SIMULATION TEST CONDITION

- Growth promotion tests should demonstrate that the medium supports recovery and growth of low numbers of microorganisms, i.e. 10-100 CFU/unit or less.
- Growth promotion testing of the media used in simulation studies should be carried out on completion of the incubation period to demonstrate the ability of the media to sustain growth if contamination is present.

- Clarity: The medium should be clear to allow for ease in observing turbidity.
- Medium Concentration: Recommendations of the supplier should be followed unless alternative concentrations are validated to deliver equal results.
- Filterability: If a filter is used in the aseptic manufacturing process, the medium should be capable of being filtered through the same grade as used in production.
TEST PERFORMANCE: PROCESS SIMULATION TEST CONDITION

**Incubation Conditions:**

- It is generally accepted to incubate at 20-25°C for a minimum of 7 days followed immediately, or after a first reading, by incubation at 30-35°C for a total minimum incubation time of 14 days. Other incubation schedules should be based on supporting validation data.

- Prior to incubation the containers with the microbiological growth medium should be inverted or otherwise manipulated to ensure that all surfaces, included the internal surface of the closure, are thoroughly wetted by the medium. The containers should not be completely filled with medium in order to provide sufficient oxygen for the growth of obligate aerobes.

- The microorganisms present in the containers of the simulation test should be identified to genus but preferably species level to aid determination of the possible sources of the contamination.
TEST PERFORMANCE: PROCESS SIMULATION TEST CONDITION

Reading of the Test

• When inspecting the containers they should be compared to a known sterile container for comparison as some microbial growth shows up as a faint haze which is difficult to detect unless there is a control container to compare against.

• Personnel should be trained for this task.
TEST PERFORMANCE: PROCESS SIMULATION TEST CONDITION

Test Frequency:

- The manufacturer based on his individual circumstances should ultimately decide if more or more frequent tests are required than requested.
- It should be distinguished between “start-up” and “on-going” simulation tests.
- A “start-up” simulation test consists of three consecutive satisfactory simulation tests per shift and should be carried out before routine manufacturing can start.
- “Start-up” simulation tests are performed for example for new processes, new equipment or after critical changes of processes, equipment or environment as for example significant personnel changes (a new shift), modifications in equipment directly in contact with the product or modifications in the HVAC system.
TEST PERFORMANCE:
PROCESS SIMULATION TEST CONDITIONS

Test Frequency:

• “on-going” simulation test consists of one satisfactory simulation test per shift and is mainly performed for the periodic monitoring of aseptic conditions during routine manufacturing but also for example after less critical changes of processes, equipment or environment or if processing lines stand idle for more than 6 months.

• “On-going” simulation tests should be performed with each shift of each process line at least twice per year under the condition that there were no changes in the normal production procedures and no action limits were exceeded.

• Exceeding an action level demands a re-validation. Depending on the result of the follow-up investigation this re-validation may require the inclusion of one to three satisfactory process simulation tests.
TEST PERFORMANCE: PROCESS SIMULATION TEST CONDITIONS

INTERPRETATION OF DATA

• After the incubation period of the media-filled containers they are visually examined for microbial growth. Contaminated containers should be examined for evidence of container/closure damage which might compromise the integrity of the packaging system. Damaged containers should not be included as failures (positives) when evaluating results.

• The number of containers used for media fills should be sufficient to enable a valid evaluation. For small batches, the number of containers for media fills should at least equal the size of the product batch. The target should be zero growth and the following should apply:

• When filling fewer than 5000 units, no contaminated units should be detected.
TEST PERFORMANCE: PROCESS SIMULATION TEST CONDITIONS

INTERPRETATION OF DATA

• When filling 5,000 to 10,000 units: a) One (1) contaminated unit should result in an investigation, including consideration of a repeat media fill;  
   b) Two (2) contaminated units are considered cause for revalidation, following investigation.

• When filling more than 10,000 units:  
   a) One (1) contaminated unit should result in an investigation;  
   b) Two (2) contaminated units are considered cause for revalidation, following investigation.
For any run size, intermittent incidents of microbial contamination may be indicative of low-level contamination that should be investigated. Investigation of gross failures should include the potential impact on the sterility assurance of batches manufactured since the last successful media fill.

All contaminating microorganisms whether or not an alert or action limit has been exceeded should be identified to at least genus and preferably species where practicable to determine the possible source of contamination.
TEST PERFORMANCE:
PROCESS SIMULATION TEST CONDITION

INTERPRETATION OF DATA

• Here practicable to determine the possible source of contamination.

• If a process simulation test fails then due account should be taken of products filled between the last successful test and the test failure. Recording of any deviations during the simulation test is important to trace later on the exact cause and to evaluate the consequences.

• The investigation should identify batches that could be affected during this time period and the disposition of the affected batches should be re-assessed.
ENVIRONMENTAL AND PERSONNEL MONITORING

Non-viable monitoring:

• The location chosen for monitoring should be checked to ensure that the positions reflect the worst case. For room monitoring, the counts should be performed in locations where there is most operator activity. For the filling environment the counts should be performed adjacent to the filling zone and where components are exposed in such way as to detect operator activity within these areas.

• Monitoring with sampling probes located in such a way that they monitor the air from the HEPA filter rather than the air immediately surrounding the critical zones should be avoided. However the location of the sample device should not compromise the laminarity of the air flow in the critical zone.

• Initial validation should be checked to confirm that worst case positions have been adequately identified. These may be reconfirmed during process simulation tests.
Microbial Monitoring:

• Microbial monitoring should be performed in and around areas of high operator activity. It is not unusual to see settle plates and air sample locations well away from such areas. A typical example is where settle plates are located well to the rear of the filling machine where there is little or no operator activity. The same may be true for air sampling. It is important, therefore, to observe operator activity over a period of time and ensure that the monitoring sites are so located as to monitor operator activity.

• The process simulation test provides an ideal opportunity to confirm that worst case locations have been identified by the use of additional monitoring during the test.

• Additional monitoring around the affected area prior to disinfection may provide useful information as to the cause.
INTERVENTION MONITORING

• It is essential to include in a simulation test the various interventions that are known to occur during normal production runs, e.g. repair or replacement of needles/tubes, replacement of on-line filters, microbial sampling by monitoring personnel and sampling device, duration of stops on the line, filling and handling of stoppers etc.

• The process simulation test should last long enough to accommodate all possible interventions and a “worst case scenario”, which may include several unfavourable conditions which are occurring during routine processing.
STAFF TRAINING

• The routine training of personnel who work in a controlled environment needs special emphasis as people are potentially one of the main sources of microorganisms in the environment. Included are not only operators but also other personnel working in a controlled environment as staff responsible for monitoring, equipment maintenance, engineering, washing and preparation.

• A formal personnel training program is needed for all activities in each clean room. This means the program has to be planned, documented and repeated at adequate intervals to ensure that the once trained individual meets the ongoing requirements for the work in a controlled environment.

• This training encompasses subjects like basic microbiology, good manufacturing practice principles, hygiene (disinfection and sanitisation), aseptic connections, alert and action limits, and gowning procedures.
STAFF TRAINING

• Environmental monitoring personnel need a thorough understanding of the sources of contamination risks (e.g. inadequately disinfected / sterilised sampling equipment) that are involved with the sampling methods.

• Periodic process simulation tests (SKILL TEST) are required to ensure that the training of the personnel in charge of filling is effectively maintained. The competence of an individual should be formally assessed after attending the training courses and active participation of a process simulation test.

• The evaluation of filled containers of a simulation test should be done by personnel who are especially trained. They should have routine eye sight tests. This training should include the inspection of filled containers interspersed with contaminated units.

• Staff responsible for equipment maintenance, washing and preparation require regular retraining.
Container/Closure Integrity Testing

• The integrity of particular container/closure configurations should be assured by:

• Validation of the closure system by filling the container with sterile growth medium and inserting the container in a broth containing approx. 10^6 cfu/ml of a suitable micro-organism. The container is removed after submersion for a recognised period of time, disinfected and then incubated for 14 days. Growth would indicate a failure of the closure system.
Container/Closure Sterilisation:

- Problems are rarely encountered with sterilization of containers. However sterilization of stoppers might cause problems:

- Lack of air removal and adequate steam penetration: stoppers should not be packed too densely into trays or bags since this may prevent adequate air removal during the vacuum phase of the autoclave cycle. During the vacuum phases of the autoclave cycle stoppers may clump together to form a tightly bound mass. Pairs of stoppers may become attached to each other with the base of one stopper becoming attached to the top of the other stopper.
Equipment Cleaning and Sterilization

• Manual cleaning of equipment can be a problem but procedures should be checked to ensure that O-rings and gaskets are removed during cleaning otherwise there can be a build up of product residues and/or dirt.

• If equipment is steam sterilised in an autoclave then the following points should be addressed:

  • Sterilization of filters, housings and tubing might cause problems.

  • Problems are usually identified by slow heat up times inside the equipment compared to the chamber temperature. If there is a temperature lag of several minutes then this is usually indicative of entrapped air.
Equipment Cleaning and Sterilisation:

- The steam will heat up the entrapped air but sterilising conditions will not be obtained as saturated steam will not be present.

- Equipment should be wrapped and loaded into the autoclave in such a way to facilitate the removal of air from items in the load.

- Only porous load steam autoclaves with a vacuum system to withdraw entrapped air should be used for sterilising equipment.

- Passive displacement autoclaves (no vacuum to withdraw entrapped air) would normally not be appropriate because of the difficulties in air removal from the load.
Clean-in-place/sterilise-in-place (CIP/SIP).

- Validation of these systems may be difficult because of the potential incompatibilities in requirements for the design of CIP and SIP facilities. All systems have dead legs to a greater or lesser extent and the required orientation of the dead legs differ for CIP and SIP.

- The orientation for CIP dead legs is slightly sloping so that the cleaning solution can enter and also drain away. The dead leg for SIP is vertically up so that steam can downwardly displace the air.
Desinfection:

• There should be documented procedures describing the preparation and storage of disinfectants and detergents. These agents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilized.

• Disinfectants and detergents used in Grade A and B areas should be sterile at the time of use. If spray bottles are used they should be sterile before being filled and have a short in-use shelf life.

• Sporicidal agents should be used wherever possible but particularly for “spraying-in” components and equipment in aseptic areas. The effectiveness of disinfectants and the minimum contact time on different surfaces should be validated.
Filter Validation

• Whatever type of filter or combination of filters is used, validation should include microbiological challenges to simulate “worst case” production conditions. The selections of the microorganisms to perform the challenge test (e.g. *B. diminuta*) has to be justified. The nature of the product may affect the filter and so the validation should be performed in the presence of the product.

• Where the product is bacteriostatic or bactericidal an alternative is to perform the test in the presence of the vehicle (product without the drug substance). The filter integrity test limits should be derived from the filter validation data. The filter manufacturer should also evaluate the maximum permitted pressure differential across the filter and this should be checked against the batch documentation to ensure that it is not exceeded during aseptic filtration.
• In addition to the validation of the filter type the integrity of each individual product filter used for routine production should be tested before and after use

**Vent Filters:**

• It is important that the integrity of critical gas and air vent filters is confirmed immediately after the filling and if it fails, the disposition of the batch determined. In practice vent filters fail the integrity test more frequently than product filters as generally they are less robust and more sensitive to pressure differentials during steam sterilization.
Equipment Maintenance and Testing:

- Aseptic holding and filling vessels should be subject to routine planned preventive maintenance. Gaskets and O-rings should be checked regularly.

- Sight-glass gaskets are rarely checked routinely and after a number of autoclave cycles may become brittle and allow bypass of room air. All vessels should be subject to regular leak testing (pressure hold or vacuum hold).

- Standard Operating Procedures should be checked so as to ensure that any faults or failures of equipment identified by examination, testing or during routine cleaning of equipment are notified immediately to Quality Assurance.
STERILITY TEST

The sterility test can provide useful information on the validation status of aseptic process. It is important to compare the retest rate for aseptically processed products against that for terminally sterilized products. If aseptically processed products have a higher rate then this may be indicative of sterility problems not identified during validation.

This is not an unusual situation as validation cannot take into account all the possible permutation sand combinations in equipment, personnel and processes. A typical example of where the sterility test can identify a problem is in the case of damaged O-rings on aseptic holding vessels.
Sterility Test:

• However the number of retests should decrease due to the revised Sterility Test in the European Pharmacopoeia. The revision has been made in order to have a harmonised method in the European, the United States and the Japanese Pharmacopoeias. It means that retesting only is allowed if it can be clearly demonstrated that the sterility test was invalid for causes unrelated to the product to be examined.

• The conditions for considering the method invalid are given in the method. If retesting is allowed it should be made with the same number of containers as in the first test.

• Provision should be made to sample a sufficient amount of product from the same location of the load in case of retesting is performed.
QUESTIONS??????

THANK YOU

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