Technical Monograph No. 2

VALIDATION OF ASEPTIC FILLING FOR SOLUTION DRUG PRODUCTS

PARENTERAL DRUG ASSOCIATION, Inc.

PREFACE

THIS IS THE SECOND Technical Monograph dealing with the subject of sterile process validation[i]. In this treatment, our intent is to provide guidance to the generally more elusive and demanding process of aseptic filling of solution drug products. Because the final stage of this process is not subject to the more explicit parameters of time-temperature as is terminal steam sterilization, the method of assigning a level of sterility assurance becomes more open to controversy. Task Group 15, under the chairmanship of Michael Anisfeld, has provided a reasonable approach to accomplish this aim.

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Chairman,
Research Committee

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

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CHAPTER 1. SCOPE

This guide focuses on the aseptic filling of solution drug products. The scheme outlined should be effective, regardless of the dosage form being considered (ampul, vial, syringe, etc.).

The applicability of the techniques and philosophies discussed in this document to other parenteral drug formulations (powders, suspensions, lyophilized product, etc.), will have to be determined, on an individual basis, by the drug manufacturer.

Submitted solely as a guide, this technical monograph is not intended to establish mandatory standards.

It is the judgment of the Task Group compiling this monograph that by utilizing the techniques stated—or other equivalent methods—the probability of sterility achieved using an aseptic filling process can be validated. The recommended levels of probability of sterility represent a level of assurance that this Task Group believes is achievable based on current technology and industrial expertise.

This monograph provides examples of equipment and materials that may be used to assist with the validation process. The choice of equipment and materials listed in no way represents an endorsement of the items listed, but represents materials and equipment with which the Task Group is acquainted. Alternate equipment and materials of equal or better utility may exist; the user is advised to use the product that best suits the desired objectives.

CHAPTER 2. INTRODUCTION

Although terminal sterilization using heat will sterilize drug and drug container/delivery systems to acceptable levels of sterility assurance, the process may degrade drug products or render parenteral drug container/delivery systems unsuitable for their intended use over the product life. For those systems that must be sterile and are not terminally sterilized, an alternative is processing by aseptic technique. Aseptic processing (or sterile technique) requires the pre-sterilization of all components of the drug product and its container. Then, all of the components are brought together in a controlled environment to create the finished entity—drug product sealed within its container closure system.

This aseptic filling process is one of the most exacting operations in the pharmaceutical industry. Bearing in mind the stringent requirements demanded for the end use of the material being processed, all efforts must be expended to systemize the aseptic process to yield reproducible validated standards of product sterility.

This monograph defines one system for validating aseptic filling operations. We do not suggest that this is the only method of performing the validation process nor do we imply that other techniques are not acceptable for the validation process. Rather, we present this as a guide to a systematic approach to the validation of aseptic filling. This monograph includes an indication of levels of probability of sterility assurance that should reproducibly be attained.

Users of this monograph should realize that assurance of product sterility relies upon each step in the aseptic process being validated to known levels of sterility assurance. These process steps include:

- Container/Closure Integrity
- Container Sterilization
- Closure Sterilization
- Drug Product Sterilization
- Drug and Container Contacting Equipment Sterilization
- Aseptic Filling
- Aseptic Sealing

The level of sterility assurance attained is a cumulative function of all the process steps involved in the product's manufacture. The final level of sterility assurance claimed for the product cannot be greater than that of the processing step providing the lowest probability of sterility.

In addition to processing operations, environmental factors must be validated to demonstrably support the aseptic process. These environmental factors include but are not limited to:

- Facility Design and Operating Parameters
- Room Pressure Differentials
- Room Air Change Frequencies
- Humidity and Temperature Levels
- HEPA Filter Efficacy

The impact of human involvement in aseptic processing cannot be overstated, and it should be realized that the levels of sterility assurance claimed are subject to the random variable of the human factor. The staff should be trained to minimize variation on the system. Specific training programs should be prepared relating to:

- Personal Hygiene
- Gowning Technique
- Manipulative Technique
- Safety
- Cleaning Procedures

Adherence to the program outlined in this monograph will enable the user to accomplish the validation objective of ensuring that all the steps in the chain of the aseptic process are collectively functioning; and, yielding a product which can be claimed 'sterile' at a known level of sterility.

CHAPTER 3. DEFINITIONS

The following terms, used in this monograph, are intended to convey the following meanings:

Containers

All final packaging components which contact (or will contact during use) the drug product. Includes closures, where these are distinct from the "holding" entity, and delivery systems such as needles of prefilled syringes.

Critical Area

Any area within the aseptic filling room where product and/or containers are exposed to the environment.

Critical Surface

Any surface which contacts previously sterilized product and/or containers (e.g., product transfer piping downstream of sterile filters).

Probability of Sterility

The likelihood that a product is sterile.

Process Simulation Test

A test of the ability of the manufacturing process to aseptically combine previously sterilized product and containers (using growth supporting medium in a place of drug product). This test is a method of determining the probability of microbial contamination of the product manufactured under normal process manipulation. (This test is sometimes referred to as a Media Fill or a Media Run.)

Smoke Test

A test using smoke, generated by smoke sticks or other suitable smoke sources, to visually observe the patterns of air flow (laminar or turbulent) in a clean room. This test does not include DOP testing for leaks in HEPA filters.

Validation

A systematic process of demonstrating that a process will reproducibly meet its claims.

CHAPTER 4. THEORY OF VALIDATING THE ASEPTIC FILLING PROCESS

Aseptic processing, one of the most demanding operations in the pharmaceutical industry, requires validation to assure batch-to-batch consistency of product quality. For aseptic processing, the major concern regarding product quality centers about the sterility assurance for the drug product, and the ability to consistently produce that level of sterility assurance.

The validation scheme, outlined in this monograph, attempts to establish that the aseptic process lives up to its claim—that it is indeed an "aseptic" process.

Some basic steps for validation of the system are:

- Properly designing the facilities and systems;
- Challenging the system (testing those systems and methods "in place");
- Monitoring the system (recording the effects of the challenge);
- Assessing the system (evaluating the data to determine whether the "in place" system can indeed perform to the standards (confidence levels) claimed).

When validating the system, it is necessary to agree on the acceptable validation criteria *prior to conducting the challenge.*

Criteria for acceptable validation can be derived from many sources; these sources include, but are not limited to:

- Published Standards (e.g., NASA Specifications, WHO, FDA Guidelines, USP);
- Previously gathered on-site data.

The challenge tests used and the interpretation of results must be consistent if the validation exercise is to be meaningful.

The sequence of activities outlined in this monograph, when correctly carried out, is a scientifically sound validation program. However, the validation process is an integral part of the overall

facility quality profile and must be correlated with all other usual testing and monitoring activities. Some of the activities performed during validation can be considered as "extreme-worst case" challenges whose benefits lie in their diagnostic potential for highlighting if, and where, (and possibly to what extent), problems or system compromises exist.

4.1 "Start-Up" Validation

For a new facility or new production process, one approach to validation would be:

a) Check the facility to ensure that all equipment installed (both production and utility) satisfy the engineering/quality design criteria intended and are functioning properly;
b) Evaluate the air flow patterns and air quality in critical areas using smoke sticks, particle counters and microbial air monitors to ensure proper air quality and flow patterns exist. This evaluation may be performed for the four following situation combinations (or those applicable) to demonstrate the sequential impact of increasing activity and provide a base for evaluating activity loadings. Or, it may be performed only on the fourth situation to demonstrate that the entire system functions adequately.

- The critical area, with no process equipment present, and no personnel present
- The critical area, with process equipment present (but not running), and no personnel present
- The critical area, with process equipment running (ideally with movement of containers and closures), and no personnel present
- The critical area with process equipment running (and filling product) and personnel present performing normal operations.

The critical area should be monitored for viable particles during the above activity. This monitoring of facility air quality should be continued until a steady-state capability has been demonstrated;

c) Perform process simulation tests (as outlined in Chapter 5) and continue monitoring the critical area (air and exposed surfaces) for viable particles;

d) Review the data generated from the above activities and determine if the facility operational criteria have been met. An ancillary effect of this review might be to redefine/reset facility limits in the light of what has been demonstrated as achievable.

At least two consecutive acceptable process simulation tests should be completed prior to release of normal production to market.

4.2 "On-Going" Validation

An on-going facility/process will, by definition, have a bank of historical performance data. This data should minimally consist of:

- Viable organism counts taken routinely in the critical area during normal operations and with personnel present;
- Results of sterility testing of aseptically produced drug product.

The need for re-validation of an "on-going" facility/process should be seriously considered if any of the following incidents (or combination of situations) occur:

- Environmental data shows an increasing trend for viable organisms in the critical area;
- Facility guidelines are exceeded for viable organisms;
- An increased incidence of product sterility test failures occurs.

When these incidents occur, a review of the process and any changes that may have occurred since the previous validation, as well as a scrutiny of the environmental data and sterility test data, should be performed.

The current situation should be compared to previous validation data and to facility guidelines currently in force.

Based on this evaluation, appropriate corrective action can be taken to restore the facility to its "validated state." Process sim-

ulation testing might be used to assure that the "validated state" has been re-established. (Validation scheduling is further elaborated in Chapter 6.)

CHAPTER 5. METHODS OF VALIDATING THE ASEPTIC FILLING PROCESS

Validation of the aseptic filling process involves consideration of two situations. The first situation is the immediate product environment (container, drug product, critical surfaces), all of which have been previously sterilized. The second situation is the "environment" where these previously sterilized materials are brought together and manipulated (processed) into the finished dosage form.

This Chapter discusses methods for monitoring the "filling room environment," the "immediate product environment," and for validating the aseptic filling process.

The sections on Microbial Monitoring and Evaluation of Air/Surfaces details equipment and techniques applicable to evaluating the air and sanitation systems in the filling area. The Section on Process Simulation Testing details techniques used to validate the efficacy of the aseptic process including the steps prior to filling and the filling process itself. Both sets of data must be evaluated together to arrive at valid conclusions relating to facility capabilities.

5.1 Environmental Monitoring

The fill room environment is the last occasion, 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 microorganisms suspended in the air; or manipulative procedure, where actual component handling leads to contamination.

5.1.1 Microbial Monitoring and Evaluation of Air

Equipment used for microbiological evaluation and monitoring of air should be subject to routine calibration. Growth support of the media should be tested and demonstrated as being effective (to include the sampling/handling conditions to which the media will be exposed).

The user should establish action levels for microbial counts

which, when exceeded, will cause a planned sequence of corrective actions to occur. These corrective actions should be designed to return the facility to the originally validated state and, if possible, to ascertain the cause of the deviation from normal levels.

The locations where air sampling is performed should be those places in the process in which the product has the greatest potential for becoming contaminated from the environment. Smoke testing may help identify these areas.

Listed below are methods for microbial monitoring and evaluation of air. The user is cautioned that some of these methods could, by the very nature of the bulk of the sampling equipment and of the sampling manipulations required, alter the airflow patterns and the environment of the very systems that are the subject of the monitoring. These disturbances should be minimized. The user should select those methods most appropriate to his situation and needs.

a) *Qualitative Method*

Settling Plates

This is one of the most widely used methods for monitoring viable organisms in the air in aseptic filling areas. Petri dishes containing sterile microbiological growth media are exposed to the environment. Viable organisms which settle on the media surface will grow after suitable incubation. Any organisms present should be counted, identified, and evaluated in relation to the user established action levels.

This basic qualitative technique is simple, portable, and inexpensive; does not require sub-culturing procedures; and allows for continuous monitoring. Collection efficiency is affected by air currents and particle size, and is random in nature.

Location of the settling plates is important. At least two plates should be exposed continuously during the validation process. One plate should be located as close as possible to the filling equipment; the location of the other plate would depend on the history established for the area, selecting the "worst case" location anticipated. [If

no such history exists, the British Standards Institute recommendations (BS 5295, see Appendix C) may be used as a starting point to detect if differences occur within the aseptic filling area.] Media should be demonstrated as growth supporting for the time and conditions under which exposure occurs. Plate counts should be zero after 30 minutes exposure with occasional counts of one or possibly two organisms. Counts of three or more on one plate, recurring counts of one or two in the same critical area, or counts of one or two in more than one critical area are an indication that investigative and possible corrective action may be required.

b) Quantitative Methods

Sampling rates and action levels should be established by the user, but as a guide, counts of 0.1 organism/ft^3 of sampled air should be attainable for laminar flow air (NASA Standard NHB 5340.2, see Appendix C).

1. Slit-to-Agar Impact Sampler

The slit-to-agar impact sampler employs a revolving plate containing a suitable growth support media containing agar, 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 sub-culturing 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, such as 70% alcohol. Portions can be removed and separately sterilized. This instrument cannot be used during dry powder filling operations without precautions or modifications to keep powder from causing damage to the instrument.

2. Reuter Centrifugal Sampler

This battery operated device draws air at 40 L/min

through its rotating blades. Particles in the air are hurled by centrifugal force against a nutrient agar strip substrate (obtainable 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), and does not require a vacuum or power source. Portions of the unit (impeller blade assembly and the open end drum) can be steam sterilized.

3. *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, highly efficient, and quantitative. 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. The glass units are easily cleaned and sterilized, but should be handled with care due to their fragile construction. A vacuum source is required for operation of the device. The collecting fluid (usually normal saline solution) may require the addition of an antifoaming 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. Due to basic differences between Liquid Impingement techniques and Agar Impaction techniques of air sampling, results obtained by the two methods may not be directly comparable.

4. *Membrane Filtration*

Air is drawn across a membrane filter surface by vac-

uum for a period of time related to anticipated room contamination levels. The membrane filters are then mounted on a nutrient media to promote growth of colony forming units. The method is economical and allows sampling at virtually any location. It is suggested that the manufacturer's recommended methods of sampling and membrane handling be followed.

With these membrane filtration techniques, gelatin membranes can be used to keep viable organisms from drying out. In addition, since 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.

5. *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 is incubated and colonies counted.

The device is portable, fairly efficient, and does not require sub-culturing procedures. The unit requires a vacuum source for operation. It should be noted that the high air velocity used with the device tends to dry out the growth supporting media.

5.1.2 Microbial Monitoring and Evaluation of Surfaces

a) Contact Plates

Contact plates are prepared with an appropriate media, containing agar, in a manner that results in the surface of the media protruding above the sides of the plate. After removal of the plate cover, the sterile media surface is applied to a smooth, flat 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 surfaces such as equipment, floors, walls, and 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 media residue is removed from the surface tested by cleaning with an appropriate agent such as sterilized alcohol. If disinfectants are used to sanitize the equipment, the media can be inactivated. To avoid this, a suitable neutralizer such as Lecithin or Tween could be added to the media (see Appendix A).

It is advisable to test surfaces as closely as possible to the critical portions of the aseptic operation (e.g., filling machines, stopper hoppers). Floor and wall surfaces in the critical area should be monitored sufficiently during validation and periodically thereafter to assure adequate sanitation and microbial kill. For critical surfaces, counts should be zero. Repeated counts of one or two on one or more surfaces should be questioned. For floors and walls consistently low counts should be attained. Additionally, the monitoring of operator's gloves is recommended.

b) *Swabs*

Sterilized swabs are immersed in a suitable liquid culture media, or sterile diluent. Using aseptic techniques, the swabs are then rubbed over the test surface and placed in an appropriate incubation medium. The number of colony forming units, per unit area, can be quantified using standard microbiological techniques such as plate counting.

Swabs are suitable for irregular surfaces. The technique is more suited for qualitative than for quantitative assessment. Surface swabbing should be standardized to a consistent area (4 $in.^2$ is a recommended area). Counts should usually be zero on critical surfaces.

As in the case of contact plates, when media is used, proper cleanup is essential.

c) *Agar-Overlay*

A representative surface material is mounted in a suitable

location (generally on or near the filling machine) for a certain period of time and then completely immersed in a suitable medium. This method is accurate in terms of recovery and allows for quantitative examination of the residual effects of surface sanitizers; however, this technique can be cumbersome to use.

5.2 Process Simulation Testing

Simulation of aseptic manufacturing processes using a microbiological growth medium is a sensitive and valuable tool in the overall assessment of the microbiological acceptability of a manufacturing process. This testing method allows for evaluation of all previous steps in the process and, therefore, provides a realistic indication of the capability of the overall process from a microbial control viewpoint. A second attribute of this method is that the results can be useful in detecting and identifying process or procedural weaknesses which can lead to microbiological contamination of product.

Although there are distinct advantages in this approach to validation and monitoring, there are some limitations. Since a microbiological growth medium is used to simulate the product, there is a potential risk to the clean room if medium spillage occurs. This critique can, however, be an asset in that it verifies the effectiveness of facility cleaning and sanitation methods.

Despite its limitations, the information afforded by the process simulation test makes it one of the most valuable monitoring tools currently available for the validation of an aseptic filling process.

Process simulation tests should be used to verify the acceptability of all aseptic manufacturing operations.

A manufacturing operation can consist of:

1. An operation requiring unique equipment or handling procedures, e.g., lyophilized products, ointments, powders;
2. Operations for manufacturing different packaging configurations, e.g., syringes, ampuls, vials;
3. Similar operations performed in separate filling suites;
4. Similar operations requiring separate operating personnel.

5.2.1 Medium Considerations for Use in Product Simulation Tests

a) Type of Medium

A number of general microbiological growth media are available and may be used in a process simulation program. 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 organisms including fungi and yeasts.

Clarity—The medium should be clear to allow for ease in observing turbidity.

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

Soybean casein digest (SCD)* is currently one of the most frequently used medium (see Appendix C—'Proceedings of the Second PMA Seminar on Validation of Sterile Manufacturing Processes), due to its low selectivity and relatively low cost; however, a partial listing of acceptable medium would also include the following:

- Tryptone Glucose Yeast Extract (TGYE)*;
- Brain Heart Infusion (BHI)*;
- Alternate (NIH)* Thioglycollate—(If an anaerobic growth medium is desired).

b) Medium Concentration

The medium, of manufacturer's recommended concentration, should be used when preparing media for process simulation tests unless alternate concentrations can be shown empirically to be equivalent.

c) Medium Utilization

In conducting process simulation tests, there are two basic

* See Appendix A for formulae.

alternative techniques available:

1. Use unsterilized medium and filter the medium through the normal sterilizing membrane hooked directly to the filling equipment. The media may be prefiltered to reduce bioburden and increase filtration efficiency;

2. Pre-sterilize the medium in a separate operation. After verification of medium sterility (such as examining the bulk medium for absence of growth), use the medium in the process simulation test. For the test, pass the sterilized medium through normal processing equipment.

d) Medium Sterilization

Medium for use in a process simulation test can be rendered sterile using either moist heat (autoclaving) or filtration. The method chosen depends on the availability of suitable equipment, and the information desired from the study.

1. Sterilization with Steam

When using this approach it is recommended that:

- The medium should be solubilized and dispensed into vessels with suitable closures to allow for filtered gas exchange, and for subsequent dispensing at the filling line. The vessel should, if possible, be identical to regular production equipment;
- The medium should be exposed to steam under pressure in a validated sterilization cycle to achieve at least a 10^{-6} probability of survival of organisms within the medium;
- Medium should be cooled slowly to prevent excessive boiling;
- Medium is ready for use immediately upon cooling. It should be inspected for clarity prior to use.

2. Sterilization by Filtration

When using this approach it is recommended that:

- Medium be solubilized at an elevated temperature (50 °C) to facilitate dissolution of the solids;
- Filtration be conducted under normal production conditions using a sterilizing grade of filter with adequate pre-filtration to increase final filter throughput and life;
- Medium may be stored in bulk vessels following filtration to insure that adequate aseptic technique was used.

5.2.2 *Process Simulation Test Parameters*

a) Scheduling

Process simulation tests are conducted independent of production runs, with the filling line set up specifically for the test. These tests should be conducted at various times during the normal production shifts.

b) Number of Containers

The quantity of containers filled during a process simulation test should meet one or more of the following criteria (where possible):

- The number of containers required per process simulation test may be defined as the number of containers normally filled in a given fill period when operating the equipment at normal filling speeds;
- The number of containers filled per media run may be defined in terms of a pre-determined time period when operating the equipment at normal filling speeds. For example, the media test may be defined as one hour's normal production output;
- The process simulation test should be of such duration that sufficient containers* are filled to properly determine the contamination rate.

* To determine the number of units that need to be tested in order to have a probability of 95% of detecting at least one failure; the following formula applies: $P(x > 0) = 1 - e^{-NP} > 0.95$. The formula is valid where the failure rate is no larger than 0.1%. At the 0.1% failure rate, $N = 2996$ or about 3000 units need be tested to be assured of 95% probability of detecting at least one contaminated unit. At the 0.1% failure level, the test is deemed successful if no more than three contaminated units are observed out of 3000 tested.

c) *Filling Speed*

Equipment should be operated at normal production speeds.

d) *Filling Volume*

The volume of medium filled in each container during a process simulation test should be equivalent to the volume of product normally filled. This is necessary to simulate wetting of the container by the medium as well as to simulate the exposure conditions experienced during normal production.

If lesser volumes of medium are filled, the equivalency of this deviation from the normal production situation should be documented.

5.2.3 Media Incubation Parameters

a) *Technique*

The filled container with medium should be gently rotated immediately prior to incubation so that all surfaces, including the closure (if any), are wetted by the medium. The container should be incubated in an upright position with the closure uppermost. This posture minimizes the migration of closure ingredients which might affect the growth promoting characteristics of the medium.

b) *Time*

Media, in the sealed container as delivered from the production line, should be incubated for a minimum of 14 days.

c) *Temperature*

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

The temperature should be monitored throughout the test period and should be maintained within the specified range for the test period. Deviations from the specified range should be evaluated and countered with appropriate action.

d) Positive Controls

These should be incubated under identical incubation conditions as the test containers.

5.2.4 Test 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.

a) Microorganisms

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

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

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 be used as controls. In all cases, however, microorganisms used in growth promotion testing should include both bacterial and fungal species.

b) Challenge Parameters

Challenge levels not to exceed 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

* Use only if testing for anaerobiosis of thioglycollate medium.

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 identical to those of the test medium.

c) *Interpretation of Results*

Medium is acceptable if growth is observed in at least one of the two test containers for all of the challenge microorganisms.

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

5.3 Analysis and Interpretation of Production Simulation Testing

5.3.1 Test Read-out

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. When damaged containers are found, an investigation into probable causes should be conducted and appropriate corrective action taken.

The identity of the microorganism within contaminated containers should be determined. This identification, at a minimum, should include colony and cellular morphology as well as Gram stain characteristics.

5.3.2 Test Limits and Remedial Action

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

$$\% \text{ Contamination level} = \frac{\text{no. of undamaged containers with microbial growth}}{\text{(total no. containers filled} - \text{no. of contaminated damaged containers)}} \times$$

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 is the responsibility of each manufacturer to establish acceptable process simulation testing end-points. A widely accepted end-point contamination level for process simulation testing is 0.3% (WHO, 1973); however, it is suggested that a manufacturer should strive for a contamination level of less than 0.1%.

Following initial validation studies, if a process simulation test failure occurs during routine monitoring, a follow-up program consisting of a review by production as well as quality control/assurance and other functional groups should be initiated to review:

- Environmental data;
- Sterilization records;
- Operational logs;
- Other data associated with the process simulation tests.

A repeat process simulation test should be initiated as soon as possible.

If the contamination level of the repeat test is greater than the established end-point, the process is no longer considered validated at that level.

Investigative action should be initiated immediately to identify the cause of the increased contamination level and appropriate corrective action should be taken. The process cannot be considered revalidated until at least two consecutive acceptable process simulation tests are completed.

CHAPTER 6. VALIDATION SCHEDULE

6.1 "Start-up" Validation or Revalidation of a Process

Prior to the release of a new manufacturing process for production use, acceptable results from consecutive process simulation tests should be achieved to demonstrate the reproducibility of the process.

When conducting validation studies, these process simulation tests should be conducted on separate days simulating different times during the normal working period, and should include normal facility/equipment operations and clean-up routines.

Acceptable results from consecutive process simulation tests should be obtained following any process change of such scope that previous validation studies would be invalidated. Examples of such changes might include:

a) Modifications in equipment directly contacting product or immediate product containers. (Interchanging parts does not constitute need for re-validation.);

b) Modifications in equipment or facilities which potentially affect air quality or air flow surrounding the operation;

c) Major changes in production personnel (e.g. new production crews);

d) Initiation of evening or night shift production.

6.2 "On-going" Product Simulation Testing

Process simulation tests simulating each manufacturing operation should be performed at least semi-annually.

For those operations where multiple pieces of similar equipment are available, monitoring on a rotational basis can be considered. Where this is used, each operation should be validated at least annually. Depending on individual circumstances, more frequent monitoring may be necessary.

Operations used less frequently than once every six months should be re-validated with a single acceptable process simulation test prior to re-commencement of the manufacturing process.

APPENDIX A: FORMULAE FOR GROWTH SUPPORTING MEDIA USED IN ENVIRONMENTAL MONITORING AND PROCESS SIMULATION TESTING

Note: 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 hereunder) for the given situation.

Soybean-Casein Digest Agar Medium (USP XX) (SCD Agar)

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Water	1000 ml

pH after sterilization 7.3

Soybean-Casein Digest Medium (USP XX) (SCD Broth)

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soybean Meal	3.0 g
Sodium Chloride	5.0 g
Dibasic Potassium Phosphate	2.5 g
Dextrose	2.5 g
Water	1000 ml

pH after sterilization 7.3

Fluid Thioglycollate Medium (USP XX) (FTM)

L-Cystine	0.5 g
Sodium Chloride	2.5 g
Dextrose	5.5 g
Agar, granulated (moisture content not in excess of 15%)	0.75 g
Yeast Extract (Water-Soluble)	5.0 g
Pancreatic Digest of Casein	15.0 g
Sodium Thioglycollate	0.5 g
or Thioglycollic Acid	0.3 ml
Resazurin Sodium Solution (1 in 1000), freshly prepared	1.0 ml
Water	1000 ml

pH after sterilization 7.1

Nutrient Agar

Peptone	5.0 g
Beef Extract	3.0 g
Agar	15.0 g
Water	1000 ml

Final pH 6.8

Brain Heart Infusion (BHI)

Infusion from Calf Brain	200.0 g
Infusion from Beef Heart	250.0 g
Peptone	10.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g
Dextrose	2.0 g
Water	1000 ml

Final pH 7.4

Brain Heart Infusion Agar

Infusion from Calf Brain	200.0 g
Infusion from Beef Heart	250.0 g
Peptone	10.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g
Dextrose	2.0 g
Agar	15.0 g
Water	1000 ml

Final pH 7.4

Lecithin Agar

Peptone	5.0 g
Dextrose	1.0 g
Beef Extract	3.0 g
Lecithin	1.0 g
Polysorbate 80	7.0 g
Agar	15.0 g
Water	1000 ml

Final pH 7.0

Tryptone Glucose Extract Agar (TGEA)

Tryptone	5.0 g
Beef Extract	3.0 g
Glucose	1.0 g
Agar	15.0 g
Water	1000 ml

Final pH 7.0

Alternative Thioglycollate Medium (USP XX) (NIH)

L-Cystine	0.5 g
Sodium Chloride	2.5 g
Dextrose	5.5 g
Yeast Extract (water soluble)	5.0 g
Pancreatic Digest of Casein	15.0 g
Sodium Thioglycollate	0.5 g
or Thioglycollic Acid	0.3 ml
Water	1000 ml

pH after sterilization 7.1

APPENDIX B: GROWTH SUPPORTING MEDIA—TYPICAL APPLICATIONS/USES IN ENVIRONMENTAL MONITORING AND PROCESS SIMULATION TESTING

1. For use in settling plates:
   (a) Soy-Bean Casein Digest Agar (SCD)
   (b) Tryptone Glucose Extract Agar (TGEA)
2. For use in Slit-to-Agar sampler:
   Soy-Bean Casein Digest Agar (SCD)
3. For use in Reuter-Centrifugal-sampler
   Nutrient Agar
4. For use in Liquid Impingement techniques:
   (a) Soy-Bean Casein in Digest Agar (SCD)
   (b) Tryptone Glucose Extract Agar (TGEA)
5. For use in Membrane Filtration techniques:
   Soy-Bean Casein Digest Agar (SCD)
6. For use with Cascade/Sieve Impactors:
   Soy-Bean Casein Digest Agar (SCD)
7. For use with contact plates:
   Soy-bean Casein Digest Agar (SCD)*
8. For use with swabs:
   Soy-Bean Casein Digest Agar (SCD)*
9. For use with agar overlay:
   Soy-Bean Casein Digest Agar (SCD)
10. For use in process simulation tests:
    (a) Soy-Bean Casein Digest Broth (SCD)
    (b) Tryptone Glucose Yeast Extract (TGYE)
    (c) Brain Heart Infusion (BHI)
    (d) Alternate (NIH) Thioglycollate Medium

* Note: It may be necessary to add neutralizers to media used for contact plates(7) and swabs (8), to counteract the effects of any residual surface disinfectant.

APPENDIX C: LITERATURE REFERENCES ON VALIDATION OF ASEPTIC PROCESSES

The following references may be found of value by users of this monograph for further background:

Austin, P. A. and Timmerman, S. W., "Design and Operation of Clean Rooms," Business New Publishing Co., Detroit, 1965.

Anisfeld, M. and Lovejoy, C., "Design for a Small-Volume Parenteral Manufacturing Facility," *J. Parenteral Drug Assoc.*, **32,** 285 (1978).

Borneff, J., Werner, H. P., and Duppre, M., "Investigations on the Efficacy of Surface Disinfection and Surface Cleaning Procedures. III. Evaluation of the Results of In-use and Laboratory Tests," *Hyg. Inst., Univ. Mainz.*

British Standards Institute, "Environmental Cleanliness in Closed Spaces," B.S. 5295 (Parts 1, 2, and 3) (1976).

Buhlman, X., Gay, M., Gubler, H. U., Hes, H., Kabary, A., and Knusel, F., "Microbiological Quality of Pharmaceutical Preparations, Guidelines and Methods," *Am. J. Pharm.*, **144,** 165 (1972).

Byers, T. E., "GMP's and Design for Quality," *J. Parenteral Drug Assoc.*, **32,** 22 (1978).

Dirksen, J. W. and Larsen, R. V., "Filling Vials Aseptically While Monitoring for Bacterial Contamination," *Am J Hosp Pharm.* **32,** 1031 (1975).

Gibbs, G. A., and Rountree, P. M., "A Laminar-Flow Sterile Products Department. An Example of Modern Biomedical Engineering," *Med. J. Aust.*, **1,** 253 (1973).

Griffin, J. C., and Pauli, W. A., "Design Concepts for a Sterile Products Production Facility," *Bull. Parenteral Drug Assoc.* **30,** 293 (1976).

Health Industries Manufacturing Association, "Microbial Control in the Manufacturing Environment," (*HIMA*) *Report, 78*—4.3, June 1978.

Hortig, H. P., "Laminar Flow in the Pharmaceutical Industry," *Bull Parenteral Drug Assoc.* **27,** 30 (1973).

Horwarth, W. R., "Environment Control in Parenteral Filling Operations," *Bull. Parenteral Drug Assoc.* **26,** 147 (1972).

Kiritsy, P. A., and Engvall, R., "Sterile Filling Facility—Its Design, Construction and Operations," *Bull. Parenteral Drug Assoc.*, **27,** 279 (1973).

Macek, T. J., "Biological Indicators—A U.S.P. Review," *Bull. Parenteral Drug Assoc.*, **26,** 18 (1972).

NASA, "Standards for Clean Room and Work Stations for Microbially Controlled Environment," NHB 5340.2, August 1967.

Pharmaceutical Manufacturers Association, "Proceedings of the PMA Seminar Program on Validation of Sterile Manufacturing Processes," Reston, Va., March 15–16, 1978.

Pharmaceutical Manufacturers Association, "Proceedings of the Second PMA Seminar Program on Validation of Sterile Manufacturing Processes: Aseptic Processing," Atlanta, Ga., March 1–2, 1979.

Raiman, H. L., "Panel Discussion: Environmental Sampling in an Aseptic Environment. I. Microbiological Environmental Monitoring," *Bull. Parenteral Drug Assoc.*, **28,** 253 (1974).

Schnorf, U., "Production and Control in Pharmaceutic Industry. Claims and Principles in Production of Sterile Preparations. Production in Bigger Plants," *Pharm. Acta Helv.*, **48,** 83 (1973).

Sherman, N. E., "Panel Discussion: Environmental Sampling in an Aseptic Environment. II. Particulate Contamination Control," *Bull. Parenteral Drug Assoc.*, **28,** 260 (1974).

Werner, H. P., Swinke, U., and Werner, G., "Development of a New Test Method for Surface Disinfection Procedures. III. The Impression Method: Influence of the Test Surface Material and the Types of Microorganisms on the Recovery Rate (Author's Transl.)," Hygiene-Institute der Universitat Mainz.

World Health Organization, "Sterility and Sterility Testing of Pharmaceutical