CENTER FOR DRUG EVALUATION AND RESEARCH

Guidance for Industry

The FDA published Good Guidance Practices in February 1997. This guidance was developed and issued prior to that date.

Additional copies are available from:
Office of Training and Communications
Division of Communications Management
Drug Information Branch, HFD-210
5600 Fishers Lane
Rockville, MD 20857

(Tel) 301-827-4573
(Internet) http://www.fda.gov/cder/guidance/index.htm

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION

GUIDELINE ON STERILE DRUG PRODUCTS PRODUCED BY ASEPTIC PROCESSING

June, 1987

(Reprinted June, 1991)

Prepared by: Center for Drug Evaluation and Research
Center for Biologics Evaluation and Research, and
Office of Regulatory Affairs,
Food and Drug Administration

Maintained by: Division of Manufacturing and Product Quality (HFD-320)
Office of Compliance
Center for Drug Evaluation and Research
Food and Drug Administration (FDA)
5600 Fishers Lane
Rockville, Maryland 20857

I. PURPOSE

This guideline informs interested persons on certain practices and procedures for the preparation of sterile drug products by aseptic processing that constitute acceptable means of complying with certain sections of the Current Good Manufacturing Practice (CGMP) regulations for drug products (Title 21 Code of Federal Regulations, Parts 210 and 211). For biological products regulated under 21 CFR Parts 600 through 680, it should be noted that sections 210.2(a) and 211.1(b) provide that where it is impossible to comply with the applicable regulations in both Parts 600 through 680 and Parts 210 and 211, the regulation specifically applicable to the drug product in question shall apply. Therefore, the sterility testing of biological products, and the culture media employed for such testing, must conform to the requirements under section 610.12.

II. INTRODUCTION

This guideline is issued under 21 CFR 10.90, and as such, it states principles and practices of general applicability that are not legal requirements but are acceptable to the Food and Drug Administration (FDA). A person may rely upon this guideline with the assurance of its acceptability to FDA, or may follow different procedures. When

different procedures are chosen, a person may, but is not required to, discuss the matter in advance with FDA to prevent the expenditure of money and effort on activity that may later be determined to be unacceptable.

This guideline may be amended from time to time as the agency recognizes the need through its regulatory efforts and through comments submitted by interested persons.

There are certain differences between the production of sterile drug products by aseptic processing and by terminal sterilization. Terminal sterilization usually involves filling and closing product containers under conditions of a high quality environment; the product, container, and closure are usually of a high microbiological quality but are not sterile. It is important that the environment in which filling and closing is achieved be of a high quality in order to minimize the microbial content of the product and to help assure that the subsequent sterilization process is successful. The product in its final container is then subjected to a sterilization process--usually using heat or radiation. In aseptic processing, the drug product, container, and closure are subjected to sterilization processes separately and then brought together. Because there is no further processing to sterilize the product after it is in its final container, it is critical to the maintenance of product sterility

that containers be filled and closed in an environment of extremely high quality. In addition, there are usually more variables attendant to aseptic processing than to terminal processing, a factor that can make it more difficult to attain a high degree of assurance that the end product will be sterile. For example, before aseptic assembly, different parts of the final product may have been subjected to different sterilization processes -- such as dry heat for glass containers, steam under pressure for rubber closures, and filtration for a liquid dosage form -- each requiring thorough validation and control, each with the possibility of error. (For the terminally sterilized drug product, on the other hand, there is generally only one sterilization process, thus limiting the possibilities for error.) Furthermore, any manipulation of the sterilized dosage form, containers, and closures immediately prior to aseptic assembly involves the risk of contamination and thus must be carefully controlled.

These processing differences have led to several questions on aseptic processing regarding what FDA believes are acceptable ways of complying with certain sections of the CGMP regulations for drug products. The sections most frequently questioned concern buildings and facilities, components, containers/closures, production time limitations, validation, laboratory controls, and sterility testing. Because most of the questions have concerned process validation in

particular, this guideline addresses this area extensively. This guideline is intended to respond to these questions and clarify certain technical aspects of aseptic processing of sterile drug products. It should be noted that this document does not address several other important aspects of aseptic processing--such as employee hygiene, aseptic gowning, and clean room design. These and other aspects will be covered in future revisions of this guideline as needed. This guideline does not address terminally sterilized drug products, although some portions may be applicable to their preparation also.

In this guideline stated CGMP requirements of certain sections of 21 CFR Part 211 are followed by discussions of practices and procedures which FDA considers as acceptable means of meeting the requirements. It should be noted that not all portions of the regulations which apply to the preparation of aseptically processed sterile drug products are identified -- only those portions for which pertinent questions have been raised. The guideline also includes a list of references which may be of value to the reader.

Definitions

Critical areas - Areas where sterilized product or container/closures are exposed to the environment.

Critical surfaces - Surfaces which come into contact with sterilized product or containers/closures.

D value - The time at a given temperature needed to reduce the number of microorganisms by 90%.

Overkill sterilization process - A process which is sufficient to provide at least a 12 log reduction of microorganisms having a minimum D value of 1 minute.

Sterilizing filter - A filter which, when challenged with the microorganism *Pseudomonas diminuta*, at a minimum concentration of 10^7 organisms per cm^2 of filter surface, will produce a sterile effluent.

Validation - Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

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

III. BUILDINGS AND FACILITIES

Requirements

Section 211.42 (design and construction features) requires, in part, that there be separate or defined areas of operation to prevent contamination, and that for aseptic processing there be, as appropriate, an air supply filtered through high efficiency particulate air (HEPA) filters under positive pressure, and systems for monitoring the environment and maintaining equipment used to control aseptic conditions.

Section 211.46 (ventilation, air filtration, air heating and cooling) requires, in part, that equipment for adequate control over air pressure, microorganisms, dust, humidity, and temperature be provided where appropriate and that air filtration systems, including prefilters and particulate matter air filters, be used when appropriate on air supplies to production areas.

Guidance

In aseptic processing there are various areas of operation which require separation and control, with each area needing different degrees of air quality depending on the nature of the operation. Two exposure areas are of particular importance to drug product quality--critical areas and controlled areas.

CRITICAL AREAS

A critical area is one in which the sterilized dosage form, containers, and closures are exposed to the environment. Activities that are conducted in this area include manipulations of these sterilized materials/product prior to and during filling/closing operations. These operations are conducted in what is typically called the "aseptic core" or "aseptic processing" area.

This area is critical because the product is not processed further in its immediate container and is vulnerable to contamination. Therefore, in order to maintain the quality and, specifically, the sterility of the product, the environment in the immediate proximity of the actual operations should be of the highest quality.

One aspect of environmental quality is the particulate content of the air. Particulates are significant because they may enter a product and contaminate it physically or, by acting as a vehicle for microorganisms, biologically. It is therefore important to minimize the particle content of the air and to effectively remove those particles which are present. Air in the immediate proximity of exposed sterilized containers/closures and filling/closing operations is of acceptable particulate quality when it has a per-cubic-foot

particle count of no more than 100 in a size range of 0.5 micron and larger (Class 100) when measured not more than one foot away from the work site, and upstream of the air flow, during filling/closing operations. The agency recognizes that some powder filling operations may generate high levels of powder particulates which, by their nature, do not pose a risk of product contamination. It may not, in these cases, be feasible to measure air quality within the one foot distance and still differentiate "background noise" levels of powder particles from air contaminants which can impeach product quality. In these instances, it is nonetheless important to sample the air in a manner which, to the extent possible, characterizes the true level of extrinsic particulate contamination to which the product is exposed.

Air in critical areas should be supplied at the point of use as HEPA filtered laminar flow air, having a velocity sufficient to sweep particulate matter away from the filling/closing area. Normally, a velocity of 90 feet per minute, plus or minus 20%, is adequate, (Refs. 1 and 2) although higher velocities may be needed where the operations generate high levels of particulates or where equipment configuration disrupts laminar flow.

Air should also be of a high microbial quality. An incidence of no more than one colony forming unit per 10 cubic feet is considered as attainable and desirable (Ref. 3).

Air is not the only gas in the proximity of filling/closing operations which should be of a high particulate and microbial quality. Other gases, such as nitrogen or carbon dioxide, which contact the product, container/closure, or product contact surfaces, e.g., purging or overlaying, should be sterile filtered. In addition, compressed air should be free from demonstrable oil vapors.

Critical areas should have a positive pressure differential relative to adjacent less clean areas; a pressure differential of 0.05 inch of water is acceptable.

CONTROLLED AREAS

The controlled area, the second type of area in which it is important to control the environment, is the area where unsterilized product, in-process materials, and container/closures are prepared. This includes areas where components are compounded, and where components, in-process materials, drug products and drug product contact surfaces of equipment, containers, and closures, after final rinse of such surfaces, are exposed to the plant environment. This environment

should be of a high microbial and particulate quality in order to minimize the level of particulate contaminants in the final product and to control the microbiological content (bioburden) of articles and components which are subsequently sterilized.

Air in controlled areas is generally of acceptable particulate quality if it has a per-cubic-foot particle count of not more than 100,000 in a size range of 0.5 micron and larger (Class 100,000) when measured in the vicinity of the exposed articles during periods of activity. With regard to microbial quality, an incidence of no more than 25 colony forming units per 10 cubic feet is acceptable (Ref. 3).

In order to maintain air quality in controlled areas, it is important to achieve a sufficient air flow and a positive pressure differential relative to adjacent uncontrolled areas. In this regard, an air flow sufficient to achieve at least 20 air changes per hour and, in general, a pressure differential of at least 0.05 inch of water (with all doors closed), are acceptable. When doors are open, outward airflow should be sufficient to minimize ingress of contamination.

Gases other than ambient air may also be used in controlled areas. Such gases should, if vented to the area, be of the same quality as ambient air. Compressed air should be free from demonstrable oil vapor.

In addition to these production areas, there may be certain pieces of equipment which should be supplied with high quality filtered air. This is especially important where the air in the equipment will contact sterilized material or material which should have a low microbial or particulate content. For example, bacterial retentive filters should be used for lyophilizer vacuum breaks and hot air sterilizer vents to ensure that air coming in contact with a sterilized product is sterile. Likewise, air admitted to unpressurized vessels containing sterilized liquid should also be filtered. Air in tanks used to hold material which must be of a high microbial quality should be filtered too, and the filters should be dry to prevent wetting by condensation with subsequent blockage or microbial grow-through (two ways of achieving this are providing heat to the filter and use of hydrophobic filters.) It is important that these equipment air filters be periodically integrity tested.

An acceptable system for maintaining air quality includes testing HEPA filters for integrity. Integrity testing should be performed initially when the units are first installed in order to detect leaks around the sealing gaskets, through the frames or through the filter media. Thereafter, integrity tests should be performed at suitable intervals. Usually it is sufficient to perform such testing at least twice a year for critical areas; however, more frequent testing may be needed when air quality is found to be unacceptably low or as part of an investigation into a finding of non-sterility in a drug product.

One acceptable method of testing the integrity of HEPA filters is use of a dioctylphthalate (DOP) aerosol challenge. Inasmuch as a HEPA filter is one capable of retaining 99.97 percent of particulates greater than 0.3 micron in diameter, it is important to assure that whatever substance is used as a challenge will have a sufficient number of particles of this size range. An acceptable DOP challenge involves introducing a DOP aerosol upstream of the filter in a concentration of 80 to 100 micrograms/liter of air at the filter's designed airflow rating and then scanning the downstream side of the filter with an appropriate photometer probe at a sampling rate of at least one cubic foot per minute. The probe should scan the entire filter face and frame at a position about one to two inches from the face of the filter (Ref. 1). A single probe reading equivalent to 0.01 percent of the upstream challenge is considered as indicative of a significant leak which should be repaired.

Use of particle counters without introducing particles of known size upstream of the filter is ineffective for detecting leaks.

The reader should note that there is a difference between filter integrity testing and efficiency testing. Integrity testing is performed to detect leaks from the filter media, filter frame and seal. The challenge is a polydispersed aerosol usually composed of particles ranging in size from one to three microns. The test is done in place and the filter face is scaned with a probe; the

measured downstream leakage is taken as a percent of the upstream challenge. The efficiency test, on the other hand, is used to determine the filter's rating. The test uses a monodispersed aerosol of 0.3 micron size particles, relates to filter media, and usually requires specialized equipment. Downstream readings represent an average over the entire filter surface. Therefore, leaks in a filter may not be detected by an efficiency test.

It is also important to monitor air flow velocities for each HEPA filter according to a program of established intervals because significant reductions in velocity can increase the possibility of contamination and changes in velocity can affect the laminarity of the airflow. Airflow patterns should be tested for turbulence that would interfere with the sweeping action of the air.

IV. COMPONENTS

Requirements

Section 211.80 (general requirements) requires, in part, the establishment of written procedures for the storage, handling and testing, and approval or rejection of components.

Section 211.84 (testing and approval or rejection of components, drug product containers, and closures) requires, in part, that components liable to microbiological contamination that is objectionable in view of their intended use be subjected to microbiological tests before use.

Guidance

One of the most important aspects of components used in sterile drug products made by aseptic processing is microbiological quality. A finished drug product produced by aseptic processing may become contaminated through use of one or more components which contain microorganisms. Therefore, unless an overkill sterilization process is applied to components, it is important to routinely characterize the microbial content of each component liable to contamination and to establish appropriate acceptance/rejection limits based on this bioburden. Knowledge of this bioburden is especially significant in attaining a high degree of sterility assurance when the component is subjected to a non-overkill sterilization process.

In aseptic processing, each component may be individually sterilized or several components may be combined, with the resulting mixture sterilized. There are several methods to sterilize components, and each can be acceptable when properly validated. A widely used method

is filtration of a solution formed by dissolving the component in a solvent such as USP water for injection; the solution is passed through a sterilizing membrane or cartridge filter. This method can be useful where the component is likely to be adversely affected by heat. A variation of this method involves subjecting the filtered solution to aseptic crystallization and precipitation of the component as a sterile powder. However, this method involves more handling and manipulation than other methods and therefore has a higher potential for contamination during processing.

If a component is not adversely affected by heat, and it is soluble, it may be made into a solution and subjected to steam sterilization either in a separate autoclave or within a steam-jacketed pressurized preparation vessel.

Dry heat sterilization is a suitable method for components that are heat stable and may be insoluble. However, this method can pose problems of inadequate heat penetration and distribution. For example, the treatment of powders by this method necessitates suitable heat penetration and distribution studies because of the powders' insulating effects.

Ethylene oxide exposure is another method of sterilizing components. However, its effectiveness as a primary method is questionable because of a lack of consistent penetration of the sterilant to the crystal core of a powder. Ethylene oxide may be useful for the surface sterilization of powders as a precaution against potential microbial contamination during aseptic handling.

For products intended to be pyrogen free, there should be written procedures for acceptance or rejection of components which are susceptible to pyrogens. Those components found to be contaminated with pyrogens should be rejected or processed to remove the pyrogenic properties provided that the resultant components will meet appropriate standards, specifications, and characteristics.

CONTAINERS/CLOSURES

Requirements

Section 211.94 (drug product containers and closures) requires, in part, that drug product containers and closures be clean and, where indicated by the nature of the drug, sterilized and depyrogenated. Standards and testing methods and, where indicated, methods of cleaning, sterilizing and processing to remove pyrogenic properties must be written and followed.

Guidance

In the case of sterile drug products made by aseptic processing, preparation of containers and closures prior to filling and closing operations should go beyond mere cleaning to remove surface debris. It is critical to the integrity of the final product that containers and closures be rendered sterile and, in the case of injectable products, pyrogen free. The type of processes used to sterilize and depyrogenate will depend primarily on the nature of the material which comprises the container/closure. Any properly validated process can be acceptable.

In the case of glass containers, presterilization preparation usually involves a series of wash and rinse cycles. Not only is it important that these washes effectively remove debris, it is also important that the final rinse water be of a high quality. Final rinse water is acceptable if it meets the requirements of USP water for injection. Depyrogenation may be accomplished by a variety of methods; for example, by initial washings with chemical solutions followed by rinses with water for injection. Dry heat may be used to sterilize and depyrogenate glass containers. Validation of dry heat sterilization/depyrogenation should include heat penetration and heat distribution studies as well as use of representative process cycles

and loading configurations to simulate actual production practices. Whatever depyrogenation method is used, the validation data should demonstrate that the process will reduce the endotoxin content by 3 logs.

One method of assessing the adequacy of a depyrogenation process is to simulate the process using containers having known quantities of standardized endotoxins and measure the level of reduction. However, FDA is aware of one potential problem where challenge endotoxins are used to assess certain washing processes. The problem stems from applying the powdered endotoxin challenge directly to the surface being tested, rather than first resolubilizing the material and air drying it onto the surface. The powdered material may be much more soluble in the wash and rinse water than the reconstituted air-dried material and more so than endotoxins that may normally be present on container/closure surfaces. This could result in the perception of the process under consideration as being much more efficient at endotoxin removal than it really is. Therefore, endotoxin challenges should not be easier to remove from the target surfaces than the endotoxins that may normally be present.

Plastic containers, subjected to uncontrolled handling and storage may be a source of pyrogens and should, therefore, be depyrogenated. Plastic containers may be sterilized with ethylene oxide gas. Biological indicators can be useful to monitor such processes, along

with monitoring and control of temperature, pressure, humidity, and ethylene oxide concentration. The potential for residues, such as ethylene oxide and its degradation products, to remain on or in the container should be assessed.

Rubber compound stoppers pose another potential source of microbial and (of concern for products intended to be pyrogen free) pyrogen contamination. They are usually cleaned by multiple cycles of washing and rinsing prior to final steam sterilization. The final rinse should be with USP water for injection. It is also important to minimize the lapsed time between washing and sterilizing because moisture on the stoppers can support microbiological growth and the generation of pyrogens. Because rubber is a poor conductor of heat, proper validation of processes to sterilize rubber stoppers is particularly important.

VI. TIME LIMITATIONS

Requirements

Section 211.111 (time limitations on production) requires, in part, the establishment of time limits for completion of each phase of production, when appropriate, to assure the quality of the drug product.

Guidance

In aseptic processing of sterile drug products the establishment of time limitations is generally appropriate for several operations. The total time for the product filtration and filling operations, for example, should be limited to an established maximum in order to prevent contamination of the filtrate by microorganisms growing or passing through the filter over a period of time. Such a limit should also prevent a significant increase in the number of microorganisms on the upstream side of the filter, which increase could lead to pyrogen formation.

VII. PRODUCTION AND PROCESS CONTROLS; VALIDATION

Requirements

Section 211.113 (control of microbiological contamination) requires, in part, the establishment and adherence to appropriate written procedures designed to prevent microbiological contamination of drug products purporting to be sterile. Such procedures must include validation of any sterilization process.

Guidance

In order to assure the sterility of products purporting to be sterile which are prepared by aseptic processing, it is most important that two types of operations in particular be adequately validated, namely sterilization and filling/closing under aseptic conditions. The objective of the most effective sterilization processes can be defeated if the sterilized elements of a product -- the drug, the container and closure -- are brought together under conditions that contaminate those elements. Conversely, product sterility may be compromised where those conditions add no contamination whatsoever, but where the product elements are not sterile at the time they are assembled.

Questions have arisen as to acceptable ways of validating the aseptic assembly of sterile product elements, and the sterilization of those elements. However, the former operation is considered by some people to be the most difficult and has generated more questions. Therefore, the guidance presented places greater emphasis on validating the aseptic assembly (i.e., filling/closing) operations.

Aseptic Assembly Operations

An acceptable method of validating the aseptic assembly process involves the use of a microbiological growth nutrient medium to simulate sterile product filling operations. This has been termed "sterile media fills". The nutrient medium is manipulated and exposed to the operators, equipment, surfaces, and environmental conditions to closely simulate the same exposure which the product itself will undergo. The sealed drug product containers filled with the media are then incubated to detect microbiological growth and the results are assessed to determine the probability that any given unit of drug product may become contaminated during actual filling/closing operations. Media filling in conjunction with comprehensive environmental monitoring can be particularly valuable in validating the aseptic processing of sterile solutions, suspensions, and powders. Filling liquid media, as part of validating the processing of powders, may necessitate use of equipment and/or processing steps that would otherwise not be attendant to routine powder operations. However, such additional efforts are valuable and important in characterizing exposure of powders to contamination.

Several questions about media fills have been raised concerning contaminating equipment with media, frequency and number of runs, size of runs, the medium itself, environmental conditions, and test results.

1. Contamination with media - Some drug manufacturers have expressed concern over the possible contamination of the facility and equipment with the nutrient media during media fill runs. However, if the medium is handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary, sterilization of equipment, then media fill operations should not compromise the quality of product subsequently processed using the same facility and equipment.

2. Frequency and number of runs - When a process is initially validated each separate media fill should be repeated enough times to assure that the results are consistent and meaningful. This is important because a single run may be faulty, and widely divergent results of multiple runs may signal a process that is not in control. FDA believes that, in many cases, at least three separate runs are needed: this minimum number has been recognized as a general validation principle in the industry (Refs. 6 and 7). The frequency of additional media fills needed after initial validation has been completed will vary depending

upon a number of events and changes that may affect the ability of the aseptic process to exclude contamination from the sterilized product elements. For example, facility and equipment modification, significant changes in personnel, anomalies in environmental testing results, and end product sterility testing showing contaminated products may all be cause for revalidating the system. In the absence of such changes or events, however, a generally acceptable frequency is at least twice each year for each shift for each filling/closing line. All personnel should take part in a media fill at least once a year. Depending upon the reason for revalidating the process using a media fill, the number of separate media fill runs per revalidation need not be as great as in the initial validation. For example, a single run may be sufficient if results are consistent with the initial validation, and if environmental monitoring data demonstrate that the quality of the filling environment is within established limits. However, additional confirmatory media fill runs are warranted where findings are adverse or indicate the proccess may not be in control.

3. Size of runs - The number of units in the media fill should be large enough to yield a high probability of detecting low incidences of contamination. It has been reported that at least 3,000 units are needed to detect, with 95% probability (confidence), a contamination rate of one in one thousand (Ref. 4).

The duration or time of aseptic processing operations should also be a primary consideration in determining the size of the run. Specifically, the duration of the run should be sufficient to cover all manipulations that are normally performed in actual processing. The number of test units should reflect "worst case" situations concerning exposure time at filling rates which are equivalent to or slower than actual production filling speeds.

It is also important that each unit be filled with enough medium to contact the inside container surface (e.g., by swirling the container of medium), and to permit the visual detection of microbial growth.

4. Media - The most important aspect of media is their ability to promote microbiological growth. Before any medium is chosen for validation runs, it should be demonstrated capable of supporting microbiological growth. In this regard, it is valuable to incubate positive control units along with media fill runs. Generally, a microbiological growth medium that supports the growth of a broad spectrum of aerobic microorganisms, such as soybean-casein digest medium, is acceptable. The microorganisms referenced in the United States Pharmacopeia/National Formulary--USP XXI/NF XVI for sterility test growth promotion tests are acceptable for this purpose.

In selecting suitable media, consideration should also be given to their ability to grow the types of microorganisms that have specifically been identified by environmental monitoring and by positive sterility test results.

It is also important to incubate the media sample units for a sufficient time (a period of not less than 14 days is acceptable) at a sufficient temperature to detect organisms that may not grow in other incubation conditions because of the possible shock administered to them by sampling methods and environmental conditions.

5. Environmental Conditions - Media fills should be conducted under environmental conditions that simulate actual and preferably "worst case" conditions established as quality limits for production. To the extent such stressful conditions are permissible within standard operating procedures, it is vital that they exist during some media fills used to assess the process covered by those procedures. An inaccurate assessment (making the process appear "cleaner" than it may in fact be) may result from conducting a media fill under extraordinary air particulate and microbial quality, and under production controls and precautions taken expressly in preparation for the media

fill. Rather, the system should be challenged at the established limits for such things as number and activity of personnel, temperature, and humidity.

6. Test results - Test results should show, with a high degree of confidence, that the probability of a product unit becoming contaminated during aseptic processing is very low. In general, tests results showing a probability of contamination of no more than one in one thousand are acceptable. FDA's acceptance of this level of probability in test results does not mean that an aseptically processed lot of drug product purporting to be sterile may contain one non-sterile unit per thousand count. A manufacturer is fully liable for the shipment of any non-sterile unit. FDA merely recognizes that there are scientific and technical limits on how precisely and accurately validation can characterize a system of controls intended to exclude contamination.

Sterilization Operations

Filtration

Filtration is a common method of sterilizing drug product solutions which are then placed in sterilized containers under aseptic conditions. A sterilizing filter is one which, when challenged with

the microorganism Pseudomonas diminuta (P. diminuta), at a minimum concentration of 10^7 organisms per cm^2 of filter surface, will produce a sterile effluent. Such filters usually have a rated porosity of 0.22 micron or smaller. In some instances where products having high viscosity or colloidal properties which inhibit filtration through 0.22 micron porosity, it may be possible to exclude microorganisms by using 0.45 micron filters in series. However, it is preferable to use other sterilization methods where such alternatives provide a greater level of sterility assurance than filtration without adversely affecting other product attributes.

Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate "worst case" production conditions, particularly regarding the size of microorganisms in the material to be filtered. The microorganisms should be small enough to both challenge the filter's nominal porosity and simulate the smallest microorganism that may occur in production. The microorganism P. diminuta is acceptable in this regard because it is one of the smallest bacteria (0.3 micron mean diameter) and, when properly grown, harvested and used, can pass through oversized pores of a 0.22 micron rated filter. The number of microorganisms in the challenge is important because a filter may contain a number of pores larger than the nominal rating which may allow passage of microorganisms (Ref. 8). The probability of such passage increases as the number of organisms (bioburden) in the

material to be filtered increases (Ref. 9). An acceptable challenge concentration of P. diminuta is at least 10^7 organisms per cm^2 of filter surface. It is important to assure that actual influent bioburden does not contain microorganisms of a size and/or concentration that would reduce the targeted high level of filtrate sterility assurance.

Addition of P. diminuta to products having inherently bactericidal activity or to oil-based formulations would not present a meaningful filter challenge. In such cases, the challenge fluid should simulate the product as closely as practical in terms of viscosity and other physical characteristics that are not antagonistic toward the microbial challenge.

Challenge conditions for filter validation should simulate other aspects of production as well as influent bioburden. For example, pH and viscosity of the material to be filtered, flow rates, pressures, temperature, compatibility of the material with the filter itself, and the effects of hydraulic shock are factors of production which can affect filter performance and which should be simulated during validation of filtration processes (Ref. 8). Many of these factors are significant relative to their effect on the filter's method of microbial capture, i.e., sieve retention and/or adsorption.

Filter validation experiments, including microbial challenges, need not be conducted in the actual manufacturing areas. However, it is vital that laboratory experiments simulate actual production conditions, as discussed above.

The agency recognizes that some of the more complex filter validation tests may be beyond the capabilities of some filter users. In such cases, it is acceptable to have the tests conducted by outside laboratories or by filter manufacturers. FDA considers it the responsibility of the filter user to have the test data available. The data should be applicable to the user's products and conditions of use because filter performance may differ significantly for various conditions and products.

Where families of drug products share similar attributes and processing conditions that affect filter efficiency, it is not necessary to perform validation studies on each individual product within a family. Rather, it is acceptable to extrapolate to related products, validation findings from a product having attributes and processing conditions which are the most challenging of the group. The justification for such extrapolation should be documented.

After a filtration process is properly validated for a given product, process, and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. One way of achieving this is to

correlate filter performance data with filter integrity testing data. Normally, integrity testing of the filter is performed after the filter unit is assembled and sterilized prior to use. More importantly, however, such testing should be conducted after the filter is used in order to detect any filter leaks or perforations that may have occurred during the filtration. "Forward flow", "bubble point" (Ref. 10) and "pressure hold" tests are acceptable integrity tests .

Equipment

Equipment surfaces which contact sterilized drug product or sterilized container/closure surfaces should, of course, be sterile. It is just as important in aseptic processing to properly validate sterilization processes applied to these equipment surfaces as it is to validate such processes for the drug product and container/closurers.

Where equipment, such as filling equipment, connecting lines, and filter holders, is steam sterilized in autoclaves, it is important that established loading patterns in the autoclave are considered because different configurations may affect patterns of heat distribution and the ability to achieve sterilization. (One way of assuring replication of the validated conditions is to follow established loading configuration diagrams which are part of the processing record.)

Where equipment, such as large tanks and immobile piping, is sterilized in place by the passage of pressurized steam it is important that validation consider temperature and pressure at various locations in order to identify potential "cold spots" where there may be insufficient heat to attain sterility. For example, some in-line filters in piping systems cause a significant pressure differential across the filter, resulting in a significant temperature drop on the downstream side. One method of determining if such a drop in temperature will adversely affect the sterilization procedure involves the placement of suitable biological indicators at appropriate downstream locations; validation should also include measurements of temperature and pressure at various points.

VIII. LABORATORY CONTROLS

Requirements

Section 211.160 (General Requirements) requires, in part, the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials and drug products conform to appropriate quality standards.

Guidance

In aseptic processing, one of the most important laboratory controls is the establishment of an environmental monitoring program. Samples should be collected from areas in which components and product are exposed to the environment, such as mixing rooms and component preparation areas. It is especially important to monitor the microbiological quality of the aseptic processing area to determine whether or not aseptic conditions are maintained during filling/closing activities. The monitoring program should include routine sampling and testing of the environment including room air, floors, walls, and equipment surfaces. Such a program can establish the effectiveness of cleaning and sanitizing of equipment and product contact surfaces and can determine if potential contaminants are held to a reasonable level. It is important to assure that disinfectants retain their efficacy against the normal microbial flora.

The written monitoring program should have a scientifically sound sampling schedule, including sampling locations and frequency. In addition, maximum microbial limits should be established along with a definitive course of action to be taken in the event samples are found to exceed the limits.

There are several acceptable methods of monitoring the microbiological quality of the environment. One method is the use of passive air samplers such as settling plates--Petri-type dishes

containing nutrient agar which is exposed to the environment. These devices have limited value in quantitative monitoring because they do not detect microorganisms that do not settle onto the agar surface. However, settling plates can be valuable as qualitative indicators if they are positioned in critical areas posing the greatest risk of product contamination, and if they are able to effectively capture microorganisms during exposure. Data from such samplers can be useful when correlated with data from other types of air samples.

Another acceptable method of assessing the microbial quality of air involves the use of more "active" devices such as slit to agar samplers, centrifugal samplers, and those using liquid impingement and membrane filtration. Each device has certain disadvantages, although all allow a quantitative test by detecting the number of organisms per volume of air sampled. The use of such devices in aseptic areas is considered essential and they should be used at least daily during production. Where there are multiple production shifts, daily monitoring should cover each shift.

Environmental monitoring should include testing of critical surfaces for microbiological quality. Commonly used for such tests are touch plates, swabs, and contact plates. Other surfaces in controlled areas should be tested periodically to indicate the adequacy of cleaning and sanitizing procedures as well as to detect contamination caused by personnel.

The environmental monitoring program should include the routine identification of the recovered microorganisms. Identification should be sufficient to differentiate between the "normal flora" and incidental contaminants in order to permit reasonable assessments of the microbiological quality of the environment. Although every isolate need not be identified as to genus and species, it is nonetheless important that the characterization be specific enough to establish a valid data base and to demonstrate that cleaning and sanitizing continue to be effective. It is also important to characterize the isolates sufficiently to establish a potential relationship to organisms which may be found during sterile media fills and product sterility testing. Such a correlation can be highly valuable in investigating sterility failures.

Microbiological culture media used in environmental monitoring should be capable of detecting molds and yeast as well as bacteria, and should be incubated at appropriate conditions of time and temperature.

The environment should be monitored for particulate quality of the air in addition to microbiological quality. Critical areas should be monitored for particulates on at least a daily basis while the areas are in active use. Particle detection devices should sample the air as it approaches the working area.

Periodic monitoring should be carried out to detect any significant changes in particle count from the normal level. Excessively higher numbers of particulates obtained from a given location would indicate something abnormal which should be investigated and promptly corrected.

IX. STERILITY TESTING

Requirements

Section 211.167 (Special Testing Requirements) requires, in part, that for each batch of drug product purporting to be sterile there is appropriate laboratory testing to determine sterility.

Guidance

Certain aspects of sterility testing are of particular importance. These include control of the testing environment, understanding the test limitations, interpretation of positive results, and retesting.

The testing laboratory environment should employ facilities and controls comparable to those used for filling/closing operations. Poor or deficient sterility test facilities or controls can result in a high rate of sterility test failures. If production facilities and controls are significantly better than those for sterility testing,

there may be the danger of attributing the cause of a positive sterility test result to the faulty laboratory even when the product tested may have, in fact, been non-sterile. Therefore, some deficiency in processing may go undetected.

In general, sterility tests are limited in their ability to detect low levels of contamination. For example, the sampling requirement of the USP sterility test procedure described in USP XXI/NFXVI is such as, "to only enable the detection of contamination in a lot in which 10% of the units are contaminated about nine times out of ten in making the test" (Ref. 5). This limited sensitivity makes it necessary to ensure that for batch release purposes an appropriate number of units are tested and that they uniformly represent the batch. Furthermore, considering the limited sensitivity of the test, any positive results (growth observed in test units) should be thoroughly evaluated.

The evaluation of a positive sterility test result should include an investigation to determine insofar as possible whether the growth observed in the test arose from product contamination or from laboratory error. Although it is recognized that such a determination cannot be reached with absolute certainty, it is usually possible to acquire persuasive evidence one way or the other. When persuasive evidence showing laboratory error is absent, or when available evidence is inconclusive, firms should err on the side of safety and batches should be rejected as not conforming to sterility requirements.

The investigation should consider all relevant factors concerning the manufacture of the product and testing of the samples. In this regard it is inappropriate to attribute an initial positive result to laboratory error merely because no growth is detected in repeated tests. Rather, persuasive evidence of the origin of the contamination should be based upon at least the following:

1. The identification (by at least genus) of the organism in the sterility test. If the organism is seldom found in the laboratory environment then product contamination would be more probable. If the organism is common to both laboratory and production environments product contamination should not automatically be ruled out.

   Sensitivity of the organism to sterilization methods as well as sensitivity to any preservatives in the product can also help determine the source of contamination. Intrinsic product contamination should be considered more likely the case when the organism is resistant to the sterilization methods or preservative. On the other hand, absent contrary indications as to the source of contamination, laboratory error may be a more likely avenue of contamination where the organism is not resistant.

2. The laboratory's record of tests over time. Careful review of trends in laboratory findings can help to exonerate or implicate the laboratory as the source of contamination. In FDA's experience, it should be normal for a laboratory to find an initial positive sterility failure in less than 0.5% of all sterility tests. Failure rates, for the first tests, of higher than 0.5% can indicate laboratory or production problems and thus should be investigated. If the rate of initial positive results shows an increasing trend then an investigation should be initiated. Upward trends in conjunction with other elements in the overall investigation can corroborate a suspected causative agent. Similarly, upward trends in the rate of false positives should be investigated; such increases can be harbingers of production or laboratory problems. In order to more accurately monitor potential contamination sources, it is important that trends be separated according to distinctions that can influence those sources. For example, it is useful to keep separate trends by product, container type, filling line, and degree of sample manipulation. Where sample manipulation is the same for a terminally sterilized product and an aseptically processed product, higher rates of initial sterililty failures for the latter should be taken as indicative of production problems.

Microbial monitoring of the laboratory environment over time can reveal trends that are informative. Upward trends in the microbial load in the laboratory should be investigated as to cause and corrected. However, such trends are more indicative of laboratory error as a source of sterililty test failures.

3. Monitoring of production area environments. Of particular importance is trend analysis of viable organisms in critical areas. Upward trends tend to implicate the product as the source of a sterility failure. It is important that consideration of environmental microbial loads not be limited to results of monitoring the production environment for the lot, day, or shift associated with the suspect lot. Results showing low levels of viable organisms may be misleading especially when bracketed by findings of higher loads that may be part of a trend. Therefore, it is important to consider a broad range of environmental monitoring data.

4. Product presterilization bioburden. Trends in product bioburden may show problems which can be associated with the sterility test failure. Upward bioburden trends occurring at the same time as the test failure would be more persuasive of product contamination.

5. Production record review. Complete batch and production control records should be reviewed to detect any signs of failures or anomalies which could have a bearing on product sterility. For example, records of air quality monitoring for filling lines may show a time at which there was an unusually high particulate count.

6. Sterility retest. An acceptable retest includes the collection of at least twice the number of test units used for the original test, taken to be representative of all parts of the batch. A finding of no growth in the retest should be accorded less weight than other parts of the investigation. However, if growth is observed in the retest, the batch should be rejected unless a new exhaustive investigation conclusively shows that the contamination arose in the sampling or testing procedure. In such instances, a second retest may be warranted. The sample size of the second retest should be twice as large as in the first retest. If growth is observed in the second retest, the batch should be rejected and no additional retesting for batch release purposes is acceptable.

Twice the number of test units is not required on the first sterility retest of biological products. This is necessary if a second retest is warranted (21 CFR, Section 610.12). A second retest is not permitted for bulk biological material.

X. REFERENCES

1. Federal Standard 209B, Clean Room and Work Station Requirements, Controlled Environment, April 24, 1973.

2. Technical Order 00-25-203, Contamination Control of Aerospace Facilities, U.S. Air Force, December 1, 1972.

3. NASA Standard for Clean Room and Work Stations for Microbially Controlled Environment, Publication NHB 5340.2 (August 1967)

4. Technical Monograph No. 2, "Validation of Aseptic Filling for Solution Drug Products", Parenteral Drug Association, Inc., 1980.

5. Pharmacopeial Forum, July-August 1980, p. 354, "Commentary on the Sterility Tests and Sterilization Chapters of the U.S. Pharmacopeia," Aubrey S. Outschoorn, Sr. Scientist, U.S.P. Drug Standards Division

6. Report No.: 78-4.1, June 1978, "HIMA MEDICAL DEVICE STERILIZATION MONOGRAPHS: Validation of Sterilization Systems," Health Industry Manufacturers Association.

7. AAMI OPEO-P 8/80, "GUIDELINE FOR INDUSTRIAL ETHYLENE OXIDE STERILIZATION OF MEDICAL DEVICES: Process Design, Validation, Control of Routine Sterilization (Proposed)," Association For the Advancement of Medical Instrumentation, 19 August 1980 Revision.

8. "Validation of Bacterial - Retention Capabilities of Membrane Filters," Timothy J. Leahy and Mary J. Sullivan, Pharmaceutical Technology, Nov. 1978.

9. "Particulate Retention by Bacteria Retentive Membrane Filters," David B. Pall, Erwin A. Kirnbauer and Barrington T. Allen, Pall Corporation Colloids and Surfaces, 1 (1980) 235-256, Elsevier Scientific Publishing Company, Amsterdam.

10. "An Assessment of Test Criteria for Evaluating the Performance and Integrity of Sterilizing Filters," Adrian R. Reti, Millipore Corp., Bull. Parenteral Drug Association, July - August, 1977 Vol. 31, No. 4.

3464C