Technical Report No. 22 (Revised 2011) Process Simulation for Aseptically Filled Products

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Process Simulation for Aseptically Filled Products PDA Task Force

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1.0 Introduction

This document replaces the original PDA *Technical Report No. 22, Process Simulation Testing for Aseptically Filled Products*, published in 1996. The intent of the current effort is to update that document to reflect the continuing changes that have occurred in aseptic processing technology within the global industry. We have attempted to address the subject as fully as possible recognizing the notable contributions by other organizations, regulators, compendia and individuals who have worked in this area. In addition the report provides guidance where risk based approaches may be applied.

This technical report was disseminated in draft for public review and comment prior to publication. Many of the submitted comments have been included in the final document. We believe this approach accomplished the widest possible review of the document and ensures its suitability as a valuable guide to industry in the area of process simulation for aseptic processing operations.

This technical report should be considered as a guide; it is not intended to establish any mandatory or implied standard. The reader must recognize that there may be additional requirements imposed because of new or localized regulatory expectations that are not included in this document. This technical report does not provide a universally appropriate template for the execution of process simulation studies. Each company must determine the appropriate rationale and approaches applicable to their unique operations.

A recurring theme in this report is the consideration of risk to product sterility and patient safety as criteria for the design of the aseptic process simulation studies. Regulatory authorities have issued recommendations for aseptic process study design and companies should be aware of these recommendations when planning their studies. However, the use of relative risk and scientific evaluation as a means to provide information used to make decisions on study design may be of benefit because it should result in better understanding of the aseptic process and its capabilities. The use of risk assessments and related information may result in studies which go beyond the recommendations of regulatory authorities. It may also result in studies which differ from those recommendations. However, it should not result in studies which are less effective than those recommended by regulatory authorities.

1.1 Scope

This technical report addresses process capability assessment for aseptic processing. Such assessments consist of one or more aseptic process simulations (APS) during pharmaceutical and biopharmaceutical formulation and filling activities (referred to as secondary manufacturing in many parts of the world). Aseptic operations required in the preparation of sterile bulk materials and biotechnology inoculums, and feed materials are not a part of this document; refer to PDA *Technical Report No. 28: Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals* ***(1)***.

While the focus of this document is on aseptic processing in the pharmaceutical and biopharmaceutical industry, application of the concepts and principles to the preparation of sterile medical devices and diagnostics may be appropriate.

1.2 Previous PDA Publications

PDA has published previous reports on the aseptic filling process: *Technical Monograph No. 2: Validation of Aseptic Filling for Solution Drug Products; Technical Report No. 6: Validation of Aseptic Drug Powder Filling Processes,* and the 1996 edition of this report, *Technical Report No. 22: Process Simulation Testing for Aseptically Filled Products* ***(2,3,4)***.

1.3 Reason for Revision

Since the above reports were issued, there have been continued advances in facility and equipment design, such as the use of barrier, isolation and blow-fill-seal technologies. The understanding and philosophies of aseptic process qualification, validation and control have evolved as well. In addition, global regulatory and standards authorities have revised their own guidance's on aseptic processing ***(5,6,7,8).***

The 2011 version of Technical Report No. 22 features the following new or revised information.

- Risk Management: This report frequently references the use of quality risk management concepts in the design of APS programs
- Concepts and Principles: There have been clarifications and enhancements of **Section 3.0,** in keeping with current scientific knowledge, experience and regulatory expectations
- Lyophilized Products: The section on "Lyophilization of Dilute Medium" has been removed as this APS approach is generally not considered appropriate.
- Freezing of Media: For similar reasons references to freezing of media have been removed.
- Powder Filling: Certain APS approaches for powder filling have been removed, including:
  - — On-line powder fill followed by off-line liquid fill
  - — Non-aseptic liquid fill, sterilized, and followed by on-line powder fill
  - — Off-line liquid fill followed by on-line powder fill
- Isolators, restricted access barrier systems (RABS), blow-fill-seal (BFS): Updated information include in **Section 4.8**
- Elements of APS: Enhanced information is included for: filling speed, interventions, and duration and number of units filled
- Interventions: This report differentiates aseptic process interventions as either "inherent" or "corrective," a distinction that is helpful in understanding their relationship to microbial contamination and the design of the APS program.
- Acceptance Criteria: Section 10 includes background, current recommendations and good practices in setting acceptance criteria for the APS
- On-going Process Evaluation: Formerly referred to as 'Validation Maintenance,' this section has been updated to reflect that the state of control is an ongoing process.

Note: This 2011 revision of Technical Report No. 22 represents a significant update of the content of the report. This version should be treated as a full replacement of the 1996 version. As such, readers/users should fully review this edition. With the publication of the 2011 version of Technical Report No. 22, PDA no longer supports or considers valid the 1996 version.

1.4 Purpose

Aseptic process simulation (sometimes referred to as a media fill) is a useful tool to evaluate the capability of aseptic processing activities. For the results to be meaningful, engineering and manufacturing controls, maintenance activities, quality systems, employee training, written procedures, environmental control, environmental monitoring, strict adherence to aseptic technique, and intervention controls should be in place. APS simulates the aseptic process from the point of product and component sterilization to closure of the container (including any process/handling

steps subsequent to sealing that might impact container integrity), substituting a microbiological growth medium for the sterile product.

The aseptic process simulation also provides a means for the evaluation of changes made to an aseptic processing operation which might impact the sterility of the final product. An aseptic process simulation can be useful in identifying potential weaknesses in an aseptic process that might contribute to the microbiological contamination of the product during processing.

The purpose of an aseptic process simulation is to:

- Assess the capability of an aseptic process under a given manufacturing environment and process controls
- Demonstrate that appropriately designed and implemented process changes are acceptable
- Evaluate the proficiency of aseptic processing personnel
- Demonstrate compliance with current Good Manufacturing Practice
- Demonstrate the appropriateness of operating practices used in support of aseptic processing
- Challenge the aseptic process for microbial contamination vulnerabilities

The successful completion of an APS cannot be considered a validation of aseptic processing in the same sense that a performance qualification effort involving biological challenge and temperature measurement can support a steam sterilization process. Aseptic processing relies heavily on personnel intervention practices, equipment features, facility design/control and procedures that in combination serve to exclude microorganisms from sterile components and products. These elements of aseptic processing cannot be as rigorously controlled as a sterilization process; resulting in a higher risk of contamination. The aseptic process simulation is only a demonstration of the capability of the process to produce sterile products aseptically at the time of its execution using the defined process, materials, facility, equipment and personnel.

The APS does not provide information which relates directly to the sterility of a specific product batch. Therefore, the fact that a specific APS does not meet the required acceptance criteria does not necessarily indicate a sterility problem for any particular production batch. However it is an indication that some event has occurred during the APS leading to contamination of one or more units. The potential impact of the event on production materials must be determined.

Similarly, the successful performance of a high-risk aseptic intervention, technique or practice in a simulation does not in itself justify its use or acceptability during production. The aseptic process simulation is one tool for evaluating the processing steps used to manufacture a sterile product. The APS provides supporting data demonstrating the on-going capability of producing sterile product by aseptic processing

A holistic approach must be used to control aseptic processes. An aseptic process incorporates many systems to assure and control sterility of the materials produced. These systems include:

- Product, equipment and component sterilization
- Personnel training and certification of aseptic gowning and aseptic techniques
- Equipment and facility sanitization programs
- Environmental system: microbial levels, differential pressure, air pattern, velocity, temperature and humidity, air supply
- Personnel, material and equipment flows

- Standard operating procedures/work instructions or their equivalent.
- A underlying quality system approach to process control

These systems must be routinely monitored to provide verification of their continued acceptable performance, by which the sterility assurance of a manufactured product can be established. Therefore, it is important to validate all of the related sanitization and sterilization processes independently, such as sterilization/depyrogenation of the product, container, closure and all product contact and indirect product contact surfaces (e.g., stoppers, hoppers).

2.0 Glossary of Terms

Action Level (environmental monitoring)
An established microbial or non-viable particle level that, when exceeded, should trigger appropriate investigation and corrective action based on the investigation.

Action Plan
A written plan consisting of elements to be accomplished to achieve a specific result. The plan describes responsibility for each element and a target date for completion.

Aerobic Microorganism
A microorganism that utilizes oxygen as the final electron acceptor during metabolism; a microorganism that will grow primarily in the presence of oxygen. For the purpose of this report, this definition encompasses facultative anaerobes.

Alert Level (environmental monitoring)
Established microbial or non-viable particle level giving early warning of potential drift from normal operating conditions; not necessarily grounds for definitive corrective action but typically requires follow-up investigation.

Anaerobic Organism
A microorganism that does not utilize oxygen as the final electron acceptor during metabolism; microorganism that will grow only in the absence of oxygen.

Aseptic Filling
The part of aseptic processing where a pre- sterilized product is filled and/or packaged into sterile containers and closed.

Aseptic Processing
Handling sterile materials in a controlled environment, in which the air supply, facility, materials, equipment and personnel are regulated to control microbial and particulate contamination to acceptable levels.

Aseptic Processing Area (APA)
Controlled environment, consisting of several zones, in which the air supply, facility, materials, equipment and personnel are regulated to control microbial and particulate contamination to acceptable levels.

Aseptic Processing Simulation (APS)
A means for establishing the capability of an aseptic process as performed using a growth medium.

Note: Aseptic processing simulations are understood to be synonymous with media fills, process simulations, simulated product fills, broth trials, broth fills, etc.

Barrier System
A system of physical partitions that affords ISO 5 protection by partially separating its interior from the surrounding environment utilizing airflow.

Bioburden
Total number of viable microorganisms on or in a health care product prior to sterilization.

Campaign
A series of consecutive production batches manufactured without intervening cleaning and sterilization.

Change Control
A formal program that describes evaluation and actions to be taken if a change is proposed or completed to facilities, materials, equipment, and/or processes used in the fabrication, packaging, and testing of drugs, or a proposed or completed change that may affect the operation of the quality or support systems.

Colony Forming Unit (CFU)
One or more microorganisms that produce a visible, discrete growth entity on a semi-solid, agar-based microbiological medium.

Compounding
A process in which a bulk drug substance is combined with one or more excipients and/or another bulk drug substance to produce a drug product.

Contamination Rate
The percentage of units filled in a process simulation that are positive for microbial growth after incubation.

Critical Area
An area designed to maintain sterility of sterile materials. Sterilized product, containers, closures, and equipment may be exposed in critical areas

Environmental Flora (isolates)
Microorganisms associated with a processing environment.

Environmental Monitoring Program
Defined, documented program which describes the routine particulate and microbiological monitoring of processing and manufacturing areas. (**Note:** The program should reference a corrective action plan in cases where action levels are exceeded.)

Growth Promotion Test
Test performed to demonstrate that media will support microbial growth.

Integrity Test
Test to determine the functional performance of a membrane filter or container/closure system.

Intervention
An aseptic manipulation or activity performed by personnel that occurs within the critical area. This technical report regards interventions as either corrective or inherent.

Intervention, Corrective
An intervention that is performed to correct or adjust an aseptic process during its execution. Examples include such activities as: clearing component misfeed, adjusting sensors, and replacing equipment components.

Intervention, Inherent
An intervention that is an integral part of the aseptic process and is required for set-up or routine operation and/or monitoring, e.g., aseptic assembly, container replenishment, environmental sampling, etc. Inherent interventions are required by batch record, procedure, or work instruction for the proper conduct of the aseptic process.

ISO 5
Environmental operating conditions defined in ISO 14644-1, "Cleanrooms and associated controlled environments" ***(5)***. (**Note:** For total particulates, ISO 5 approximates the Class 100 description from the now obsolete U.S. Federal Standard 209, and is roughly comparable to Grade A as defined in European GMP Annex 1 – "Manufacture of Sterile Medicinal Products.")

Isolator, Closed
A decontaminated unit meeting ISO 5 conditions that provides uncompromised, continuous, isolation of its interior from the surrounding environment. Any air exchange with the surrounding environment takes place only through microbially retentive filters.

Isolator, Open
A decontaminated unit meeting ISO 5 conditions that provides uncompromised, continuous, isolation of its interior from the surrounding environment. It may transfer air directly to the surrounding environment through openings (e.g., "mouse holes") that preclude the ingress of microbial contamination.

Media Fill
See Aseptic Processing Simulation.

Microbiological Identification
Biochemical characterization of isolated colonies to determine the isolate genus and, where feasible and appropriate, the species.

Positive unit
Unit filled in an aseptic process simulation that exhibits detectable microbial growth after incubation.

Restricted Access Barrier System (RABS)
RABS are aseptic processing systems (ISO 5) intended to substantially reduce human borne contamination within the aseptic environment where sterile product, containers, closures and equipment are exposed by the use of separative devices and defined mechanical features and operating procedures.

Shift
Scheduled periods of work or production, usually less than 12 hours in length, staffed by alternating groups of workers.

Sampling Frequency
Established period for collecting samples.

Sterile
Absence of life; usually refers to absence of viable microorganisms.

Note: In practice, no such absolute statement

regarding the absence of microorganisms can be proven (see sterilization).

Sterility Test

Test performed to determine if viable microorganisms are present.

Sterilization

Validated process used to render a product free of viable microorganisms.

Note: In a sterilization process, the nature of microbiological death or reduction is described by an exponential function. Therefore, the number of microorganisms which survive a sterilization process can be expressed in terms of probability. While the probability may be reduced to a very low number, it can never be reduced to zero.

Validation

Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. (**Note:** There has been wide- spread evolution in the concept and definition of validation in recent years. Readers should refer to the U.S. FDA, EC and other related regulatory definitions and guidance regarding validation.)

Worst Case

A set of conditions encompassing upper and lower processing limits and circumstances, including those within standard operating procedures, that pose the greatest chance of process or product failure (when compared to ideal conditions). Such conditions do not necessarily induce product or process failure.

3.0 Process Simulation Concepts and Principles

The validation of an aseptic processing operation should include aseptic process simulations using a microbiological growth medium in place of the product. This aseptic process simulation, or media fill, normally includes exposing the microbiological growth medium to product contact surfaces of equipment, container closure systems, critical environments, and process interventions to closely simulate the same exposure that the product itself will undergo. Results are then interpreted to demonstrate the potential for a unit of drug product to become contaminated during actual operations.

3.1 Number and Frequency of Simulations

The number and type of aseptic process simulations performed should be based on an assessment of risks posed by the process or significant changes to the process. New facilities and processes are regarded differently based on the determination of those risks.

For a new facility or production process, process simulations are performed as part of the overall validation activities. Initial process simulations are generally conducted after completion of:

- Equipment qualification
- Facility environmental system qualification
- Sterilization process validation
- Implementation of environmental decontamination procedures
- Personnel training and gowning certification
- Environmental monitoring
- Room qualification
- Standard operating procedures

The aseptic process simulation supports that a new facility, line or process is operating under the desired state of control. Historically, there has been a regulatory expectation that at least three consecutive successful process simulations are performed when qualifying a new facility, filling line or process ***(6,7,8)***. This may be an appropriate activity to utilize risk management approaches.

There is a regulatory expectation for at least semi-annual simulations for a qualified facility, line or process ***(6,7,8,9)***. Additional process simulations may be required based on a risk assessment to assist in the evaluation of any major changes to procedures, practices or equipment configuration (See **Section 12.0 – Ongoing Process Evaluation**). Flexibility in media fill design may be appropriate for isolators which offer robust separation (built in by design) and a consistently heightened level of product protection.

3.2 Worst Case

A useful technique in the validation of pharmaceutical processes is the employment of "worst case" scenarios. The use of "worst case" situations is intended to challenge the process under conditions that may be on the edge of normal operating conditions. If, under the circumstances of the worst case challenge, acceptable results are achieved, then there is greater confidence in the reliability of the system under more routine conditions. Worst case does not mean creation of artificial conditions or environments which exceed allowed operating conditions and which can force a system failure.

Worst case conditions vary depending on the operations or risk being considered. For example, executing the APS using the maximum number of personnel may be worst case at certain times as gowned personnel are the greatest source of microbial contamination in an aseptic process. In other situations worst case may include executing the process with fewer people if this results in more movement by the process operators.

Other examples of "worst case" practices may include:

- Using room/equipment at the maximum time period after completion of sanitization/sterilization (clean hold time)
- Using the slowest fill speed for the largest container (maximum opening)
- Using the highest fill speed for the smallest container (handling difficulty)

The worst case conditions selected for inclusion in an APS should be predefined based upon characteristics of the operation. The identification of appropriate worst case conditions should be accomplished by conducting an assessment of the APS covering the relevant variables and their microbiological impact on the process. Such assessments can benefit from the application of risk management principles. The assessment conclusion should outline the variables selected as worst case and considerations/rationale for their selection.

3.3 Risk Assessment

Risk is defined as the combination of the impact of a hazard or unwanted event and its likelihood of occurring and harming the patient ***(10,11).*** The hazard associated with aseptic processing in the context of this technical report is the loss of sterility assurance or potential for pyrogens. The use of risk assessment principles should be of benefit in making decisions related to aseptic processing and its simulation. It would be beneficial to take into account risk to product quality and patient safety when confirming the design of the APS study.

A number of risk assessment methods specific for aseptic processing have been defined ***(12,13,14,15,16,17,18).*** A risk assessment may be performed to determine, identify, and evaluate the aseptic process steps and interventions that can potentially adversely affect the sterility assurance risk to the product. Risk assessments can also be used to determine the worst case manufacturing scenarios related to container size, configuration, line speed, batch size, and operating conditions. Where feasible, efforts should be made to mitigate identified risk by eliminating or changing risky process steps, and improving facility, equipment, and process design. The risk assessment should be documented and communicated to stakeholders and management in the organization including the Quality Unit.

3.4 Ongoing Evaluation

An assessment of ongoing controls and changes to the aseptic process, personnel, equipment, computerized systems, facility and critical utilities may be performed to determine the risk of such changes to the assurance of sterility. This assessment may be used to justify the type of response or simulation of the process change needed to assure that the change has not adversely affected the aseptic process. Risk assessment may be beneficial to present a rationale for the need and extent of aseptic process simulation as a result of change control. The risk assessment and risk management decisions should be recorded, approved and incorporated into the change control documentation.

4.0 Process Simulation For Sterile Dosage Forms

The conduct of process simulations for aseptically produced parenteral products entails simulation of the process from the point of sterilization of the product and product contact surfaces, including containers and closures, through sealing of the filled container. Any aseptic activities performed during dosage form compounding are a necessary part of the APS. The following section summarizes considerations to be made in the design and performance of process simulations for aseptically produced solutions, lyophilized products, suspensions, ointments and powders.

The aseptic process is simulated through the use of production operations in which a sterile medium and/or placebo is handled in a manner which approximates as closely as possible the methods used in routine production. The application of this principle to a specific aseptic process or procedure may require adaptation of the methods described in this report to that process or procedure. Such adaptations should be accomplished in a manner which will not reduce the effective challenge of the simulation and, as a result, appear to improve the results of the simulation relative to routine production operations.

It is important to note that where media is utilized in the process simulation its sterilization need not be performed in a manner identical to that utilized for the product being simulated (See **Appendix 13.2).** For example, aseptic process simulations do not support the filtration validation of the product/process being simulated, so differences in the filter area, filter media, etc., are acceptable providing these changes do not enhance the aseptic process or eliminate a process step which could adversely affect the sterility assurance of the product.

This document does not provide a universally appropriate template for the execution of process simulation studies. The following are general approaches to common aseptic processing simulations. Unique product configurations, presentations, and processes may require modification from the information provided in this section. A company must determine appropriate rationale and approaches applicable to their unique operations.

The steps in the execution of an aseptic process simulation are listed in **Appendix 13.3.**

4.1 Aseptic Compounding Activities

The aseptic compounding process simulation may be performed as a stand-alone activity or be fully integrated with the aseptic filling process. Where a separate simulation is performed the methods, practices and acceptance criteria described in *PDA Technical Report No. 28: Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals* may be appropriate ***(1).*** When integrated with the filling process no adjustment for the compounding is made in the acceptance criteria.

Process simulation studies should assess all aseptic operations performed subsequent to the sterilization of the materials utilized in the process:

1. Aseptic steps for products that are solutions may be limited to set-up, sampling, and in-situ filter integrity testing.
2. Suspensions, ointments and other non-filterable formulations may require a substantial number of aseptic steps (e.g., sterile powder addition).
3. Processes requiring the addition of sterile powders should employ an acceptable placebo material in containers identical to those utilized in the process being evaluated (See Appendix 13.1).
4. Blending, milling and subdivision processes performed at a sterile powder facility require similar attention.

Each of these processes should be supported by simulation studies that incorporate the aseptic activities. The complexity and number of activities should be comparable to that required in the process being supported by the simulation. Sterilization of equipment and components, and their post-sterilization integrity, is validated independently of process simulation. Media hold studies as performed in support of fermentation/bioreactor operations and product stability in the bulk state are part of the validation of those non-aseptic processes and are not relevant to the aseptic processing simulation.

4.2 Solutions

Compounding Operation – After sterilization, the medium should be passed through the equipment train as though it were an actual production batch, and all routine aseptic procedures used in the manufacture of a batch performed, e.g., sampling, aseptic connection, etc. Any aseptic manipulations performed during and at the end of the holding period should be simulated as well, e.g., sampling, re-filtration, and product recirculation.

Filling Operation – The containers, and closures (if necessary), product contact equipment and filling parts should be prepared using standard operating procedures (SOPs). The filling machine should be operated at the pre-determined fill rate for the container size being utilized (See Section 3.2 for guidance on "worst case" conditions). The containers should be sealed and the medium-filled units collected in sequentially numbered trays or boxes. If possible note the time of collection since it allows contaminated units to be linked with the approximate time and the activity being simulated during the media fill. The filled units should be briefly inverted and swirled after filling to assure all internal surfaces contact with the medium.

The process simulations should be observed and/or video recorded to assure proper intervention challenge is performed as well as to provide further insight into problem resolution should positive growth units be subsequently observed. All activities which take place on the filling line should be a part of the simulation, e.g., weight/volume adjustments, replenishment of containers, addition of components. An expanded discussion of these and other considerations in the conduct of process simulations is presented elsewhere in this document (See **Section 5.0**).

4.3 Lyophilized Products

Most lyophilized products are aseptically filled solutions which are transferred to sterilized lyophilization chambers after filling. Within the industry, various container-closure systems are used, e.g., vial with fluted stopper, vial with combination stopper and crimp, multi-chambered vial, pre-filled multi-chamber syringe. The less common packages may require further adaptation of the methods described in this section.

Compounding Operation – See **Section 4.2** on compounding of solution products.

Filling Operation – See **Section 4.2** on filling of solution products presented earlier.

Lyophilization Operation – The methods employed for lyophilization process simulation are generally similar to those used for solution fills with the addition of the transport, loading, simulation of freeze-drying, stoppering, unloading, and closing steps. Presented below are several possible means for evaluation of these activities. Other approaches are possible.

4.3.1 Simulated Load/Unload with Shortened Hold Time

Containers are filled with medium, and stoppers are partially inserted. The containers are loaded into the lyophilizer at ambient temperature. A partial vacuum, insufficient to cause boiling of the

medium, is drawn on the chamber and this level is held for a pre-determined time. The chamber is then vented and the stoppers are seated within the chamber. The stoppered units are removed from the aseptic processing area and sealed.

Advantage(s)

The medium is not frozen. Therefore, there are fewer concerns with regard to microbial survival in a freezing process or the ability of the medium to support growth.

Focuses on loading and sealing activities, which are presumed to be the greatest source of potential contamination.

4.3.2 Simulated lyophilization

Containers are filled with (full strength) medium, and stoppers are partially inserted in the necks. The containers are transported and loaded into the lyophilizer. A partial vacuum, insufficient to cause boiling of the medium, is drawn on the chamber at ambient temperature, and maintained for the duration of a normal lyophilization process. The stoppered units are removed from the lyophilizer and sealed.

Advantage(s)

The medium is not frozen. Therefore, there are fewer concerns about microbial survival in a freezing process or the ability of the medium to support growth.

Simulates duration of the lyophilization process.

Disadvantage(s)

Time-consuming to perform, extending the simulation through the entire lyophilization cycle.

4.3.3 Special Considerations unique to the Production of lyophilized Products

4.3.3.1 Freezing of Media

In order to maximize microbial recovery, freezing of media is not recommended.

4.3.3.2 Vacuum Levels and Duration

In the simulation of a lyophilization process, the depth of vacuum drawn on the chamber and the period of time for which this vacuum is held are important considerations. The vacuum must not be so low as to permit the medium in the container to boil out, thereby invalidating the simulation.

4.3.3.3 Anaerobic Conditions

In the production of lyophilized products it is common practice to use sterile inert gases to break the vacuum on the chamber. These gases can remain in the product containers after sealing. Where Soybean-Casein Digest Medium is used for the conduct of the process simulation, air should be used rather than an inert gas to assure aerobic conditions for the process simulation. The introduction of air and the elimination of the inert gas introduction should not enhance the aseptic process or represent a 'better-than-production' process condition. (See **Section 7.3**.) The use of an inert gas and anaerobic medium (e.g., Alternate Fluid Thioglycollate Medium) would be appropriate where the persistent presence of strict anaerobic organisms has been confirmed in either environmental monitoring or, more likely, during end product sterility testing. Where anaerobes have not been detected in the environmental monitoring or sterility testing, lyophilizer process simulations should utilize Soybean-Casein Digest Medium and air.

4.4 Suspensions

While sterile suspensions are not as common as solutions, they are used for the administration of insoluble sterile materials such as some antibiotics, vaccines and corticosteroids. Process simulation for suspension filling requires the use of procedures which mimic those used in the manufacture and filling of suspensions.

Compounding Operations – The simulation procedures should include the particular aspects of suspension manufacturing including sterilization of the vehicle, addition of the sterile powder and homogenization of the suspension. The most basic adaptation of the standard liquid process simulation is the addition of a sterile placebo powder to a tank of medium. This simulates the critical difference in the production of suspensions: the addition of a sterile solid under aseptic conditions.

Note: See **Appendix 13.1** for a description of the placebo material selection, sterilization and evaluation.

Filling Operations – These should be carried out in a manner similar to that described for solution fills, with the introduction of any routine changes in the filling set-up to accommodate suspension filling. Where recycle lines, surge tanks, agitators and other modifications are employed to fill suspensions, they should be employed in the simulated fill.

4.5 Ointments/Creams/Emulsions/Gels

Sterile ointment, cream, emulsion and gel production processes can resemble either solution or suspension products, depending upon the solubility of the active and inactive materials in the bases. The simulation should use the actual procedures used by the firm in their operations.

Compounding Operations – Follow the procedures previously described for either solution or suspension compounding, using whichever method more closely simulates the actual compounding procedure used for the product being simulated. It may be necessary to formulate the media such that it increases the viscosity of the medium to more closely resemble the product's filling characteristics to enable it to be processed in the filling equipment without difficulty.

Filling Procedures – Filling of sterile ointments generally is performed on a filling machine quite different from one employed for vials, syringes or ampoules. The differences in equipment design and operation aside, the basic approach to the conduct of the fill is very similar to that employed for other dosage forms.

Special Considerations Unique to the Production of Sterile Ointments, Creams, Emulsions and Gels – Inspection of units – The post-incubation inspection of filled process simulation tubes may require extra care. When opaque containers are filled, it is acceptable to extrude the material from the individual tubes into glass containers for individual inspection. Care should be taken in the extrusion and inspection, to assure growth will be detected. Alternatively, special tubes which do not contain the opacifying agent may be purchased for the process simulation. The use of thickening agents in the medium may be required to allow for filling on equipment intended for viscous fluids.

4.6 Powders

The production of sterile powders requires processes and equipment quite different from that used for the production of other aseptically produced sterile dosage forms. Presented below are several means for evaluation of powder filling activities. Other approaches are possible.

Compounding Operations – These activities should be included if sterile bulk actives are blended with sterile buffers, preservatives or other sterile materials prior to filling. Blending, milling, subdivision and other procedures carried out at the filling site can be simulated using an appropriate placebo powder using the same methods as those employed for the process.

Note: See **Appendix 13.1** for a description of the placebo material selection, sterilization and evaluation.

Note: The simulation of aseptic processes utilized for the manufacture and isolation of sterile bulk powders is not part of this document. The aseptic production of sterile bulk pharmaceutical chemicals is addressed in PDA *Technical Report No. 28: Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals* ***(1).***

Filling Operations - The filling of dry powders utilizes equipment quite different from that used for filling liquids. In order to perform a process simulation for a powder filling procedure, adaptations to the filling practices must be employed. It should be noted that utilization of medium in the evaluation of a dry powder fill process often requires two individual filling operations (one each for the liquid medium and the placebo powder). The individual contamination contribution from each of these individual filling steps may increase the overall risks of contamination. Process controls must take these risks into account. Presented below are several possible means for evaluation of these activities. Other approaches are possible.

4.6.1 Liquid Medium Filled by the Powder Filling equipment

A limited number of sterile powder filling machines are capable of liquid filling with little or no modification. While these units may not fill liquids to the same degree of consistency with which they fill powders, their flexibility greatly simplifies the process simulation. In this procedure, the liquid medium is introduced as a direct substitute for the sterile powder in the fill hopper. The methods used to introduce the medium, of course, are different from those utilized when powder filling, but that is a minor adaptation to the process when contrasted with the modifications necessary for other fillers. The conduct of the process simulation is essentially the same as that described in **Section 4.2,** integrating all of the fill line activities during the simulation.

Advantage(s)

Only a single fill machine is required; a separate liquid filler is not necessary. This greatly simplifies the conduct of the process simulation.

Additional media controls for the liquid fill machine are not required (see below).

Disadvantage(s)

Feed set-up may differ from that used for powder fill.

4.6.2 Dry Powder Filler with Supplementary Liquid Fill Capability

Some manufacturers of dry powder fillers offer adaptations to their machines which can add a supplementary liquid filling capability. The liquid filling capability of these machines is not equivalent to conventional liquid fillers, and is used only in process simulations. In this manner, the same filling machine could be used for both the liquid and solid filling operation.

Advantage(s)

Single filler; no additional line modifications required

Disadvantage(s)

May need to be operated at lower speeds due to the design of the filler. Some designs may not fill liquid into every vial which receives powder.

4.6.3 On-line liquid Fill Followed by On-Line Powder Fill

In this approach, a liquid filling machine is added to the filling line prior to the powder filler. A volume of medium (in the range appropriate for a liquid fill) is added to the container, followed by a fill of a sterile placebo material. The choice between this method and that in **4.6.4** is governed largely by the dictates of space on the filling line.

Advantage(s)

All processes on-line, no additional handling of containers.

Disadvantage(s)

Second filler to set up and qualify.

This process subjects the open units to additional exposure time.

4.6.4 On-Line Powder Fill Followed by On-Line Media Fill

This method is used when the physical addition of a liquid filler before the powder filler is not possible.

Advantage(s)

All processes on-line, no additional handling of containers.

Disadvantage(s)

Second filler to set up and qualify.

This process subjects the open units to additional exposure time.

Potential for powder aspiration from the container when the liquid is filled last.

4.6.5 Special Considerations unique to the Simulation of Aseptic Filling of Sterile Powders

Negative Controls – With the exception of the methods in **Sections 4.6.1** and **4.6.2,** all of the dry powder process simulations entail the filling of both a sterile liquid and a sterile placebo powder. It may be appropriate to fill some number of containers solely with liquid. For use as negative controls. The intent of this liquid fill procedure is to evaluate the potential for the liquid fill system as the contamination source, should the combined fill demonstrate contamination. The liquid units generally are filled before starting the powder fill. This assures that if the liquid filler cannot be operated successfully, the remainder of the fill is canceled.

Liquid controls are not required, but may be performed at the firm's discretion. The absence of negative controls on the liquid fill may adversely affect investigations of APS failures.

Where liquid controls are used, the detection of contamination in any control requires investigation to isolate the additional fill process as the most probable cause. If this is the case, then the simulation may be invalidated.

Positive controls consist of growth promotion test in placebo or placebo with liquid fill.

4.7 Other Dosage Forms and Device/Drug Combinations

This document presents validation approaches for the more common sterile dosage forms. There are other, less common dosage forms, e.g., inhalants, aerosols, device/drug combinations, and implants which are produced. The aseptic processes for manufacturing these products can be simulated by adapting the methods described above. Evaluation of their acceptability may require sterility type tests for the device portion of the combination.

4.8 Other Aseptic Processing Technologies

Properly designed and operated aseptic processing technologies designed to eliminate human intervention, such as Restricted Access Barrier Systems (RABS), Form-Fill-Seal (FFS), Blow-Fill-Seal (BFS), and isolation technology, can provide enhanced environments in which the processes described in this report may be performed. Use of isolators and highly automated processes with rare interventions during processing mitigate contamination risk and allows for greater flexibility in media fill design (number of vials needed for media fill, duration of fill, and accounting for multiple shift changes). Relative risk and unique aspects of these technologies should be taken into consideration when assessing the design of aseptic process simulation studies. The information in this section of the technical report is not appropriate for "barrier systems" or "open Restricted Access Barrier Systems" that cannot be fully operated and/or decontaminated while sealed.

4.8.1 Restricted Access barrier Systems

Restricted Access Barrier Systems limit the access of personnel and materials into the critical environment by defined mechanical and barrier systems. These systems are intended to substantially reduce human-borne contamination within the aseptic environment. Restricted Access Barrier Systems are subjected to high-level disinfection prior to use. The performance capability of Restricted Access Barrier Systems varies with details of their design. Closed Restricted Access Barrier Systems can frequently be decontaminated/operated in a manner essentially identical to isolators.

4.8.2 Form-Fill-Seal and blow-Fill-Seal

BFS and FFS aseptic processes involve some unique process conditions, which should be considered in the design of the APS study.

Aseptic process simulation considerations should include:

- Ability to view contamination in translucent or opaque plastic containers.
- Interventions involving manual manipulation in forming, cutting and adjustment/removal area, fill nozzle exposure area, aseptic connections, and manipulations post-filtration.
- Affect of de-flashing operation on creation of post filling leakers.
- Foaming characteristics of media which could affect sealing of containers.
- Fill volume including lower than normal volume may affect the formation of the container,
- Heat exposure to media, and open container dwell time,
- Effect on container integrity post-fill when separating units

4.8.3 Isolation Technology

Isolators eliminate direct access of personnel into the critical environment by defined mechanical

and barrier systems. Isolators are intended to eliminate human-borne contamination within the aseptic environment where sterile product, containers, closures and equipment are exposed. Isolators are decontaminated and material entry is accomplished using validated transfer systems. Unless otherwise noted, the principles and methods presented in this document apply to the simulation of aseptic processes using isolation technology. The performance of these systems should be considered in the design of the APS.

5.0 Documentation

Documentation is an important element of a process simulation program, since it serves as a record of the simulation's rationale, and its performance. Subsequently this record can be of assistance in sterility failure investigations as well as for regulatory bodies' review of the adequacy of the simulation ***(19)***. Therefore process simulation studies should be well documented.

Documentation provides instructions for performance of the study, criteria for acceptance of the study, historical references, study results, and proof that the studies were conducted. The documentation should include procedures, protocol deviation investigations, final report, and positive unit investigations that are reviewed and approved by the Quality Unit.

An overall aseptic process simulation policy or procedure presents the requirements for scheduling, conducting, and documenting process simulation studies. This will include the rationale for "worst case" container and line configuration, intervention and process step inclusion, and re-evaluation frequency. A master plan may also be developed to present requirements and rationale for conducting APS studies for a specific product, facility, or manufacturing line.

5.1 Process Definition

The processes to be simulated are defined as any and all manufacturing steps which occur after product equipment and container/closure sterilization and can adversely affect the sterility assurance of the product. In some cases, this may include process/handling steps subsequent to sealing of the container (e.g., leak detection, automated inspection, etc.) where damage from handling can adversely affect product container integrity. Processes may include (but are not limited to) the post sterilization handling of the drug product, transfer and holding of sterile drug product, transfer and handling of sterilized container and closure, loading and removal of product from a lyophilizer, the filling of product to the point the drug product is sealed and capped, and any subsequent inspection or handling steps which may affect sterility assurance of the product.

5.2 Protocol/Procedure Preparation

A formal written protocol or procedure should be prepared, approved, and issued prior to the start of the study. The document should be identified for traceability and should be approved prior to execution by representatives of the Quality Unit. Other stakeholders may review and approve the document at the discretion of the company. The document should include but not be limited to the following information:

- Groups responsible for execution, microbial testing, and approval of study
- Rationale for the "worst case" parameters chosen as appropriate simulation of routine operations
- Identification of the process to be simulated
- Identification of the room or rooms to be used
- Identification of the filling line and equipment to be used including fluid path configuration details if multiple configurations are available
- Type of container/closure to be used
- Line speed
- Minimum number of units to be filled
- Number and type of interventions and stoppages
- Identification of units to be excluded from incubation and rationale
- Number, identity and specific roles of people participating
- Media to be used

- Volume of medium to be filled into the containers
- Incubation time, temperature and duration for the filled units
- Environmental monitoring to be performed
- A copy of the batch record to be used
- Accountability requirements
- Acceptance criteria for all activities
- Description of the documentation required for the final report
- Duration of the aseptic process simulation
- Duration of routine production fills being simulated
- Definition of conditions that may cause the simulations to be invalidated and decision-making authority.

Other factors may have to be considered due to the nature of the process to be simulated. The protocol should require that prior to execution of the process simulation study critical support system qualifications and process validations have been verified to be successfully completed and approved.

5.3 APS Execution Record

Execution of the protocol may be performed through the instructions noted in a batch record. The batch record gives detailed instructions on how to perform the process simulation. It should be writ- ten in the same format as a normal production batch record and contain all the routine data and sign-off requirements. All information applicable to the process simulation which normally would be attached to a batch record also should be attached to the simulation batch record, e.g., cleaning and sterilization records for pieces of equipment used, release stickers for the containers and closures, etc. All interventions, whether inherent (an integral component of the process) or corrective (required to maintain operation), and stoppages should be documented in the batch record as to the type of intervention, time the intervention occurred, aseptic operators involved with the duration of the intervention or stoppage, and the number of the box or tray being filled. The executed batch re- cord should include information which is relevant to the performance and completion of the study. This includes but is not limited to:

- Names of individuals participating in the simulation
- Number of units filled
- Number of units incubated
- Filled unit incubation time, temperature and duration
- Number of units positive and box or tray number of any positive unit(s)
- Number of units rejected for cause during pre-incubation inspection (e.g.,, damaged container, defective seal)
- Growth promotion of medium (after incubation)
- Filled unit accountability
- Media sterilization
- Filter identification and membrane integrity test results
- Environmental and personnel monitoring results
- Record or log of routine and non-routine occurrences including those in the filling room, which may have an affect on the outcome of the study
- The description and resolution of any discrepancies or deviations to the protocol

The executed batch record should be approved, signed and dated.

5.4 Final Report

The final report is an evaluation of the data from the batch record and environmental control records. Based upon this information, a conclusion is formulated regarding the acceptability of the aseptic process simulation as adequate simulation of the manufacturing process.

The final report should note the results of the study, whether the study met the acceptance criteria, the resolution of discrepancies or deviations to the protocol, the conclusion of the study (is the study successful), and any follow up actions.

Note that any media fill positives should be investigated and, if possible, a root cause determined, regardless of whether the simulation meets the acceptance criteria. This investigation and cause should be documented (See **Section 10.0**). Any "aborted" media fills conducted for that line or conducted during the overall process simulation study should be noted in the report.

The final report and completed documents should be approved by representatives of the Quality Unit. The protocol, batch record, final report, investigations, and any and all relevant support documentation should be retained in accordance with the firm's policies and regulatory requirements.

5.5 Process Simulation Observation

The process simulation should be observed to assure that all planned activities are properly executed and represent an appropriate challenge to the process capability. Observation may also be used to augment aseptic conduct and technique training.

Observation should commence upon the initiation of the process simulation, including equipment set-up, and continue until the process simulation has completed. Monitoring of the simulation should be performed by individuals having the knowledge and competency to assess if operators have used proper aseptic conduct as well as to assure that aseptic interventions have been executed properly so as to provide for realistic assessments of sterility control.

Process simulation observation should be documented and/or video recorded. The use of video recording has advantages as process simulation activity can be reviewed in detail to assist with training or failure investigation.

6.0 Microbiological Environmental Monitoring

It is important to determine and understand the environmental conditions present during the process simulation study.

A properly defined, controlled and executed environmental monitoring program provides increased assurance of sterility by demonstrating that environmental conditions conducive to the production of sterile product are being met over the duration of the aseptic process and that appropriate systems and utilities are functioning as intended.

The elements and processes that define and detail the establishment and maintenance of an effective environmental monitoring program (including sample site selection, sampling frequency, alert and action levels, methodology and interpretation of data) can be found in *PDA Technical Report No. 13: Fundamentals of a Microbiological Monitoring Program* ***(20).***

Process simulation should be carried out using the routine environmental monitoring operating procedures and sampling requirements. This should include the set-up period, set-up interventions and set-up personnel. Any changes to the routine environmental monitoring requirements during process simulation (e.g., additional sampling or change in sampling location) should be explained and documented.

The results of the environmental and personnel monitoring are used to assess whether suitable processing conditions were maintained during the process simulation. Additionally, environmental and personnel monitoring results obtained during process simulation can aid in the identification of root cause if the process simulation yields any positives (See Secti**on 11.0**).

Environmental Monitoring excursion investigations should be completed and approved. Failure to meet established routine monitoring levels should be addressed according to routine monitoring investigation procedures and actions taken according to those procedures. Environmental monitoring excursions are not an automatic cause to reject the results of an APS; rather any decision should be based on the investigation results.

Note that "passing" an aseptic process simulation with environmental monitoring results that exceed action limits does not mean that the aseptic process may be routinely performed in such an environment and should not be used as justification for doing so.

7.0 Elements of Aseptic Process Simulations

This section contains general information to consider when conducting any type of process simulation. Issues such as operator intervention, fill volumes, line speeds, container sizes and run duration play a key role in effectively simulating the production process.

Careful consideration should be given to each of the parameters discussed in these sections for inclusion in the aseptic process simulation study design. Parameter selection and justification should be well documented and approved by the organization's Quality Unit.

7.1 Facility and Filling Machine Considerations

Process simulation programs should assure that aseptic operations are evaluated on a semi-annual basis as a minimum. That may necessitate multiple process simulations in a given environment to address the permutations of aseptic processing which take place there. If there is a unique process or container-closure system, different from the others in the room or on the fill machine, then a process simulation at six-month intervals should be performed for each unique process. The key consideration is that it is the aseptic filling or manufacturing process which is being evaluated, and not a specific product.

7.2 Equipment Set-Up

The set-up for an aseptic process usually entails some manual assembly of the sterilized equipment. Equipment set-up activities may require more manipulation of critical surfaces than subsequent processing operations. The process simulation should be designed to detect potential contamination from set-up activities. Personnel performing set-up operations during routine manufacturing operations should perform set-up during process simulation studies. The set-up should not enhance the aseptic process or make it better. Equipment set-ups which are similar and involve "same" activities or interventions may not require separate assessment if included in the process simulation matrix. The steps involved in the equipment set-up are a required part of the process (and thus the process simulation) and are considered inherent interventions.

7.3 Media Selection and Preparation

The most common medium for process simulation is Soybean-Casein Digest Medium (SCDM). SCDM is a general purpose growth medium well suited for the recovery of aerobic microorganisms of the types commonly associated with human borne contamination. It is very similar to SCDA which is widely utilized for microbial recovery in aseptic areas for the same reason. Replacement of the products, diluents, and buffer solutions with media is customary when performing process simulation studies.

Aseptic processing conducted in a strict anaerobic environment (one which maintains less than 0.1% oxygen throughout the process) should be evaluated with alternate Fluid Thioglycollate Medium (FTM) or other suitable medium, in addition to aerobic evaluation. An anaerobic media fill may also be considered for a typically aerobic process if anaerobic microorganisms are consistently recovered during periodic environmental monitoring (for anaerobes), or if facultative anaerobes are detected exclusively in FTM sterility test medium. In either case, oxygen is excluded from processing and parameters such as container fill volume and inert gassing may require modification to provide a true anaerobic environment for the aseptic process simulation study. (See **Appendix 13.2** for additional detail)

7.4 Inert Gassing

Nitrogen or other inert gases are used to provide a low oxygen environment for oxygen-sensitive

products. They are also used to provide positive pressure for solution transfer. Nitrogen (or other gases) for these uses does not provide a true anaerobic environment (less than 0.1% residual oxygen is needed for anaerobic conditions). In these instances, filter sterilized air should be utilized in lieu of an inert gas for process simulation studies. Air should replace the inert gas and be delivered by the same delivery system thus assuring the purge/transfer set-up and delivery considerations are fully considered in the simulation.

The sterility of the inert gas system is confirmed through filter validation, integrity testing, and sterilization of connecting lines downstream of the filter, not by means of the process simulation. The use of an inert gas with Soybean-Casein Digest Medium may inhibit growth. If it is necessary to use an inert gas for simulation of an oxygen free process, testing should confirm the ability of the inert gas/medium combination to support microbial growth.

7.5 Container Size

In general, process simulation trials should entail at least the filling of the largest and smallest containers on a given filling line based on a facility established matrix. Exceptions to this general rule occur when the same filling machine, on the same filling line is used for different product presentations. In these instances, the flexibility of the filler may make it necessary to evaluate more than one set of large and small containers, because the filling set-ups are so different. For example, if filling another size container results in a process which is significantly changed (e.g., additional manipulation or fill parts), then that size container should be included in the study.

7.6 Container/Closure Configuration

When a particular container/closure configuration provides unique operating challenges (e.g., tipping, jams) and causes increased interventions, it is recommended that a separate process simulation be performed with that particular configuration. Clear containers of identical configuration may be substituted for opaque or amber containers to aid in the detection of contamination. Closures which require additional or significantly different handling/insertion methods should be considered in the study. (See **Section 3.2** for further information)

7.7 Filling Speed

In general, the fill speed to be used for most containers should be set at the production filling speed range for that size container in commercial production. Where production filling speeds on a line are variable, if higher or lower speeds in the speed range result in the potential for greater interventions or other adverse impact such as increased product exposure to environment, that speed can be considered 'worst case' and should be considered when selecting process simulation parameters (See **Section 3.2**).

7.8 Fill Volume

The container need not be filled to its normal fill volume. The fill volume must be controlled and monitored as performed during routine filling. Where partial fills are employed, the fill speed should follow the advice given in **Section 7.7.** Regardless of the actual fill volume, the process simulation should include a fill weight/volume adjustment using methods identical to those employed during production.

While the specific amount of medium utilized in a partial fill may not be critical, there are two general criteria. First, there must be enough medium in the container to contact all the container-closure seal

surfaces when the container is inverted and swirled. Second, there must be enough medium in the container to allow for the detection of microbial growth.

The volume of headspace should be considered in the growth promoting capability of the media to support aerobic microorganisms (See **Section 7.16**).

7.9 Interventions

As a general rule during routine aseptic processing, interventions (inherent and corrective) should be minimized. Interventions that would represent an unreasonable risk of contamination should not be included in either process simulation or routine production. The choice of interventions to be considered for an APS can benefit from the use of formal risk assessment and quality risk management principles. Anticipated interventions should be assessed to determine the amount of micro- biological risk their performance poses to the product or process. Where an intervention, even if rarely performed, poses a higher risk to the product or process due to its complexity and infrequent execution, the company may consider including the intervention at a higher than normal frequency in the APS.

Intervention assessments should include the activities which occur during an aseptic filling process that could affect the sterility of the product (e.g., inherent interventions, such as weight adjustments and container/closure re-supply) as well as any permitted corrective interventions (e.g., correct for equipment and container breakage, closure jams, misalignment or part replacement). (See **Section 8.0** for expanded detail.)

7.10 Duration and Number of Units Filled

The duration and number of units filled for an aseptic process simulation should be sufficient to adequately challenge the aseptic process, the operators that perform interventions, and the capability of the processing environment to provide appropriate conditions for the manufacture of a sterile product. Inherent interventions that occur during processing, such as loading of components, environmental monitoring and equipment set-up, are an integral part of each aseptic process simulation. The frequency of inherent interventions during the APS is generally consistent with the frequency during routine production (See **Section 7.9** for discussion on risk). The duration of the APS should be long enough to capture the potential microbiological impact of performing those interventions. Corrective interventions should be performed at a frequency defined in the aseptic process simulation model. If the production process is run on a campaign basis, the aseptic process simulation should be conducted in a consistent manner (See **Section 7.11**).

The APS should also be of sufficient duration to include a representative number of interventions which might occur during an actual production filling operation. Where they are part of normal operations, gown changes, breaks and shift changes should be simulated. Justification of the selected number of units filled, duration and yield should be included in the process simulation study design.

The following are general approaches to define aseptic process simulation fill duration and number of units. Uniquely small or large batch sizes may require modification from the approaches listed below. Each company must determine appropriate rationale and approaches applicable to their unique operations.

Table 7.10.1

Production batch description	Production batch size (# filled containers)	Minimum APS batch size (# of filled containers of media)	Recommendations
Small scale	$\leq$ 5,000 units	$\leq$ 5,000 units	APS batch size should be at least equal to production batch size.
Mid-scale	5,000 to 10,000 units	5,000 to 10,000 units	APS batch size should be of comparable size to the production batch size. For high speed filling or with maximum size production batches, it may be appropriate to fill additional units in order to accommodate normal aseptic manipulations, interventions, and realistic simulation of the process.
Large scale	$>$ 10,000 units	$>$ 10,000 units A variety of approaches can be employed to evaluate the process. See guidance below.	See guidance below
Manual Fill	Any amount	Same as production batch size	APS batch size should be at least equal to production batch size. Entire manual filling operation represents an intervention which should be captured.

For large production batches the following approaches may be considered for the APS batch size and approach.

- Alternate between Media Filled and Empty Units - Fill adequate number of units to represent the manufacturing process under normal conditions (the number of units should be based on contamination risk of the process and be sufficient to accurately simulate activities representative of the manufacturing process), and include interventions at the appropriate frequency for routine production. Operate the line without filling media into all of the containers. Media should be filled periodically throughout the process including at the beginning, and end of the routine process duration, as well as during and immediately after any planned intervention. Under this approach, the aseptic personnel, procedures and processing environment are fully evaluated but the number of media-filled units produced is limited.

- Alternate between WFI Filled and Media Filled units – Follow the approach described above, except fill units with WFI when not filling with media. Filling two different liquids on an alternating basis introduces additional complexity to the fluid handling system. The impact of media dilution with WFI which could alter the growth promoting characteristics must be considered. Adequate qualification of the growth promoting characteristics of the media should be demonstrated.

- End of Process Simulation – Aseptic processing simulation is conducted after the conclusion

of a production lot without an intervening tear-down, cleaning, sanitization or sterilization of the processing equipment. This approach must be coupled with simulation of the normal production set up, start up, and commencement of filling. The product fluid pathway should be flushed with a sufficient quantity of media to remove drug product. Such flush volume should be qualified and specified in the APS protocol. Adequate qualification of the growth promoting characteristics of the media should be demonstrated. For inhibitory products, the product fluid pathway contact points should be changed with newly prepared and sterilized parts or equipment prior to conducting the aseptic process simulation

- Fill enough units to simulate interventions, as noted in **Section 8.0** of this report, and for a period of time not less than the amount of time operators are required to spend working in the clean room without leaving for break.

7.11 Campaign Operations

Note: Aseptic filling campaign operations are complex and require control and validation programs which fall outside the scope of this technical report. The following basic information is included with the recognition that additional guidance is necessary for adequate validation of aseptic campaign operations.

The use of campaigns in which a series of batches is produced following sterilization, with or without intermediate cleaning is increasingly common with BFS/FFS, RABS and isolation technology. During a campaign the environmental conditions are essentially constant. Decontamination of the system environment and/or removal, cleaning, and sterilization of filling parts is not normally performed between batches within a campaign. However, product pathway CIP/ SIP (clean in place/ sterilize in place) and filter changes may be performed between production batches. Validation of campaigns should include, in addition to other activities, start-of-campaign and end-of-campaign studies in a manner similar to that described for large batches (See Secti**on 7.10**).

In campaign modes the following situations may be possible and supportable in appropriately designed aseptic process simulations:

- Multiple product lots of the same formulation can be manufactured.
- Configuration/fill volume may change during the campaign.
- Campaign lengths substantially greater than one day are attainable.
- There is the potential to change the product formulation if cleaning and re-sterilization of delivery system can be performed aseptically.
- Production may not be continuous over the time period (days or shifts without production are possible.

Initial and periodic demonstration of campaign duration (total time or batches) should be based on an assessment of the operational elements. This is an appropriate activity for the application of risk management approaches. (See **Sections *3.3***).

7.12 Pre-Incubation Container Inspection

The normal product inspection process is qualified for the removal of non-integral containers (e.g., missing or misaligned closures, cracks in glass, poor crimps, etc.) due to the possible breach of product sterility. This inspection process should be maintained for APS filled units, with non-integral APS units removed during the pre-incubation inspection. The removal of such non-integral units is appropriate as failure to do so can lead to false-positives that may inaccurately represent the sterility

control of normal operations. (See **Section 7.6**). For the purpose of the APS, cosmetic, particulate and fill volume defects should be ignored and such units incubated and included in the APS evaluation and contamination rate.

7.13 Incubation Conditions

Prior to incubation, filled APS units should be inverted or manipulated to ensure contact of the medium with all internal surfaces of the closure system before they are incubated. APS units should be incubated for a minimum of 14 days unless supported by another qualified duration/method. The temperature chosen should be based upon its ability to recover microorganisms normally found in the environment or in the product bioburden. A single incubation temperature in the range of 20-35°C may be used ***(6).*** Data should be available to show the suitability of the selected incubation temperature to support growth. The selected temperature should be controlled and monitored continuously throughout the incubation period.

7.14 Post-Incubation Inspection

At the end of the incubation period, visual inspection of all APS units for growth is performed to determine the outcome of the aseptic process simulation. The inspection process should be performed by trained inspectors, who have demonstrated the ability to detect both low and high-level microbial growth patterns. Firms may choose to inspect units partway through the incubation period.

Since the pre-incubation inspection is expected to remove any units with container/closure defects, if such a defect is detected during the post-incubation inspection it must be appropriately investigated for cause and corrective action.

7.15 Unit Accountability and Reconciliation

As the target acceptance criterion for an aseptic process simulation study is zero contaminated units, a high level of APS unit control and accountability is necessary. Accurate counts should be performed at each step in the simulation: filling, pre-incubation inspection, and post-incubation inspection. At the conclusion of the post-incubation inspection, filled units are re-counted to verify pre-incubation accountability. In the event of a discrepancy an investigation should be performed to determine the source of the variance and potential impact on the validity of the APS study.

7.16 Growth Promotion

The growth promotion properties of the incubation media should be evaluated using pharmacopeial methods. The inclusion of tests for environmental organisms or those isolated from sterility test positives are recommended ***(6).*** Growth promotion studies are commonly performed after 14 days of incubation.

7.17 Post Simulation Cleaning

Defined procedures should be in place for removal of residual media from media/product contact equipment, surfaces, and clean room.

8.0 Interventions

8.1 Interventions

Human operators/personnel are the greatest source of microbial contamination during an aseptic process. As a consequence, activities performed by personnel in proximity to the aseptic fill zone, also called interventions, must be carefully controlled to assure they do not compromise the sterility of the materials being produced. The execution of interventions during the aseptic process simulation is critical to the process capability demonstration. To demonstrate that capability, process simulations should include all the inherent (part of the process) and corrective (problem resolution) interventions that occur during an aseptic filling process.

It is essential to include in a process simulation the interventions that are known to occur during normal production runs. Interventions that are permitted in a production operation should be specifically documented and included in process simulations at the same frequency. While interventions and/or stoppages are normally recorded in the batch record, the manner of documenting these occurrences varies. In particular, line stoppages and corrective interventions should be sufficiently documented in batch records with the associated time and duration of the event. In addition to lengthened dwell time of sterile product elements in the critical area, an extensive intervention can increase contamination risk.

8.2 Identifying Interventions Associated With an Aseptic Process

The identification of interventions and their frequency may be determined from a review of completed batch records, batch related documentation and discussions with operating personnel. The goal of this activity is to list all interventions for each circumstance. For firms with multiple aseptic operations, interventions may vary from one fill line to another, even if both are filling similar products and containers. Variations in product type may add activities that are specific to individual situations ***(21).*** The basis and number of the required simulated interventions must be documented.

8.2.1 Inherent Interventions

Inherent interventions are normal and planned activities that occur during an aseptic filling process (e.g., equipment set-up, weight adjustments, closure re-supply, container re-supply, EM sampling, etc.). Inherent interventions are not corrections to events that occur on the filling line. Rather they are a planned and documented part of the overall process and are performed during the APS at a defined frequency or point of the filling operation. While these activities may not be specifically documented within the routine production batch record; they should be recorded as interventions during an aseptic process simulation.

8.2.2 Corrective Interventions

Corrective interventions are performed to correct or adjust an aseptic process during its execution. While not part of the planned aseptic process, they are well understood operations and are recognized to sometimes occur during processing. Corrective interventions include: container break- age, tip-over of a container, stopper jam, change in filling needle, change in filling equipment, dose adjustments/samples, clearing automatically rejected units, etc. Since corrective interventions are unplanned, they should be clearly identified and documented in the associated records. The APS should include a defined and representative number of corrective interventions that can be expected to occur during an actual production filling operation. Inclusion of corrective interventions in successful process simulations can demonstrate acceptable aseptic technique and control.

A new corrective intervention (e.g., one not included in the firm's process simulation program) performed during a routine aseptic fill must be evaluated. The intervention may be determined acceptable if it is similar to a previously simulated intervention and was performed with proper aseptic tech-

nique. The evaluation of such an intervention may include an aseptic process simulation sub- sequent to the fill in which that intervention occurred. Evaluation of such a corrective intervention should be supported by a risk assessment.

Documentation of corrective interventions in the batch record will allow for identification, cataloging and trending of interventions occurring during production. Documentation of a new corrective intervention should be reviewed during the batch disposition and review process. This review should determine the extent to which the intervention was a deviation from the routine manufacturing process and the acceptability of the intervention itself. The assessment should conclude with either an acceptance or rejection of this intervention relative to the current and future manufacturing processes. If the new practice is accepted then it should be reviewed for inclusion into the list of identified interventions simulated during a scheduled APS.

8.3 Intervention Procedures

There should be an approved list of allowed interventions, both inherent and corrective, which may occur during production and in the APS. Procedures should be established that describe the methods for performance of these interventions. The procedures listing the types of inherent and corrective interventions and how to perform them should be updated, as necessary, to ensure consistency with actual manufacturing activities.

In the conduct of an intervention that requires removal of units from the process, the units to be removed must be designated by a specific number and/or location (e.g., all units from the turntable to the first fill head). This facilitates process execution where the line may not be fully populated and a fixed number of units relevant to the intervention can not be identified and removed.

8.4 Study Design

The number, type, and complexity of inherent interventions that occur with each run, as well as corrective interventions and events (e.g., maintenance, stoppages, and equipment adjustments) should be incorporated into the aseptic process simulation study design ***(6).*** When the aseptic simulation procedures are consistent with those used for routine production, the simulation will be a more authentic representation of the routine process. For filling lines that utilize similar equipment set- up configurations the 'worst case' configuration may be used in the study design to support similar configurations. See Sect**ion 3.2** for more detail regarding 'worst case' considerations.

Anticipated interventions for inclusion in the APS protocol should be considered in terms of both expected frequency and of microbiological risk they pose to the product or process. Tracking the intervention frequency in production allows development of an APS program which reflects both intervention frequency and risk. In general, interventions that commonly occur should be routinely simulated, while those occurring rarely can be simulated periodically.

The performance of interventions should be accomplished by qualified personnel, including maintenance personnel, following defined procedures. The ability of the operator/mechanic to intervene in the process to fix a "mechanical failure" should be reflected in the APS. For ex- ample, a firm may choose to simulate an equipment breakdown. However, it is difficult to predict the frequency of occurrence of breakdowns, part replacements, or other non-routine corrective interventions. If these types of corrective interventions do not occur naturally during a process simulation study, the activities associated with them must be simulated to qualify their performance during routine operations.

Defined intervention procedures provide for controlled process activities and help avoid uncontrolled,

personnel-dependent activities. Intervention procedures that are demonstrated acceptable can normally be performed by other trained and qualified individuals. Note: This is not the case for highly complex activities such as line set-up where only individuals who have performed that activity successfully (as supported by acceptable aseptic process simulation results) are considered qualified (See Sect**ion 9.0**).

8.5 Handling of Intervention-Related Containers

If written procedures and batch documentation adequately describe the removal of units not filled or sealed during an intervention, then those units do not need to be incubated. However, in no case should more units be removed or a larger zone cleared during a media fill intervention than would be cleared during a production run. Units that would normally remain on the line as acceptable units must be incubated to verify that appropriate control and segregation is maintained. Units which are closed prior to an intervention, but would otherwise not be included in production should be incubated and included in the evaluation of the study.

9.0 Personnel Qualification

Each person in the aseptic filling suite (e.g., operations, engineering, quality) has the potential to introduce microbiological contamination; however, the risk to product may vary with the specific job function.

Personnel working in the clean room should be capable of adequately performing their job function, properly trained in their work function, and qualified to perform those functions. Work functions include aseptic process gowning, clean room practice, aseptic technique, as well as specific operational functions. Operational functions may include filtration and fill system set-up, adjustment, repair, maintenance, cleaning, sanitization, operation, component and product handling, transfer, sampling, monitoring, and other inherent and corrective interventions. The requirements for the qualification of clean room personnel should be written in a formal procedure and the results documented.

9.1 Personnel Prerequisites

Personnel must successfully meet the firm's gowning certification requirements. They should have completed all relevant training, including but not limited to GMP training, procedure training, gowning training, clean room practices training, training in basic microbiology and specific clean room operation, function and relevant intervention procedure training.

9.2 Initial Qualification

Personnel should:

1) Demonstrate their proficiency in aseptic technique by successfully performing a qualification test entailing manual media manipulation not associated with an APS (See Sect**ion 8.4**) ***(22);***

or

2) Participate in a successful aseptic process simulation run in which they perform the same function(s) to the extent that they will perform it during actual production.

9.3 Periodic Qualification

Personnel should participate in a successful aseptic process simulation run in which they perform the same function(s) to the extent that they will perform it during actual production at least once per year.

9.4 Access Without Prior Qualification

There may be situations where nonfilling personnel must enter into an aseptic processing area during an aseptic process to observe or perform non-aseptic process activities. It is recommended that the company have a procedure for this situation. It is recommended that individuals who have not successfully completed qualification be closely supervised and accompanied while in the clean room and not be present during critical aseptic process steps, and that their access to the aseptic processing area be restricted to the specific function required (e.g., equipment maintenance, audit, etc.).

9.5 Loss of Qualification Status

Previously qualified personnel may be considered to have lost that qualified status if one or more of the following occurs:

1) They fail to qualify as presented in **Section 9.2.**
2) They participate in a failed media fill, where the cause of the failure is related to their performance.
3) They perform in the clean room or the workplace in a manner deemed unacceptable in relation to clean room or aseptic process operations or functions.
4) They fail to maintain gowning certification.

The individual's qualification can be reestablished once the specific deficiency is properly remedied.

9.6 Personnel Monitoring

Postgowning personnel monitoring during the aseptic process simulation, including gown and glove sampling should be performed to at least the extent that it is performed during actual production. Additional monitoring may be conducted. For further information on personnel monitoring techniques and approaches, please refer to *PDA Technical Report No. 13: Fundamentals of an Environmental Monitoring Program* ***(20)*****.**

10.0 Acceptance Criteria

10.1 Background

The ultimate goal for the number of positives in any process simulation should be zero. This is true regardless of the number of units filled during the APS or the number of positives allowed. A sterile product is, by definition, one which contains no viable organisms. Regulatory authorities have provided guidance on process simulation acceptance criteria and these should be well understood before developing internal requirements ***(6,7,8).***

However, there are numerous technical problems in achieving this goal. For example, media and simulated product do not completely mimic real products in terms of their processing characteristics and microbiological growth supporting properties. There may be differences in solubility, pH, filtration rates and filterability and viscosity. With powdered products, the process simulation involves reconstituting powdered media or simulated product, introducing extra processing equipment or manipulation, with the inherent risk of contamination. Since a microbiological medium is designed specifically to support or stimulate the growth of microorganisms, it is a more rigorous challenge than processed products, which often provide neutral and sometimes hostile microbial growth environments. For these reasons acceptance criteria with a limit of some low number of positives, other than zero, are often chosen, consistent with applicable regulatory requirements.

This section offers guidance which can be used to establish appropriate limits and acceptance criteria for aseptic process simulations.

10.2 Recommendations

The following recommendations may be used to establish appropriate process simulation limits and acceptance criteria:

- The methodology must simulate the process as closely as practical.
- Rationale for the chosen methodology and limits must be justifiable and documented. It should be based on an assessment of the relative risks of the aseptic process.
- The methodology should be sensitive enough to confirm a low process simulation contamination rate. The selected limit must be routinely achievable.
- Any positive unit is significant, regardless of run size, and should result in a thorough, documented investigation. Following the investigation, appropriate corrective action may be taken based on scientific evaluation and risk assessment.
- Process simulation contamination rates approaching zero should be achievable using well designed and controlled aseptic filling operations, especially those involving automated production lines in well designed aseptic processing facilities, blow-fill-seal; form-fill-seal and in isolator-based systems.
- Recurring positive units in successive process simulations indicate a problem and should be investigated and resolved even when the acceptance criteria are met for each individual simulation.

11.0 Considerations for Investigation

All positive units should be identified to at least the genus, and to the species level when practical. A comprehensive sampling and identification scheme is critical in the investigation and determination of the contaminant source. When positive units are encountered, all possible sources of contamination should be investigated. A detailed history of the investigation should be maintained.

The identification of the contaminating organism should be compared to the database of the organisms identified within the facility through the environmental monitoring program. The biochemical and/or genetic profile of the contaminating microorganisms should also be compared to that of microorganisms obtained from testing programs including sterility tests, bioburden and environmental monitoring programs (air viables, equipment surface and personnel), in order to help identify the potential sources of the contaminant. Isolates should be checked for possible identification matches especially from areas which exceed their count limits or are trending upward. In addition, literature references describing possible sources of the contaminating organisms may be helpful in locating the point of entry into the process.

A batch production record similar to that for routine production should exist for each APS. Deviations, down times and repairs, before or during filling, should be evaluated. Filter integrity testing results and all sterilization records associated with product components and equipment should be reviewed. Cleaning and sanitization records should be reviewed.

Critical systems (e.g., HVAC, compressed air/gas, water, steam) should be reviewed for documented changes and re-qualification or acceptance criteria for those changes. Calibration records should be checked. HEPA filters in the filling area should be inspected and recertified, if warranted. Training records for all individuals (production, maintenance, cleaning) involved in the fill should be reviewed to assure proper training was provided and personnel qualification documented.

Change management and validation records related to the aseptic processing area should be reviewed for any procedure or process changes. All deviations from the original validation should have an associated justification for not performing a new validation.

Based upon the outcome of the investigation, the cause of the failure is either assignable or not assignable. If the cause is assignable, corrective action needs to be taken and documented. In the case of a media fill failure or where there is a history of intermittent incidents of positive units the root cause and the corrective action will dictate the number of process simulations required to demonstrate that the process is operating within the expected parameters. Where assignable cause cannot be determined, and considering previous process simulation results, multiple consecutive successful process simulations may be required to reaffirm process control.

The investigation report should contain:

- A summary of the occurrence
- A list of the systems investigated, not just the systems tied to the failure
- A conclusion as to root cause(s) and supporting documentation (if discovered)
- Potential effect on previous batches produced
- Corrective action(s) taken and supporting documentation
- Outcome of additional process simulations, if performed
- Appropriate signatures. In addition to the signatures of the investigators of the individual systems, the overall report should be signed by Production and Quality

The investigation should be completed in a timely fashion.

Note: This section is not intended to be all inclusive. Additional elements may need to be added depending upon the process.

12.0 Ongoing Process Evaluation

This section of the Technical Report was formerly entitled Validation Maintenance. It has been re-titled as Ongoing Process Evaluation to convey that the process of assessing a state of control must be an ongoing process and not strictly a time or event driven process.

The purpose of ongoing process evaluation is to demonstrate that a state of control for all systems that impact aseptic processing has been maintained since the last assessment. This evaluation includes the conduct of periodic APS studies based on an overall plan. Current regulatory guidance in major regions recommends aseptic process simulations as part of ongoing process evaluation once every six months ***(6,7,8).***

The ongoing process evaluation should consider information from other quality systems that provide routine information regarding the quality and control state of systems impacting the aseptic process. These systems include environmental monitoring data (including utility systems, facility systems and personnel), sterility data (including pre-filtration bioburden), sterilization validation data, and change controls. This will provide the ability to assess individual control system accept- ability in addition to the process simulation results.

Each firm should determine the frequency of and interval between ongoing APS for each process considering local regulatory requirements, as well as additional risk based criteria – such as line design and performance. A semi-annual interval between process simulations (See **Section 3.1** for selection guidance) is widely accepted in the pharmaceutical industry and expected by the applicable regulatory authorities. The APS for ongoing evaluation should not be strictly time driven. An APS is also typically performed after an extensive maintenance event, such as a facility shut down, which results in risk to the satisfactory performance of aseptic control systems. In such cases an APS will assist in verifying that the area has been returned to a qualified state and is acceptable for resumption of routine production.

There may be several different permutations of a filling process, which take place on a given filling line. If these processes differ significantly, then supporting APS should be performed for each process. In such cases, one approach may be to perform process simulations for these processes on a rotational basis, with each process challenged at least annually. Depending upon individual circumstances, however, more frequent process simulation may be necessary (See **Sections 3.2** and **7.0** for worst case selection guidance).

Activities and interventions representative of each shift, and shift changeover should be incorporated into the design of the simulation program. The APS will assess the personnel practices, facility, processing time and equipment. Therefore a firm may decide to perform a single process simulation that is split among three shifts, using personnel from each of the three shifts and performing interventions during each shift. Where production commences on a particular shift, personnel from that shift should initiate a periodic process simulation. Complex or unusual ongoing APS evaluation schemes may be reviewed with local authorities when warranted. (See Secti**on 7.0** for additional information).

Performance of process simulations prior to the scheduled reassessment may be necessary following a process change of such scope that previous simulation studies would no longer be representative or applicable. These situations should be assessed through a written change control program and reviewed by the quality unit. In such cases, the number of process simulations may vary, depending upon the extent of the change.

Examples of such changes include:

1. Modifications to the equipment (interchanging identical standard parts does not constitute an equipment modification)

2. Modification to equipment or facilities that potentially affects the air quality or airflow in the aseptic environment
3. Major changes in the number of production personnel or initiation of second (or third) shift production when the facility has been qualified only for single shift operations.
4. Major changes to the aseptic production process and/or procedures
5. Major modification to the equipment preparation or assembly techniques
6. The addition of new product containers or container-closure combinations

It also may be necessary to re-qualify a fill line with acceptable process simulations after corrective action(s) have been implemented in response to adverse trends or failures in the on-going monitoring of the facility or process, such as:

1. Continued critical area environmental monitoring results above the alert/action levels
2. Any product sterility test failure
3. Breach of asepsis in the aseptic processing area

When such incidents occur, the process and any changes that may have occurred since the previous simulation should be evaluated. Appropriate action can then be taken to restore the facility or process to its "controlled state." Process simulation may be appropriate to assure that the "controlled state" has been re-established. However, an aseptic process simulation must not be used to justify practices that pose unnecessary contamination risk.

13.0 Appendices

13.1 Selection and Sterilization of Placebo Powder/Materials

In the conduct of aseptic process simulations for suspensions, ointments, creams and dry powder fills, the use of a sterile placebo powder/material is commonplace. Care must be taken in the choice of material to be used, and in its preparation, to avoid difficulties with the process simulation program. It may be possible to use sterile dry powdered medium in the process simulation, however its utility may be hindered by the fineness of the powder and poor handling characteristics. Whichever material is utilized as a placebo, it should be packaged and handled identically to the sterile powder being simulated and the justification as its appropriateness as a substitute for the simulation should be documented.

Selection of Placebo Powder – The selection of placebo material for use in process simulation must consider several factors. The seemingly obvious choice of dry sterile media, itself, has proven less than successful because of its poor flow properties, which make its passage through conventional powder handling equipment or a typical sterile powder filling machine a considerable challenge. The principal placebo materials which have been used successfully include: lactose, mannitol, polyethylene glycol 6000 and sodium chloride. The chosen material must be easily sterilizable (using a validated method), dispersible or dissolvable in the chosen medium with minimal agitation, have no adverse effect on growth promotion, and be easily handled in the mock formulation processes or easily filled in the powder filling equipment.

Sterilization of Placebo Powder – Part of the selection process requires the identification of a suitable sterilization method for the chosen material. The material being evaluated should be subjected to a sterilization process prior to the process simulation. The validation study should include verification that the sterilization process has no significant adverse effect on the material's properties. The most common sterilization method in use is irradiation in a final container, generally a heat sealed plastic bag, identical to that used for sterile powders. Alternatively, the material can be sterilized by gas, dry heat or even by filtration, followed by bulk lyophilization. Along with the placebo material prepared for use in the filling trial, additional material in separate bags can be utilized for sterility testing after sterilization (tailgate samples). Subsequently, these samples can serve as negative controls and tested if there is any question regarding the sterility of the material.

Inhibition Testing of the Placebo Powder – Growth promotion testing, in which the chosen material is tested for potential inhibition, is performed using pharmacopeial methods ***(23).*** Consideration should be given to testing with other microorganisms commonly found in the aseptic processing area environment, such as those isolated during environmental and personnel monitoring and sterility test contaminants. The sterilized placebo material is dispersed in sterile WFI, and added to sterile medium at a range of concentrations approximating that to be utilized in the process simulation. Replicate samples at each concentration are inoculated with 10-100 CFU of each of the challenge organisms. Positive controls are prepared by inoculating replicate tubes of medium which do not contain the sterilized placebo powder. Growth must be evident in all tubes within seven days of incubation at the simulation conditions.

Solubility Testing of the Placebo Powder – The solubility of the placebo powders at the desired concentration is determined in the test medium. The amount of agitation required to dissolve or disperse the powder, as well as the time and extent of dissolution should be noted. If the powder fails to dissolve or disperse fully, it can be retested at a lower concentration or replaced.

13.2 Media Preparation and Sterilization

Media used for process simulations may be liquid or powder, depending on the type of filling process to be simulated. Media containing animal derived components should come from non-BSE/TSE origin.

The preparation of large volumes of media for aseptic process simulations poses significant challenges. In the vast majority of process simulations Soybean-Casein Digest Medium (SCDM, Trypticase Soy Broth, TSB) is used to recover and grow bacteria and fungi. SCDM is a broad spectrum medium suitable for the recovery and growth of the typical human flora which predominate in the aseptic processing environment. SCDM is also capable of recovering many spore-bearing organisms, some Gram negative bacteria, and some fungi.

The sterilization of SCDM prior to filling may be accomplished through a variety of methods. Most commonly, SCDM is sterilized by moist heat, radiation or filtration. The use of heat or radiation sterilization is preferred to eliminate potential mycoplasma contamination that might occur were filtration to be used. Consistent with its capability to grow a wide variety of environmental microorganisms, SCDM is also widely used in the microbiology laboratory for such purposes as environmental monitoring and sterility testing. However, in the laboratory the quantities of SCDM required for typical daily assays and analyses are often significantly smaller than those required for a process simulation. Moist heat sterilization processes are very reliable and can be used for large and small volumes of media. In the microbiology laboratory in almost all cases media is sterilized in an autoclave. In cases in which relatively small quantities of media are required, for example process simulations done to evaluate clinical scale or other low throughput operations, media preparation can follow typical laboratory practice. However, in larger process simulations done in support of commercial scale manufacturing, it may be impractical to produce the required large volume of media in an autoclave. Media for larger simulations may be sterilized-in-place in a suitable vessel. Alternatively, the SCDM powder can be purchased sterile and formulated in a bulk vessel. The manufacturer's recommendations regarding sterilization time and temperature should be followed to ensure the sterilized media retains its requisite growth promotion capabilities. F_0 values in the range of 15 to 20 minutes are generally sufficient. Longer or hotter sterilization cycles delivering higher levels of lethality may caramelize the medium and adversely affect growth promotion.

The filtration system used to produce sterile product is validated independently of the process simulation and does not require further validation by virtue of a process simulation. If possible, the same filtration system and vessels employed for product manufacturing can be used; however, in many cases this is not technically feasible or practical. The sterilizing-grade filter may not be identical to the one used for the product(s) being simulated, but it should be sized properly for the preparation of the required volume of media. The use of pre-filters may be required as media may contain a substantial amount of fine particles that may clog the primary sterilizing-grade filter. It is critical to design media preparation systems so that the fluid path to the filling machine and aseptic connections required effectively simulate normal and typical production operations. There have been confirmed reports of mycoplasma in filter sterilized SCDM prepared for process simulation. Thus, if there is judged to be some risk of mycoplasma contamination, appropriate countermeasures, including heat-treatment or radiation, may be indicated.

The hold time between compounding of the media and filtration of media into a sterile vessel should be minimized. Media held in less than fully sterile conditions will immediately begin to support the growth of bioburden organisms that may be present. Bioburden issues can be mitigated to some degree by preparing the media using hot water. Water temperatures above 60°C will reduce the risk of growth of vegetative bacteria and fungi, and ensure that the media dissolves more completely,

reducing the likelihood of filter blockage. When appropriate, use of a pre-filter to remove material that does not go into solution will reduce the likelihood of clogging the final sterilizing-grade filter, which usually has a 0.2 μm pore size rating. It is preferable to sterile filter the media into a sterile holding tank prior to start of the fill, rather than hold non-sterile media for several hours. Media should be allowed to cool to <35°C before use in a process simulation.

Where powdered media is employed to challenge powder filling operations this media is usually sterilized by radiation. The radiation sterilization process should be validated and each lot should have dosimetric information included in its certificate of analysis. It may be advisable to sample and evaluate media for reconstitution and growth promotion prior to use in a process simulation.

The two critical factors in media preparation for process simulations are ensuring that the media is sterile and growth promoting. Media formulation and sterilization are very different exercises from product compounding and filtration.

13.3 Aseptic Process Simulation Execution Sequence

1) Prior to initiating the simulation confirm satisfactory and currency of the qualification, validation and operation of aseptic process support and sterilization systems, including:
   a) Personnel aseptic training and qualification status
   b) Personnel aseptic gowning certification
   c) Disinfectant qualification
   d) Facility sanitization program
   e) Product, container/closure, and equipment sterilization
   f) Fill and manufacturing system qualification
   g) Container/ closure integrity
   h) Air flow, HEPA filtration, temperature and humidity control
   i) Viable and non-viable environmental control
   j) People
   k) Utility gases (product contacting)
   l) Surfaces
   m) Air
   n) Materials disinfection control
   o) Filter integrity

2) Define the routine aseptic process undergoing simulation, including:
   a) Aseptic formulation process, equipment, and operations
   b) Aseptic filling process, equipment, and operations
   c) Operation conditions
   d) Number of operators
   e) Process set-up, interventions (inherent and corrective), and stoppages
   f) Process length including breaks and operator relief
   g) Environmental conditions; air flows, temperature and humidity, pressure differential requirements

3) Define aseptic process simulation execution conditions based on appropriate stress of the routine aseptic process simulation.

4) Develop a protocol that defines the rationale, justification and acceptance criteria that establishes aseptic process simulation conditions necessary to qualify normal aseptic operations. The protocol is reviewed and approved by the Quality Unit.

5) Develop aseptic process simulation batch record that defines execution requirements. The aseptic process simulation batch record is reviewed and approved by the Quality Unit.

6) Conduct and monitor the aseptic process simulation to ensure it is executed in compliance with the aseptic process simulation protocol and batch record requirements (three runs for initial simulation and one or more runs for periodic confirmation).

7) Perform qualified container/closure integrity inspection, retain acceptable units and initiate accountability, rejecting only those units with container/closure integrity defects.

8) Prior to incubation, aseptic process simulation units are inverted to assure media contact with all internal surfaces.

9) Incubate acceptably filled units under defined temperature and duration.

10) Perform accountability and qualified inspection of filled units. Identify any units with positive microbiological growth. Investigate and establish contamination root cause for any acceptably filled units exhibiting positive microbial growth.

11) Perform growth promotion tests on aseptic process simulation media in post-incubation media filled units.

12) Document aseptic process simulation results, evaluation and conclusion in a report approved by the Quality Unit.

13) Identify and document operators that participated in the aseptic processing simulation by the executed aseptic process simulation, and the term of their qualification.

14) Identify and document interventions that were successfully demonstrated by the executed aseptic process simulation.

14.0 Suggested Readings

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