Technical Report No. 6

Validation of Aseptic Drug Powder Filling Processes

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FOREWORD

THIS IS THE SIXTH IN A SERIES OF TECHNICAL REPORTS.[1–5] This technical report was produced to serve as a guide outlining a step-by-step approach to validate the aseptic drug powder filling process. However, it does not exclude the use of alternate methods to validate the aseptic drug powder filling process. The report was directed by the Quality Control Subcommittee of the Research Committee of the Parenteral Drug Association. The task force which developed this report was chaired by John Wasynczuk, and was co-edited by Gordon Beyerle and Doris Conrad. The report includes philosophy, materials, equipment, operating parameters, validation, and revalidation approaches.

The validation approach contained herein should be treated as a guideline only and not as a requirement for validation. This technical report is only intended to provide information to the PDA membership and should not be construed as a recommendation of the PDA.

Floyd Benjamin
Chairman
Research Committee

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[1] "Validation of Steam Sterilization Cycles," Parenteral Drug Association Inc., Technical Monograph No. 1.

[2] "Validation of Aseptic Filling for Solution Drug Products," Parenteral Drug Association Inc., Technical Monograph No. 2.

[3] "Validation of Dry Heat Processes Used for Sterilization & Depyrogenation," Parenteral Drug Association Inc., Technical Report No. 3.

[4] "Design Concepts for the Validation of a Water for Injection System," Parenteral Drug Association Inc., Technical Report No. 4.

[5] "Sterile Pharmaceutical Packaging: Compatibility and Stability," Parenteral Drug Association, Inc., Technical Report No. 5.

PREFACE

In 1980, the Parenteral Drug Association published Technical Monograph No. 2 entitled, "Validation of Aseptic Filling for Solution Drug Products." Though Technical Monograph No. 2 focuses on methods applicable to validating aseptic liquid filling processes, many of the techniques, monitoring procedures, equipment, and philosophy presented also apply to the validation of other aseptic filling processes, including dry filled products. The purpose of this report is to familiarize the user with philosophy, materials, equipment, etc. used in validation of aseptic filling processes by reprinting, from Monograph No. 2, applicable sections, and to provide the user with additional guidelines and techniques which may be employed for validation of dry powder filling processes.

By employing the approaches presented in this text, or by use of alternative methodologies, the aseptic filling process can be validated. This report also provides examples of equipment and materials that may be utilized in validation studies, though alternatives of equal utility do exist.

This technical report is presented solely as a guide, and is not intended to establish any mandatory or implied standards.

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I. INTRODUCTION

The aseptic filling process, whether it is for a sterile liquid or powder drug product, is one of the most exacting operations in the pharmaceutical industry. Whereas the probability of sterility for a terminally sterilized drug and drug container/delivery system can be validated to a known level of sterility assurance, an aseptic filling process requires the pre-sterilization of all drug product components. Subsequently, all components are brought together in a controlled aseptic environment where the drug product is filled and sealed within its container/closure system. Validation of an aseptic filling process to a known and acceptable level of sterility is cumulatively dependent upon each of the operational processing steps, the facility design, the operating parameters, and the degree of personnel involvement in the operation. These parameters are outlined below.

1.1 Operational Processing Steps

The level of sterility assurance is dependent upon all processing steps involved in the manufacture of the product. The final level of sterility claimed for the product cannot be greater than that of the processing step providing the lowest level of sterility assurance. The processing steps include:

Component Sterilization/Depyrogenation
Drug Product Sterilization
Drug and Container Contact Equipment Sterilization
Aseptic Filling
Aseptic Stoppering and Vial Sealing

1.2 Facility Design and Operating Parameters

All important environmental factors should be evaluated, monitored and controlled to support the aseptic filling process. These factors may include:

Room Pressure Differentials
Room Air Change Frequencies
Humidity and Temperature Levels
HEPA Filter Efficacy
Non-Viable Particulates
Viable Particulates
Aseptic Process Area (APA) Sanitization
Air Flow Patterns

1.3 Personnel

Unless the processing excludes the human element, e.g., the use of robotics, the impact of the human element in the aseptic processing environment is a critical concern, requiring training in:

Current Good Manufacturing Practices (CGMP)
Personal Hygiene
Gowning Procedures
Aseptic Procedures
Safety Procedures
Cleaning Procedures
Sterilization/Sanitization Procedures

This report outlines several of the approaches for the validation of the aseptic dry powder filling process, and addresses some preliminary concerns.

- Process Validation without Use of Media
- Use of Sterile Diluent and Powder on Line: Approach I
- Use of Sterile Diluent off Line Followed by Sterile Powder on Line: Approach II
- Use of Sterile Powder on Line Followed by Sterile Diluent off Line: Approach III

II. DEFINITIONS

APA:
Aseptic Processing Area.

Calibration:
Demonstrating that a measuring device produces results within specified limits of those produced by a reference standard device over an appropriate range of measurements. This process results in corrections which may be applied if accuracy is required.

Critical Area:
Any area within the processing room where sterilized product, containers and closures are exposed to the environment.

Critical Surface: Any surface which contacts previously sterilized product, containers and closures.

Diluent:
The liquid used to reconstitute the process simulation powder.

Packaging Components:
All final packaging items which contact (or will contact during use) the drug product which include closures, where these are distinct from the "holding" entity, and delivery systems such as needles on prefilled syringes.

Powder:
Any dry, process simulation material used to simulate actual product during a process simulation study.

Probability of Non-Sterility:
The likelihood expressed as 1×10^{-X} that a unit is non-sterile.

Process Simulation Test:
A test of the ability of the manufacturing process to aseptically combine previously sterilized product, containers and closures under simulated operating conditions.

Revalidation:
Repetition of the validation process or a specific portion of it.

State-of-Control:
A condition in which operating variables that affect performance remain within such ranges that the system or process performs consistently and as intended.

Sterility:
The state of being free from viable microorganisms.

III. PRELIMINARY CONSIDERATIONS

3.1 Personnel

The operation of an aseptic processing area may require that the personnel perform a multitude of functions. These functions could have a major impact on the ability to produce a sterile product. Personnel involvement is the principal variable in aseptic processing. The influence of human manipulation on quality should not be underestimated.

Once a process has been established, one of the most important variables in the process is the effect of personnel in the aseptic area. Thus, pharmaceutical manufacturers stress CGMP including functional training, proper gowning procedures, proper aseptic techniques, and personal hygiene.

3.2 Monitoring of Viable Particulates

Equipment used for the microbiological monitoring and evaluation should be subject to routine calibration. The capability of the selected media to support growth of appropriate test organisms should be demonstrated. A listing of media which may be used for environmental monitoring is provided in Appendix A.

The locations where sampling is performed are usually those places in the process where the product has the greatest potential for becoming contaminated by the environment.

Listed below are several methods for microbial monitoring and evaluation. The user is cautioned that some of these methods could, by the very nature of the design of the sampling equipment and the manipulations required, alter the airflow patterns and the environment that are being monitored. Due to basic differences between techniques in sampling, results obtained by any two methods may not be directly comparable. The user should select those methods most appropriate to the situation and needs.

CAUTION: Since the preponderance of sterile dry powders are antibiotics, they may severely inhibit the growth of some microbial contaminants. When antibiotics are filled, the monitoring methods employed must be adapted to minimize this interference. Revisions to conventional methods could include monitoring in the absence of the drug substance and/or use of a neutralizer of the particular antibiotic.

3.2.1 Qualitative Methods

A method may be considered qualitative when the sample size is not controllable. For example, a settling plate located near an air supply may be exposed to more air than a plate exposed in a quiet corner of a room. Despite the variables involved, it may be possible to establish appropriate alert/action levels for a specific location(s) (see 3.2.4).

- **Settling Plates**

This technique is simple, portable, and inexpensive; does not require subculturing procedures, and allows for continuous monitoring. Collection efficiency is affected by air currents and particle size and is, therefore, variable.

Petri dishes containing sterile microbiological growth media are exposed to the environment. Most viable organisms which settle on the medium surface will grow after suitable incubation. Any organisms present should be evaluated in relation to the user established procedures and action levels.

- **Contact Plates**

This technique is simple, portable, inexpensive and does not require subculturing procedures. Contract plates may be used either qualitatively or quantitatively.

Contact plates are prepared with an appropriate medium containing agar, such that the surface of the medium protrudes above the sides of the plate. After removal of the plate cover, the sterile medium surface is applied to a smooth, flat, dry, test surface. The cover is replaced, and the plate is incubated. Any colonies present are counted to determine microbial contamination per unit area. This technique is suitable for monitoring smooth, flat, dry surfaces, such as equipment, floors, walls, and the operator's gloves and clothing. Care should be taken not to allow the agar to dry out, although confluent growth of certain microorganisms can occur if the agar is overly wet. Caution must be taken to ensure that the medium residue which may support microbial growth is removed from the surface tested by cleaning with an appropriate agent. Residual disinfectant can prevent microbial growth from appearing on the contact plates. To avoid this situation, a suitable neutralizer is added to the medium.

- **Swabs**

Sterilized swabs are immersed in a suitable sterile liquid (e.g., Water for Injection). The swabs are then rubbed over the test surface and streaked onto, or placed into an appropriate medium. The number of colony forming units, per unit area, may be quantified using standard microbiological techniques, such as plate counting.

Swabs are suitable for irregular surfaces. The technique is more suited for qualitative rather than for quantitative assessment. Surface swabbing should be standardized to a consistent unit area.

As in the case of contact plates, if a microbiological growth-supporting medium is used, proper clean-up is essential. Residual disinfectant can prevent microbial growth. To avoid this situation, a suitable neutralizer may be added to the sterile liquid.

3.2.2 Quantitative Methods

For those methods where the sample size is a reproducible and a well-defined aspect of the test, a uniform alert/action limit may be established for critical and other specified areas (NASA Standard NHB 5340.2, see Appendix E).

- **Slit-to-Agar Impact Sampler**

The slit-to-agar impact sampler employs a revolving plate containing a suitable growth medium under a fixed slit-type orifice. The volume of air sampled should be related to the expected contamination level of the room. The method is portable, efficient, qualitative as well as quantitative, provides a concentration time relationship, and does not require subculturing procedures. A vacuum source is required. Some models have built-in vacuum pumps which provide convenience; however, care must be taken in cleaning and exhausting the pump. Although the entire unit cannot be steam sterilized, it can be sanitized with appropriate agents. (*CAUTION:* This instrument can be damaged by powders; therefore, care must be taken when used in close proximity to areas where powders are present.)

- **Reuter Centrifugal Sampler (RCS)**

This battery-operated device draws air at a fixed rate through its rotating blades. Particles in the air are hurled by centrifugal force against a nutrient agar strip substrate (available from the manufacturer). The volume of air sampled should be related to the expected level of contamination of the room. The unit is portable, convenient (hand-held or suitably mounted), and does not require an external vacuum or power source. Portions of the unit (impeller blade assembly and the open end drum) can be steam sterilized. In addition, an intrinsically safe model of this unit is available for use in hazardous areas.

- **Liquid Impingement**

Air is drawn into the unit by means of vacuum. The volume of air drawn in is determined by a limiting capillary orifice. The particles and microorganisms present in the air are impinged onto the liquid at very high velocity. At the end of the sampling period, the solution onto which the air

has impinged is filtered through a membrane filter. This membrane filter is then handled and processed as described below under the section, "Membrane Filtration."

The unit is economical and quantitative as well. Because of the high velocity air flow rate, aggregates tend to break up so that the total count obtained closely reflects the true number of organisms. A vacuum source is required for operation of the device. The collecting fluid (usually normal saline solution) may require the addition of an anti-foaming agent. Depending upon the level of contamination present, plating and dilution of the collecting fluid may be required. Each type of Liquid Impingement device has its own operating characteristics for flow rates and for volumes of air being sampled.

- **Membrane Filtration**

Air is drawn across a membrane filter surface by vacuum for a period of time related to anticipated room contamination levels. The membrane filters are then mounted on a nutrient medium to promote growth of colony forming units. The method is economical and allows sampling at virtually any location.

With these membrane filtration techniques, gelatin membranes can be used to keep viable organisms from drying out. In addition, because gelatin membranes usually dissolve in media, they ensure intimate contact of the collected organisms and the medium used.

It should be noted that the membrane filtration technique can have certain adverse effects upon vegetative organisms (such as desiccation of the organisms) which should be considered when interpreting the results. It is suggested that the manufacturer's recommended methods of sampling and membrane handling be followed.

- **Cascade or Sieve Impactor**

The cascade or sieve type collectors impact particles, according to the size of the particles, on plates filled with nutrient growth media. The nutrient media are incubated and colonies counted.

The device is portable, and does not require subculturing procedures. The unit requires a vacuum source for operation. It should be noted that the high air velocity used with this device over an extended period of time may dry out the growth-supporting media.

3.2.3 Establishing Sampling Plans

The determination and selection of environmental monitoring practices are an essential prerequisite for the validation of the aseptic filling process.

Sampling frequency within the APA should encompass the multitude of variations encountered during the normal processing cycle. Selection and identification of sampling locations are important to ensure that critical locations are properly monitored. The location of the test sites monitored during the validation process will depend on the history established for the area. [The British Standards Institute recommendations (BS 5295) (see Appendix E) may be used as a starting point to determine if differences occur within the aseptic filling area if a history does not exist.]

3.2.4 Establishing Environmental Alert/Action Levels

Prior to selection of alert/action levels for any environmental monitoring variable, an environmental data base is developed. The data base will provide the user with a valuable summation of environmental conditions experienced at a particular facility. From previous validation studies, historical environmental monitoring data and previous experience with other aseptic processes, the user may be able to determine appropriate alert/action levels to assure continued state-of-control operations. Where environmental data are limited or for newly constructed facilities, it may be appropriate to select interim criteria based upon previous experience.

The user should establish appropriate alert/action levels for microbial counts which, when exceeded, will result in the initiation of an appropriately planned sequence of corrective actions. Corrective actions should be designed to return the facility to state-of-control operation and, if possible, ascertain the cause of the deviation. It is recommended that alert/action levels should also be specified for other environmental variables.

a. Analysis of Environmental Data

- Critical vs. Non-Critical
  Environmental monitoring sites should be identified as critical (defined as areas of product exposure) or non-critical. Alert/action levels may vary for critical vs. non-critical areas.

b. Data Trend Analysis

Some techniques employed for environmental data review include cumulative frequency distribution[1], negative binomial distribution[2], and control charting[3]. Though a discussion of the applicability of any method for statistical analysis of environmental data is beyond the scope of this document, it is recommended that the user review each technique with a statistician and select the most appropriate method for data trend analysis. In any case, a historical picture of environmental data will have been generated in which significant deviations from the observed distribution can be determined or predicted for the purpose of establishing alert/action levels.

3.3 Other Considerations

The fill room environment is the last opportunity, prior to sealing the drug product into its container, in which environmental contamination of the product could occur. This contamination can be due to either the settling of airborne microorganisms or manipulative procedures, where handling by personnel leads to contamination.

The manufacture of sterile products involves many other parameters besides personnel or environmental monitoring. Items such as facility design, airflow patterns, container closure integrity, must be addressed in the validation of any aseptic process. While these factors directly impact on the aseptic filling process, full coverage of each of these factors is beyond the scope of this document.

IV. METHODS OF VALIDATING THE ASEPTIC DRUG POWDER FILLING PROCESS

Validation of an aseptic drug powder filling system is somewhat different from the validation of an aseptic liquid filling process. In both processes, various components (including equipment, containers, stoppers and drug products) are sterilized according to previously established Standard Operating Procedures and introduced into the Aseptic Processing Area (APA). Subsequently, the sterilized components are assembled into the finished dosage form within the APA environment.

Validation of the aseptic liquid filling process is readily accomplished through the substitution of sterile liquid media for the drug formulation. In the case of aseptic drug powder filling, however, the additional manipulative step of filling diluent is added to the process. Because of the additional handling which must be utilized to validate a dry filling process with media, there exist two schools of thought. Some firms have chosen to utilize both liquid and powder media in this validation despite the non-representative nature of the process, while other firms have decided that to employ this additional operation presents an unreasonable challenge to the process.

This report discusses several approaches which may be utilized to validate the efficacy of aseptic drug powder filling processes, including steps which could be taken prior to, as well as during, the validation studies. Data generated from these studies are evaluated together to arrive at a valid conclusion regarding facility state-of-control.

4.1 Process Validation Without Use of Media

Recognizing the necessity of adding some non-representative manipulative steps to drug powder fill process simulations, an alternative position is to not utilize media in the testing of facilities. The concern is that the addition of a liquid filling step cannot mimic the actual dry filling process.

If a company chooses to utilize a non-media approach to validation, it is incumbent that the firm assures itself that the facility does not provide an opportunity for contamination to be introduced during the aseptic filling process. Valid certification testing of air handling systems and frequent monitoring of all control systems are necessary. Microbiological monitoring during the processing steps utilizing acceptable procedures and careful trending of results are required. Appropriate steps must be taken when action levels are reached to reduce environmental contaminants.

In the absence of a medium challenge of the filling line, an increased

reliance on the supportive validation programs becomes the principal element in demonstrating the adequacy of the facility and procedures employed during the dry fill process. These programs, in conjunction with validation of sterilization and sanitization procedures, provide the necessary sterility assurance.

Some firms have extended this concept to include a simulation of the filling process without media. During this simulation, extended environmental monitoring is performed to demonstrate conditions which serve to eliminate microbial contamination. These tests include a full operation of the line including vials, stoppers, seals, personnel, etc., with the single exception that no powder (media) is present in the operating filling machine. The environmental monitoring results obtained during this simulation should conform with the previously established Alert/Action Levels for the APA.

Recognizing the random variations in personnel practices and their critical impact on the aseptic filling process, adequate training and control of their actions must be maintained. Where possible, facility design should minimize or eliminate the personnel factor from the process.

The introduction of a liquid reconstitution/filling operation to an aseptic drug powder filling process adds additional parameters. These incremental operations further complicate the interpretation of results obtained to the extent that an otherwise acceptable facility may be discredited. It is for this reason that a number of firms have decided not to utilize media type challenges in the validation of their aseptic dry filling processes.

4.2 Process Simulation Testing with Media

Numerous attributes and limitations for performing process simulation testing with a microbiological growth-supporting medium exist, some of which have been previously described. Though the same objective applies to validation of an aseptic liquid or a drug powder filling process, validation of the latter requires some modifications. Since the physical capability of filling equipment/area may vary from one user location to another, three representative approaches toward simulating an aseptic drug powder filling process are presented. Each approach may be modified to suit the capability of the specific location.

4.2.1 Medium Considerations

a. Liquid Medium

A number of general microbiological growth supporting media are available and may be used in a process simulation program. A partial listing of liquid media may be found in Appendix A. In general,

when selecting a medium for use, the following considerations should be made:

Selectivity—The medium should have low selectivity, i.e., it should support the growth of a broad spectrum of microorganisms.

Clarity—The medium must be clear so that turbidity may be readily observed.

Filterability—Medium should not contain agar or high levels of suspended solids when a filtration process is used.

- **Medium Concentration**
  The manufacturer's recommended concentration should be used when preparing media unless alternate concentrations can be shown to be equivalent.
- **Medium Sterilization**
  Medium for use in a process simulation test can be rendered sterile using either moist heat or filtration.

b. Powder Medium

A partial listing of powders which may be used for process simulation testing is provided below:

- **Microbiological Growth Support Medium (MGSM)**
  If using a MGSM as the process simulation powder, a medium must be chosen which does not require heating to solubilize.
  —Trypticase Soy Medium[4] (Soybean Casein Digest)
  —Brain Heart Infusion
- **Process Simulation Powder**
  —Polyethylene Glycol[5]
  —Lactose
  —Mannitol
  Process simulation powders must be rehydrated with a general purpose medium. The following powder/diluent alternatives may be used:

Powder	Diluent
1) Placebo Powder	MGSM
2) Dehydrated MGSM	Water
3) A	MGSM without A

Where "A" is a component of the selected growth-supporting medium

As with microbiological growth media, when selecting a process simulation drug powder filling agent, the following considerations should be made:

- **Solubility**
  The powder must be soluble at the concentration to be used in validation trials. The selected medium and/or process simulation powder solution should be clear to allow for ease in observing turbidity.
- **Toxicity**
  The selected medium and/or process simulation powder solution should not exhibit any toxic effects on compendial microorganisms (see Positive Controls) at the concentration to be used in validation trials.
- **Sterilization**
  The powder to be used in simulation testing can be rendered sterile by use of radiation or dry heat. A suitable quantity of the selected powder should be packaged and handled in a manner which will not compromise powder sterility during transport and storage. The sterilization cycle should provide at least 1×10^{-6} probability of survival within the powder load. Also, the sterilization cycle should not affect the physical characteristics of the powder or increase the level of toxicity at concentrations to be used in validation trials.

c. Qualification of the Liquid and Powder Media

- **Dissolution**
  After determining potential fill weights and volumes, the solubility of various percentage concentrations of powder in the liquid medium can be determined. In some cases, manual or mechanical shaking may be required to assure complete dissolution.
- **Inhibition/Growth Promotion Testing**
  Once the percentage concentration range of powder in liquid medium has been determined, the growth-promoting properties of the combination should be verified. After appropriate sterilization of the selected medium and placebo powder, appropriate percentage concentrations are prepared and inoculated with compendial organisms as described in Positive Controls. In addition, during the initial qualification of the combination, a second set of Liquid/Powder combination filled containers should be inoculated after completion of the selected incubation period. This will demonstrate that the containers filled with liquid and powder retain growth-promoting properties throughout the specified incubation period. Interpretation of results are described under Positive Controls.
- **Sterility Test**
  After appropriate sterilization of the powder, a sterility test may be performed. If irradiation is used for powder sterilization, the powder may be released based upon dosimetric results.

4.3 Use of Sterile Diluent and Powder On Line: Approach I

Process simulation testing according to this approach is recommended for facilities which can accommodate both liquid and powder filling units along with aseptic filling line (see Diagram I, Appendix B). Minor filling line modifications, including the addition of a vial indexing system, may be required in order to fill vials with liquid medium.

4.4 Use of Sterile Diluent Off-Line Followed by Sterile Powder On Line: Approach II

There may be situations where it is desirable to introduce containers previously filled with the selected sterile diluent directly onto the filling line. In these cases, the selected sterile powder is filled into vials already containing the sterile diluent (see Diagram 2, Appendix B).

4.4.1 Medium Sterilization

- **Diluent Component**
  The selected diluent is filled off-line into the appropriate containers. Alternatively, the bulk diluent can be sterilized utilizing a suitable method and aseptically filled. Temporary covering for the diluent filled containers can be used at this point since the final ingredient will be added later. Sterilization of the partially-formulated medium should use a properly validated sterilization cycle. Transport of diluent filled vials should be handled utilizing appropriate aseptic techniques.
- **Powder Component**
  The powder component of the process simulation which will eventually be processed through filling equipment should be sterilized as described in section 4.2.1 b.

4.5 Use of Sterile Powder On Line Followed by Sterile Diluent Off Line: Approach III

Process simulation testing according to this approach may be used in facilities which cannot simultaneously accommodate both liquid and powder filling units along a single aseptic filling line. When using this approach, a previously sterilized powder is substituted for the powder drug product. Subsequently, the powder medium within the containers/closures is reconstituted with an appropriate amount of sterile diluent. Thus, this approach involves the additional manipulation of aseptically reconstituting powder in sealed vials (see Diagram 3, Appendix B).

This technique involves an aseptic step which is not encountered during a normal filling operation, namely, the reconstitution of a powder. This

additional step, adding a suitable volume of sterile diluent, introduces another opportunity for microbial contamination. Hence, it becomes critical to determine whether the source of contamination (should it occur) originates from the powder filling operation or the liquid reconstitution. It is recommended that suitable controls be utilized to aid in making this determination should it become necessary. For example, a statistically valid number of control samples (empty sealed, sterilized vials) should be reconstituted with the same liquid medium to simulate the reconstitution of the drug powder filled and sealed vials. These Controls should be incubated with the Process Simulation test vials. The contamination rate found in this Control is applied in the calculation to determine the actual Powder Fill Contamination Rate. The Powder Fill Contamination Rate would equal the Powder Simulation Contamination Rate minus the Control Rate.

4.6 Process Simulation Test Parameters

To insure that process simulation tests produce meaningful results, variations from normal production conditions must be minimized. Equipment preparation, filling speeds, number of personnel, environmental conditions, etc. must represent normal production conditions.

4.6.1 Scheduling

Process simulation tests are usually conducted independently of production runs, with the filling line set up specifically for the test. Though process simulation testing can be performed immediately prior to or after a normal production filling, the area should be cleaned according to standard operating procedures to prevent product/media cross-contamination. In any case, the process simulation tests should be as representative as practicable of normal production equipment and container configuration.

These tests may be conducted annually or more frequently, depending upon the individual manufacturer's requirements.

4.6.2 Number of Containers

The quantity of containers filled during a process simulation test should meet one or both of the following criteria:

- The number of containers filled per medium run may be defined in terms of a predetermined time period when operating the equipment at normal filling speeds.
- The process simulation test should be of such duration that sufficient containers are filled to properly determine the contamination rate.

Individual manufacturers should determine the number of vials to be filled by applying the following contamination rate formula:

$$P = 1 - (1 - X)^N$$

where: P= probability of detecting non-sterility
X= acceptance criteria: acceptable percent contamination rate
N= total number of vials run in the media challenge

The probability of detecting non-sterility for various combinations of acceptance criteria (percent non-sterile units), and the total number of vials filled are shown in Appendix C.

4.6.3 Filling Rate

Equipment should be operated as close to normal production speed as practicable.

4.6.4 Fill Volumes and Weights

Ideally, the quantity (weight) of powder filled into each container during a process simulation test should have relevance to the quantity of a product normally filled.

The weight of powder filled should be determined for each filling operation prior to the initiation of the simulation trial. Filling weights should be adjusted as required. Similarly, the volume of liquid media filled into containers should be verified. The fill weights and volumes used should fall within concentrations qualified during Inhibition/Growth Promotion testing.

In cases where the dehydrated growth-supporting medium must be reconstituted with a disproportionately large volume of diluent, it may be necessary to fill a limited quantity of powder into containers. When this approach is used and the dehydrated medium is thus reconstituted, the volume of liquid medium in the container should be sufficient to wet all interior surfaces when shaken or rotated upside down.

4.7 Process Simulation Media Incubation Parameters

4.7.1 Dissolution

Where dissolution of powder is required, the appropriate means of dissolution should be employed, e.g., shaking. If dissolution is not required, then the filled container should be rotated immediately prior to incubation so that all surfaces, including the closure (if any), are wetted by the medium.

4.7.2 Time

Media, in the sealed container as delivered from the production line, may be incubated for not less than 7 days, consistent with USP XXI growth promotion requirements or in accordance with the manufacturer's own sterility incubation time requirement.

4.7.3 Temperature

Process simulation test containers should be incubated at suitable incubation parameters.

The temperature should be monitored throughout the test period and maintained within the specified range for the test period.

4.8 Positive Controls

The growth-promoting ability of the medium in the final filled containers should be demonstrated using filled control containers challenged with low levels of microorganisms.

4.8.1 Microorganisms

Compendial Microorganisms—the microorganisms referenced in the USP XXI Sterility Test growth-promotion tests are suitable for use as controls. These include the following:

(i) *Bacillus subtilis* (spores) ATCC #6633
or
Micrococcus lutea ATCC #9341
(ii) *Candida albicans* ATCC #10231
(iii) *Bacteroides vulgatus* ATCC #8482*
or
Clostridium sporogenes (spores) ATCC #11437*

* Use only if testing for anaerobiosis of thioglycollate medium.

As an alternative to compendial microorganisms, isolates frequently encountered in the manufacturing environment may be used to challenge the medium.

A combination of compendial organisms and indigenous organisms may also be used as controls. In all cases, however, microorganisms used in growth-promotion testing should include both bacterial and fungal species.

4.8.2 Challenge Parameters

Challenge levels of less than 100 cells per container should be used in an attempt to simulate low level contamination.

Dilutions of actively growing, or frozen stock cultures may be used.

A viable count via a pour plate or spread plate should be obtained for the final dilution of each microorganism to verify the challenge level.

Growth-promotion studies should be carried out in duplicate for each type of microorganism and each type of container system.

Incubation parameters should be consistent with those of the process simulation test containers.

4.8.3 Interpretation of Results

The medium is acceptable if growth appears within 7 days[6].

If no growth is observed in either of the challenged containers, one repeat test may be conducted to rule out laboratory error. On the repeat test, both containers should support growth.

4.9 Analysis and Interpretation of Process Simulation Testing Results

4.9.1 Visual Examination of Test Containers

Following the appropriate incubation period, the media-filled containers should be visually examined for microbial growth.

Contaminated containers should be examined for evidence of container/closure damage which might compromise the integrity of the packaging system. Damaged containers should not be considered in evaluating results; however, they will be corrected in the % Contamination Rate equation, section 4.9.2.

One method of identifying damaged containers is to use a leak test system where containers are submerged in a suitable dye/surfactant solution and exposed to vacuum/pressure under specified parameters of exposure time and exposure severity.

It may be useful to characterize any contaminants found to determine their source in order to take remedial actions as required.

4.9.2 Test Limits

A process simulation test contamination (positive growth) rate may be determined using the following formula:

$$\% \text{ Contamination Rate} = \frac{\#\text{ of Undamaged Containers with Microbial Growth}}{(\#\text{ Containers Filled} - \#\text{ Damaged Containers})} \times 100$$

It is the responsibility of each manufacturer to establish an acceptable process simulation testing endpoint for each facility. Examples of contamination rates utilized within the industry may be found in:

- World Health Organization, "Sterility and Sterility Testing of Pharmaceutical Preparations and Biologicals," WHO/BS/73.1062 and WHO/PHARM/73.474 (1973).
- Canadian Ministry of National Health and Welfare, "Good Manufacturing Practices for Drug Manufacturers and Importers," Cat. No. H42-21-(1982).
- Australian National Biologic Standards Laboratory, "Code of Good Manufacturing Practices for Therapeutic Drug Products," Canberra, Australia (1983).
- Pharmaceutical Manufacturers Association, "Proceedings of the PMA Seminar Program on Validation of Sterile Manufacturing Processes: Aseptic Processing," Atlanta, Georgia, March 1–2 (1979). The Media Fill Action Levels Table (Table 3) on page 189 of these Proceedings (Validation of Aseptic Process by Media Fill—Survey Report and Discussion, presented by Dr. M. S. Korczynski) has been reprinted below with permission from the PMA.

PMA Survey
Media Fill Action Levels

Media Fill Action Level[1]	Action Level %	No. of Firms
1:1000	0.1	4
1.5:1000	0.15	4
3:1000	0.3	3
4:1000	0.4	1
5:1000	0.5	1
10:1000	1.0	2

[1] Number of positive units permitted relative to the number of units tested for liquid media fills.

V. VALIDATION/REVALIDATION SCHEDULE

5.1 "Start-up" Validation

Whether process simulation studies (i.e., media, media/powder challenge) or individual systems validations are performed, acceptable validation studies should be achieved prior to releasing a new manufacturing facility/equipment for production use.

When qualifying a new facility, process simulation tests may be conducted more than once, thereby simulating the multi-environments which may be encountered during production periods. Included in the process simulation studies are the standard facility/equipment operations and cleanup procedures.

When individual system validation approach procedures are used, they too should be tested employing standard operating procedures.

5.2 Revalidation

Acceptable validation studies should take place after major manufacturing equipment or procedural changes where previous validation data would be invalidated. Examples of such changes might include:

- Major modification to filling equipment or immediate product containers. (Interchanging standard parts does not constitute a major equipment modification);
- Modification in equipment or facilities which potentially affects air quality or air flow surrounding the operation;
- Initiation of second or third shift production when the facility has been validated for single shift operations.

5.3 Validation System Reappraisal

Each manufacturer should determine his own schedule for reappraisal of validated systems—e.g., semi-annually or annually. This reappraisal may consist of a review, by an interdisciplinary team, of minor manufacturing equipment or procedural changes which were deemed not to require revalidation, along with review of pertinent work orders as well as instrument calibration and environmental monitoring data associated with the system. Provided that all documentation meets the manufacturer's requirements, revalidation may consist of no more than a formally approved document concluding that the system is operating under the state-of-control conditions originally validated. Alternatively, a manufacturer may decide to perform a revalidation study even when no change is believed to have oc-

curred. In cases where the review process has detected deviations from the originally validated state-of-control, and these deviations do not satisfy the manufacturer's requirements, revalidation studies should be performed.

VI. SUMMATION

It is the attempt of this technical report to provide the user with several of the many approaches which may be utilized for the validation of aseptic drug powder filling processes as well as a listing of reference material available on this subject.

Though this report does not endorse any specific endpoint for media fill testing, we have referenced several published media fill contamination rates which have been used within the parenteral industry. Since the human element is usually the most influential variable and the most difficult of all operational variables to simulate and control, the results obtained by any process simulation approach cannot be considered absolute. Each firm must judge whether component system validation or process simulation testing provide the most appropriate level of protection for each of its specific processes.

Furthermore, if one of the process simulation testing approaches is selected, each manufacturer must establish what test endpoints are appropriate for the specific process under consideration.

APPENDIX A

MICROBIOLOGICAL GROWTH-SUPPORTING MEDIA USED IN ENVIRONMENTAL MONITORING AND PROCESS SIMULATION TESTING

This listing is provided solely for user guidance with no implicit or explicit endorsement intended. Other equally or more effective growth-supporting media may exist, and the user is urged to select *and demonstrate* the suitability of any medium chosen (including those listed below) for the given situation.

Liquid Media

Soybean-Casein Digest Broth (USP XXI) (SCD Broth)
Fluid Thioglycollate Medium (USP XXI) (FTM)
Brain Heart Infusion Broth (BHI Broth)
Alternative Thioglycollate Medium (USP XXI) (NIH)

Agar Media

Soybean-Casein Digest Agar (USP XXI) (SCD Agar)
Nutrient Agar
Brain Heart Infusion Agar (BHI Agar)
Lecithin Agar
Tryptone Glucose Yeast Agar (TGY Agar)

NOTE: It may be necessary to add neutralizers to media used for contact plates, air sampling plates, and swabs to counteract the effect of any residual surface disinfectant or antibiotic where applicable.

APPENDIX B

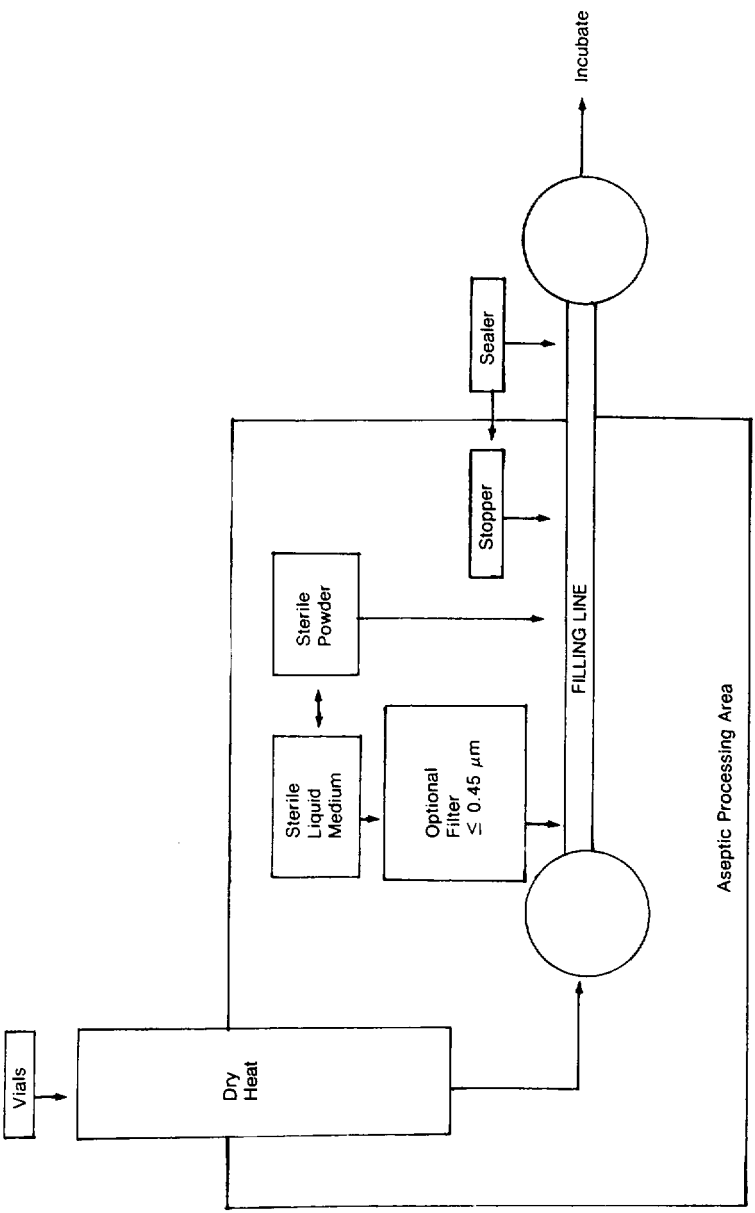

Diagram 1. Use of Liquid—Powder On Line: Approach I.

APPENDIX B (Cont'd)

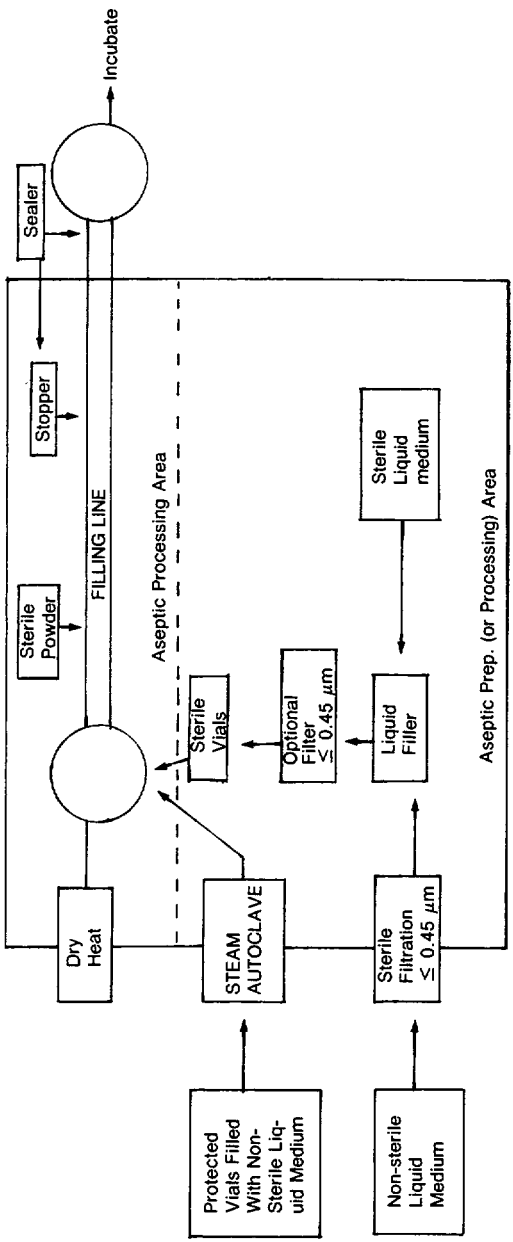

Diagram 2. Use of Sterile Diluent Off Line—Sterile Powder On Line: Approach II.

APPENDIX B (cont'd)

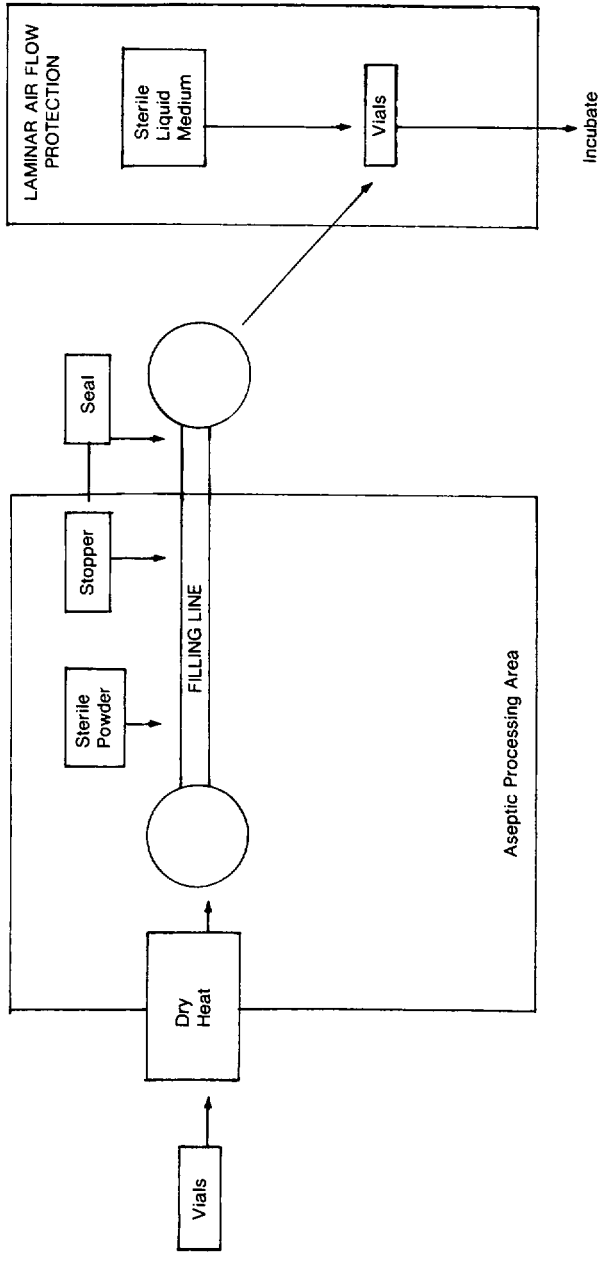

Diagram 3. Use of Sterile Powder On Line—Diluent Off Lines: Approach III.

Probability[a] of Detecting Non-sterility in Media Fills

X (%)	*N* 100	500	1000	1500	2000	3000	5000	10,000	15,000	20,000	30,000
0.01	0.010	0.049	0.095	0.139	0.181	0.259	0.393	0.632	0.777	0.865	0.950
0.05	0.049	0.221	0.394	0.528	0.632	0.777	0.918	0.993	>0.999		
0.10	0.095	0.394	0.632	0.777	0.865	0.950	0.993	>0.999			
0.20	0.181	0.632	0.865	0.950	0.982	0.998	>0.999				
0.30	0.260	0.777	0.950	0.989	0.997	>0.999					
0.40	0.330	0.865	0.982	0.998	>0.999						
0.50	0.394	0.918	0.993	>0.999							
0.75	0.525	0.977	>0.999								
1.00	0.634	0.993									
2.00	0.867	>0.999									
3.00	0.952										
4.00	0.983										
5.00	0.994										
10.00	>0.999										

[a] The probability of detecting non-sterility in media fills is determined by the following equation: $P = 1 - (1 - X)^N$: where *P = the probability; X = acceptable percent contamination rate; and N = total number of vials filled.*

APPENDIX D

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5. Prout, G., "Validation and Routine Operation of a Sterile Dry Powder Filling Facility," *J. Parent. Sci. Technol.*, **36**(5), 199 (1982).
6. The United States Pharmacopeia, 21st rev., Mack, Easton, Pa. (1984), p. 1156.

APPENDIX E

SOURCES OF FURTHER INFORMATION ON VALIDATION OF ASEPTIC PROCESSES

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