Pharmaceutical Microbiology Manual 2014

The purpose of this Pharmaceutical Microbiology Manual (PMM) is to collectively clarify, standardize, and communicate useful analytical procedures that are not specifically addressed in the microbiology methods chapters in the United States Pharmacopeia. In addition, some sections of this manual can serve as a technical reference when conducting microbiological inspections of drug, biotechnology and medical device manufacturers. The contents of this PMM were collaboration between ORA and CDER in order to maximize the efficiency of our analytical results to support CDER's goal to assure the safety and reliability of commercially distributed medical products.

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i. Introduction

The Pharmaceutical Microbiology Manual (PMM) evolved from the Sterility Analytical Manual and is a supplement to the United States Pharmacopeia (USP) for pharmaceutical microbiology testing, including antimicrobial effectiveness testing, microbial examination of non-sterile products, sterility testing, bacterial endotoxin testing, particulate matter, device bioburden and environmental monitoring testing. The goal of this manual is to provide an ORA/CDER harmonized framework on the knowledge, methods and tools needed, and to apply the appropriate scientific standards required to assess the safety and efficacy of medical products within FDA testing laboratories. The PMM has expanded to include some rapid screening techniques along with a new section that covers inspectional guidance for microbiologists that conduct team inspections.

This manual was developed by members of the Pharmaceutical Microbiology Workgroup and includes individuals with specialized experience and training.

The instructions in this document are guidelines for FDA analysts. When available, analysts should use procedures and worksheets that are standardized and harmonized across all ORA field labs, along with the PMM, when performing analyses related to product testing of pharmaceuticals and medical devices. When changes or deviations are necessary, documentation should be completed per the laboratory's Quality Management System. Generally, these changes should originate from situations such as new products, unusual products, or unique situations.

This manual was written to reduce compendia method ambiguity and increase standardization between FDA field laboratories. By providing clearer instructions to FDA ORA labs, greater transparency can be provided to both industry and the public. However, it should be emphasized that this manual is a supplement, and does not replace any information in USP or applicable FDA official guidance references. The PMM does not relieve any person or laboratory from the responsibility of ensuring that the methods being employed from the manual are fit for use, and that all testing is validated and/or verified by the user.

The PMM will continually be revised as newer products, platforms and technologies emerge or any significant scientific gaps are identified with product testing.

Reference to any commercial materials, equipment, or process in the PMM does not in any way constitute approval, endorsement, or recommendation by the U.S. Food and Drug Administration.

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Chapter 1: Antimicrobial Effectiveness Testing

Antimicrobial Effectiveness testing is described in USP <51>. Previously this chapter was known as "Preservative Effectiveness Testing". Detailed procedure for the performance of the test can be found in USP <51>.

A. Media

For the cultivation of the test organisms, select agar medium that is favorable to the rigorous growth of the respective stock culture. The recommended media are Soybean Casein Digest Agar/Broth and Sabouraud's Dextrose Agar/Broth. Add a suitable inactivator (neutralizer) for the specific antimicrobial properties in the product to the broth and/or agar media used for the test procedure if required.

B. Growth Promotion of the Media

Media used for testing needs to be tested for growth promotion by inoculating the medium with appropriate microorganisms. It is preferable that test microorganisms be chosen for growth promotion testing (Section D).

Solid media tested for growth promotion is to be set up using pour plate method in order to determine a microbial plate count (CFU) which must be ≥ 70% of the microorganism inoculum's calculated value.

C. Suitability of the Counting Method in the Presence of Product

For all product types, follow current USP methodology in chapter <51>, with the following additional instructions.

Prior to the Antimicrobial Effectiveness testing, determine if any antimicrobial properties exist by performing a Suitability testing utilizing microorganisms used for product testing (section D). Should the Suitability Test fail, the results of Suitability test are invalid and will need to be repeated with proper method modification to neutralize the inhibiting property.

If multiple samples of the same product from the same manufacturer (same amount and form) are collected, one sample may be used for method suitability for all the samples collected.

D. Test Organisms

All cultures must be no more than 5 passages removed from the original stock culture.

Candida albicans (ATCC No. 10231)
Aspergillus brasiliensis (ATCC No. 16404) (formerly *Aspergillus niger*)
Escherichia coli (ATCC No. 8739)
Pseudomonas aeruginosa (ATCC No. 9027)
Staphylococcus aureus (ATCC No. 6538)

E. Preparation of Inoculum

Preparatory to the test, inoculate the surface of the appropriate agar medium from a recently grown stock culture of each of the above test microorganisms. Use Soybean-Casein Digest medium for *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538 and incubate at 32.5 ± 2.5° C for 3 – 5 days. Use Sabouraud Dextrose medium for *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 and incubate at 22.5 ± 2.5° C for 3 – 5 days for *Candida albicans* and 3 - 7 days for *Aspergillus brasiliensis*.

Harvest the cultures by washing the growth with sterile saline to obtain a microbial count of about $1x10^8$ CFU/mL (see *Microbial Enumeration Tests* <61> and *Tests for Specified Microorganisms* <62>). For the *A. brasiliensis* ATCC 16404 culture, use sterile saline containing 0.05% polysorbate 80.

Alternatively, cultures may be grown in a liquid medium, i.e. Soybean Casein Digest Broth or Sabouraud's Dextrose Broth, (except for the *A. brasiliensis* ATCC 16404 culture) and harvested by centrifugation, washing and suspending in sterile saline to obtain a count of about $1 X 10^8$ colony forming units (CFU) per mL.

The estimate of inoculum concentration may be obtained by turbidimetric procedures for the challenge microorganisms and later confirmed by plate count.

Refrigerate the suspension if not used within 2 hours at 2-8° C.

Determine the number of CFU/mL in each suspension using the appropriate media and recovery incubation times to confirm the CFU/mL estimate.

Use bacterial and yeast suspensions within 24 hr. of harvest. The mold preparation may be stored under refrigeration (2-8° C) for up to 7 days.

Note: Alternative commercially available standardized cultures may be used in lieu of in-house prepared cultures.

F. Procedure

The procedure requires that the test be conducted with a suitable volume of product. It is advisable to begin with at least 20 mL of product. Use the original product containers whenever possible or five sterile, capped bacteriological containers of suitable size into which a suitable volume of product has been transferred. If the diluted product exhibits antimicrobial properties, specific neutralizers may need to be incorporated into the diluents or the recovery media. For purposes of testing, products have been divided into four categories:

> Category 1 – Injections, other parenteral including emulsions, otic products, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles.

Category 2 – Topically used products made with aqueous bases or vehicles, non-sterile nasal products, and emulsions, including those applied to mucous membranes.

Category 3 – Oral products other than antacids, made with aqueous bases or vehicles.

Category 4 – Antacids made with aqueous bases or vehicles.

Inoculate each container with one of the prepared and standardized inoculums and mix. The volume of the suspension inoculums used is 0.5% to 1.0% of the volume of the product. The concentration of the test organisms added to the product for Categories 1, 2 and 3 is such that concentration of the test preparation immediately after inoculation is between $1x10^5$ and $1x10^6$ colony forming organisms (CFU) per mL of product. For category 4 products (antacids) the final concentration of the test organisms is between $1x10^3$ and $1x10^4$ CFU/mL of product.

Immediately determine the concentration of viable organisms in each inoculum suspension and calculate the initial concentration of CFU/mL by the plate count method (see *Microbial Enumeration Tests* <61> and *Tests for Specified Microorganisms* <62>).

Incubate the inoculated containers between 22.5 ±2.5°C in a controlled environment (incubator) and sample the container at specified intervals. The container sampling intervals include: Category 1 products are sampled at 7, 14, and 28 days and Category 2 – 4 products are sampled at 14 and 28 days. Refer to table within USP <51>. Record any changes in appearance of the product at these intervals. Determine the number of viable microorganisms per mL present at each of these sampling intervals by the plate count method utilizing media with suitable inactivator (neutralizer). Calculate the change in log10 values of the concentration per mL based on the calculated concentration in CFU/mL present at the start of the test for each microorganism at the applicable test intervals, and express the changes in terms of log reductions.

NOTE: The USP does not require a specific volume of product to be added to each of the five sterile tubes. It is recommended that 20 mL/tube be used to standardize testing for all FDA laboratories.

NOTE: All plate counts should be performed in duplicate (2 plates per dilution), and in a dilution series to detect growth inhibited by the preservative system at the lower dilutions. Carrying the test to the 10-3 dilution would be sufficient in most cases to overcome preservative inhibition.

G. Interpretation

The criteria for microbial effectiveness are met if the specified criteria are met, see table below. No increase is defined as not more than 0.5 log10 unit higher than the previous value measured.

CRITERIA FOR TESTED MICROORGANISMS

Category 1 Products	
Bacteria:	Not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14 day count at 28 days.
Yeast and Molds:	No increase from the initial calculated count at 7, 14, and 28 days.
Category 2 Products	
Bacteria:	Not less than a 2.0 log reduction from the initial count at 14 days, and no increase from the 14 day count at 28 days.
Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.
Category 3 Products	
Bacteria:	Not less than a 1.0 log reduction from the initial count at 14 days, and no increase from the 14 day count at 28 days.
Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.
Category 4 Products	
Bacteria, Yeast and Molds:	No increase from the initial calculated count at 14 and 28 days.

Chapter 2: Microbial Examination of Non-Sterile Products

This section contains supplemental information for the quantitative enumeration of viable microorganisms and the determination of the absence of specified microorganisms in finished pharmaceutical products and raw materials, commonly referred to as Microbial Limits Testing (MLT). The detailed procedures for these tests are not addressed in this PMM chapter since they are found in USP <61> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS and <62> MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: TESTS FOR SPECIFIED MICROORGANISMS.

Methods for enumeration of microorganisms from pharmaceuticals (as described in USP <61>) include membrane filtration, conventional plate count (including pour-plate method, surface-spread method), and the Most-Probable-Number (MPN). USP Chapter <62> describes specific enrichment procedures depending on the target specified microorganism that must be absent, as required by a product monograph. Products which are insoluble or immiscible in water must be appropriately treated to obtain a suspension suitable for the test procedures.

It is important to note that even though the USP delineates methods for the recovery and identification of specified microorganisms based on monograph requirements, it is still the goal of the regulatory microbiologist to screen for any other microorganisms that may also be present in the product(s) and report these microorganisms on worksheets. In many cases, these may be opportunistic or emerging pathogens not targeted for recovery by USP <62>. Alternative methods, or the use of additional general enrichment agar plates or broth without selective properties, may better suit the screening of test samples. The application of these additional agars or methods may need to be considered based on the target population of the drug or product under analysis and may require a dialogue with the laboratory supervisor for additional instructions.

A. Product Storage and Handling

1. Samples are to be held under the same storage conditions required by the package label or insert.
2. Prior to product testing, the exterior of the unit container should be disinfected before transfer to the work station or HEPA filtered laminar flow hood. If the product container is not hermetically sealed do not soak the product container in a disinfection solution which may allow the ingress of bactericidal solution into the product.
3. The work area for opening the unit container should be either a HEPA filtered laminar flow hood or an alternate controlled environment to safeguard the exposure of open media and product to either environmental or personnel contamination.
4. If the sample is an aqueous based product, the unit(s) should be shaken prior to transfer to work area to maximize microbial dispersement.
5. All subsequent manipulation of test tubes with product or sub-culturing can be conducted on the laboratory work bench or within a Biological Safety Cabinet (BSC) if filamentous fungi are suspected.

B. Gowning Requirements

1. When conducting the testing, the analyst should wear a clean lab coat, sterile sleeves and sterile gloves. Gloves should be frequently disinfected especially between opening and handling sample (product) units.
2. Depending on the type of Laminar Flow Hood or equipment barriers in a particular laboratory, it might be beneficial to also wear a surgical mask and hair net.

C. Growth Promotion and Inhibitory Properties of the Media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients following USP <61> and <62>. Each chapter provides guidance on test strains to be used for each type of media, refer to Table 1 of USP <61> and <62>. Ensure that seed-lot cultures used are not more than five passages removed from the original master seed-lot. Test strains suspensions should be used within 2 hours, or within 24 hours if refrigerated between 2ºC and 8ºC. Spore suspensions (A. *brasiliensis*, B. *subtilis, and C. sporogenes)* refrigerated between 2ºC and 8ºC may be kept for a validated period of time. Additionally, all bacterial and spore suspensions should be prepared to yield ≤100 cfu. Growth promotion (and suitability test) plates and tubes should not be incubated in the same incubators used for product testing. If this cannot be avoided because of limited space, it is preferable to store the "spiked samples" in the lower half of the incubator below the sample inoculated plates and tubes.

D. Suitability of the Test Method

Suitability demonstrates that the products tested do not exhibit inhibitory effects on the growth of microorganisms under the conditions of the tests. Although the intent is to perform the suitability test before performing the analysis of the product, it is acceptable to run the product test and the suitability test concurrently. However, it should be noted that if the suitability test is run concurrently with the product test and the suitability test should fail, the results of the product test are invalid and the suitability test as well as the product test will need to be repeated with proper method modification to neutralize the inhibiting property.

Neutralizing agents may be used to neutralize the activity of antimicrobial agents in products, see USP <61> Table 2 for a list of potential neutralizing agents/methods. The appropriate neutralizing agent should be added preferably before sterilization of the media. Include a blank control with neutralizer and without product to demonstrate efficacy and absence of toxicity for microorganisms.

USP <61> and <62> describe the suitability tests necessary for each analysis. The correct inoculum of not more than 100 CFU is required as are specific incubation temperatures and durations. Ensure that seed-lot cultures used are not more than five passages removed from the original master seed-lot. Test strains suspensions should be used within 2 hours, or within 24 hours if refrigerated between 2ºC and 8ºC. Spore suspensions (A. *brasiliensis*, B. *subtilis, C. sporogenes)* refrigerated between 2ºC and 8ºC may be kept for a validated period of time. USP <61> and <62> require a control which is without test material to be included in the suitability test. The following viable ATCC derived cultures may be used. Please be aware that under the revised harmonized Microbial Limits chapters <61> and <62> users are allowed alternative sources of the below listed strains. The organisms below are recommended for FDA use in order to have a consistent and standard worksheet format:

Staphylococcus aureus (ATCC 6538)
Escherichia coli (ATCC 8739)
Pseudomonas aeruginosa (ATCC 9027)
Bacillus subtilis (ATCC 6633)
Candida albicans (ATCC 10231)
Aspergillus brasiliensis (ATCC 16404)
Salmonella enterica (ATCC 14028)
Clostridium sporogenes (ATCC 11437)

USP <61> and <62> each contain the acceptance criteria for their respective suitability test. For USP <61> the Results and Interpretation section requires the inoculated product to have a mean count of any of the test organisms not differing by a factor greater than 2 from the control which was without test material. USP <62> requires the specified microorganisms to be detected with the indicated reactions.

E. Test Procedure

Prepare the sample in a manner to achieve a uniform solution or suspension. This is critical because microbial contamination is not evenly dispersed throughout a lot or sample of product. Use conventional mechanical and shaking methods to the extent that original numbers and types of microorganisms are not altered in the product.

Use the following general procedures to prepare and handle samples.

1. Analyze samples as soon as possible after receipt. Inspect each unit visually for integrity of primary container and note any irregularities. Do not use the product container if it has been compromised or damaged without supervisor approval. Testing of a compromised or damaged container should be evaluated on a case by case basis. Discuss with supervisor if compromised unit containers need to be tested for forensic purposes (i.e. product tampering).

2. Identify units to be tested with Analyst's initials, date, subsample number, and sample number.

3. Cleanse outer surfaces of sample containers with sterile wipes using a validated effective antimicrobial agent. Place on a disinfected tray or surface in a properly disinfected laminar flow hood or biosafety cabinet. Allow containers to dry.

4. Aseptically open containers and perform weighing procedures in a laminar flow hood or biological safety cabinet if possible.

5. Appropriate environmental controls such as air exposure plates should be used in accordance with local quality procedures.

6. Appropriate negative controls should be run concurrently with the sample.

F. Interpretation of the Results

Regarding USP <61> the acceptance criterion for microbiological quality as it pertains to quantitative analyses has an allowable variability of the final colony forming units

(CFUs). There is a two-fold tolerance in the final results. For example, if the monograph requires a 100 cfu/ml limit, the acceptable upper limit for these results would be 200 cfu/ml. Additional information is included in the “Interpretation of the Results” section of USP <61> that should be read and understood when reviewing quantitative test results.

Chapter 3: Sterility Testing

A. Method Suitability Test

For all product types, follow current USP methodology in <71>, with the following additional instructions.

Prior to or concurrently with the sterility test, determine if any bacteriostatic or fungistatic residue has been retained on the filter membrane. The Method Suitability Test can be run concurrently with the sterility test per USP. However, it should be noted that if the Method Suitability Test is run concurrently with the product test and the Method Suitability Test should fail, the results of the product test are invalid and the Method Suitability Test as well as the product test will need to be repeated with proper method modification to neutralize the inhibiting property.

Units selected for suitability testing should be subjected to the same disinfection procedure utilized in the sample analysis.

In all cases, even if the product does not include a preservative, the product itself may have growth inhibiting properties. All products should undergo a prescribed Method Suitability test.

If multiple samples of the same product from the same manufacturer (same dosage and form) are collected, one sample may be used for method suitability for all the samples collected.

1. When to run Method Suitability:

a. Run the test prior to conducting the sterility test in accordance with USP requirements under the following conditions:

   i. If insufficient information about the product exists to judge its probable growth inhibiting activity.

   ii. In all cases, when there is sufficient analytical time available, i.e., survey type samples.

b. Run the test concurrently with product sterility tests when time is critical and problems associated with Part I above have been resolved. However, it should be noted that if the Method Suitability Test is run concurrently with the product test and the Method Suitability Test should fail, the results of the product test are invalid and the Method Suitability Test as well as the product test will need to be repeated with proper method modification to neutralize the inhibiting property.

c. If an insufficient amount of product is collected and the analysis is critical the suitability test can be conducted at the end of the 14 day incubation period. Be sure to use best judgment and maximum neutralization approach when initially conducting the Sterility test. If the suitability results indicate inhibition then the results, if negative, are invalid. However, if the product test results indication microbial presence and the suitability test show inhibition the results are still valid.

2. Method Suitability Test Procedures

Method Suitability, and any other positive control tests which require the use of viable microorganisms, should be performed outside the clean room or isolator, in a biosafety cabinet or equivalent,

a. Membrane filtration

i. Pass product fluid through filter membrane. Rinse the membrane with three 100 ml portions (or more if necessary or required) of specified rinse fluid. Do not exceed a washing cycle of five times 100mL per filter. This step hopefully will neutralize and remove any antimicrobial residue on the filter membrane.

ii. Add specified test organisms in specified numbers (less than 100 cfu) into the last 100 ml rinse fluid used.

iii. Filter the fluid and divide the filter membrane between the specified media. If conducting the sterility test using a closed canister system, rinse each canister with the inoculated rinse fluid.

iv. If the available number of test vessels is insufficient for a complete challenge test for each individual microorganism then the test organisms may be composited as necessary. However, confirmation of growth for the composited microorganisms will need to be confirmed by isolation, Gram stain, and genus/species identification after the completion of incubation.

v. Confirm composited microorganisms by Gram stain, microscopic examination, and biochemical identification after the completion of incubation.

vi. See step c. below for additional considerations.

b. Direct inoculation:

For direct inoculation, add the test organisms to separate containers of product/culture media if sufficient product is available. See step c. below for additional considerations.

c. The following test procedures apply to Direct Inoculation and Membrane Filtration:

i. Inoculate the same microorganism using the same medium without the product as a positive control.

ii. For bacteria and fungi, incubate tubes/bottles according to USP requirements. Ensure that seed-lot cultures used are not more than five passages removed from the original master seed-lot. Test strains suspensions should be used within 2 hours, or within 24 hours if refrigerated between 2ºC and 8ºC. Spore suspensions (A. brasiliensis, B.

subtilis, C. sporogenes) refrigerated between 2ºC and 8ºC may be kept for a validated period of time. Additionally, all bacterial and spore suspensions should be prepared to yield ≤100cfu.

iii. If growth comparable to that of the positive control vessel without product is obtained, then you may proceed with the sterility test. If visible growth is not obtained, the antimicrobial activity of the product has not been eliminated under the conditions of the test. Modify the test conditions and repeat the Method Suitability test.

iv. If there is not enough product material to perform method suitability test using all the specified number of organisms for the test, select the organisms most appropriate for the product based on available information. If insufficient information is available for making a judgment, perform the test using ATCC-derived organisms (or acceptable alternative sources, if necessary) in the following order:

a. *Clostridium sporogenes*, ATCC 11437
b. Candida albicans, ATCC 10231
c. Bacillus subtilis subsp. spizizenii, ATCC 6633 (Formerly *Bacillus subtilis*)
d. *Pseudomonas aeruginosa,* ATCC 9027
e. Aspergillus brasiliensis, ATCC 16404 (Formerly *Aspergillus niger*)
f. *Staphylococcus aureus*, ATCC 6538

Note: refer to ATCC documentation for the most current organism names.

d. If product is found to exhibit growth inhibiting activity when determined concurrently with product testing, the sterility test must be repeated using an additive (or increase media volume) to modify the conditions in order to eliminate the antimicrobial activity. One may need to search the literature or have the CSO contact the manufacturer and request a copy of their sterility test methodology.

e. Cultures used for the method suitability test can be purchased commercially, pre-counted and ready to use, or can be prepared and maintained locally.

B. Sample Analysis

1. Sample Containers

a. Open the sample package on a laboratory bench disinfected with a sporicidal / viricidal antimicrobial agent such as 2% glutaraldehyde or equivalent solution Refer to the AOAC and available literature for choosing suitable antimicrobial agents for use in your facility.

b. Count the number of units received. Compare this number with the number of units collected.

c. Inside the clean room preparation area located outside the certified class 100 areas (if available), remove all outer containers from sample units that will be

tested without compromising the sterile integrity of the product. Remove sample units and place them on a tray or cart disinfected with an effective antimicrobial agent.

Note: One or more units can be sacrificed for sample exploration if the number of the units received is sufficient.

d. Examine all units visually for container closure integrity, for the presence of any foreign matter and other defects present in the product. Note findings on analyst's worksheet.

e. If foreign matter is observed within the primary container, discuss with supervisor the employment of ORS procedure Document ORA-LAB.015 entitled “Screening Protocol for Direct Staining on Products with Appearance of Visible Contamination” (see QMiS for Procedure).

2. Sample Identification

If sample units are not identified by the collector, the analyst should identify unit with sample #, initials, date, and sub sample # as appropriate for sample traceability. Otherwise, date and initial each unit.

3. Unit Container Disinfection

Cleanse the exterior of all product primary containers using antimicrobial agents meeting requirements described under step 1. These suggested disinfection procedures can be performed on commonly encountered units as follows:

a. Ampoules can be wiped with lint free sterile towel/wipes saturated with disinfectant. Ampoules may be soaked in disinfectant for 1 hour.

b. Vials should only be wiped with disinfectant. Vials should not be soaked due to the possibility of migration of disinfectant under the closure and into the product.

c. Laminated Tyvek package: Disinfect polyethylene/plastic laminate with sterile towel/wipes soaked in disinfectant. Tyvek portion lightly scrubbed with sterile particle free dry wipe and air cleanse in a HEPA filtered laminar flow hood for several hours before testing.

d. Paper Package: Disinfect with UV light if possible. Scrub with sterile particle free dry wipes and air cleanse as above.

1. Depending on the clean room design, immediately move the sample to the clean room on a disinfected designated stainless steel cart or place it inside the clean room pass thru for final preparation. If conducting the sterility test in an isolator place the sample on a designated stainless steel cart. Allow the sample to react with the disinfectant for 1 hour before further handling.

2. Number of units and/or amount of product tested:
Follow the current edition of the USP to determine the correct number of units to be tested and the amount of product to be analyzed from each unit. It is

preferable to test the entire contents of each unit if possible. Follow laboratory policy if it requires testing more units than the USP requires.

If the number of units collected is less than the USP requirements, discuss with the laboratory supervisor before proceeding. Samples collected in a for-cause situation may be analyzed with a number of units less than the USP requirements.

C. Preparation for the Analysis

1. Media Preparation:

Follow current USP when preparing media used for sample analysis. Commercially purchased media may also be used for the analysis. Both prepared and purchased media must meet the requirements of the USP growth promotion test of aerobes, anaerobes and fungi. The most common media used are:

a. Fluid Thioglycollate medium (FTM) This medium should be prepared in a suitable container to provide a surface to depth ratio so that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. If more than the upper third of the medium has acquired a pink color, the medium may be restored once by heating until the pink color disappears. Care should be taken to prevent the ingress of non-sterile air during cooling.

b. Soybean Casein medium (SCD medium)

c. Alternative Thioglycollate medium NOTE: This type of media must be incubated under anaerobic conditions.

d. Media for Penicillin and Cephalosporin containing drugs. Add sufficient quantity of sterile Beta-lactamase to the media to inactivate the effect of these antibiotics.

e. Diluting and rinsing fluids. These fluid rinses must be filtered before sterilization to avoid clogging of the filter membrane during testing.

2. Media storage

For laboratory prepared media, do not use medium for longer storage period than has been validated.

For commercially purchased media, follow the manufacturer's recommended storage requirements and expiration date.

3. Media qualification:

Perform the following tests on the prepared media before use:

a. Sterility: The media batch may be used if the sterilization cycle is validated and monitored with the use of a biological indicator, and the batch passes other quality control testing. Also, if possible incubate a portion of the media at the specified temperature for 14 days.

b. Growth promotion test; follow the current USP using recommended strains of organisms (Table 1, USP <71>). Do not use cultures that are more than five passages removed from the original master seed lot. Commercially prepared and certified pre-counted cultures of the recommended organisms can also be used. Test strains suspensions should be used within 2 hours, or within 24 hours if refrigerated between 2ºC and 8ºC. Spore suspensions (A. brasiliensis, B. subtilis, and C. sporogenes) refrigerated between 2ºC and 8ºC may be kept for a validated period of time. Additionally, all bacterial and spore suspensions should be prepared to yield ≤100cfu.

4. Equipment Preparation

Analytical equipment and tools used in sterility analysis and suitability should be cleaned and sterilized using a validated sterilization procedure. Commercially purchased equipment and tools should be labeled sterile and accompanied by a certificate of analysis for sterility.

D. Clean Room Activities

1. Gowning

Personnel are critical to the maintenance of asepsis in the controlled environment. Thorough training in aseptic techniques is required. Personnel must maintain high standards each time they deal with sterile product.

a. Personnel gowning qualification should be performed by any analyst that enters the aseptic clean room to ensure proper gowning techniques are followed. Personnel gowning qualification should consist of:

i. Training of gowning techniques by a qualified trainer.

ii. Observation of trainee by trainer while gowning.

iii. General growth media touch plates utilized to analyze if the trainee gowned correctly without contaminating the sterile outer gown, sterile gloves and sterile head cover.

Some consideration should be taken before entering the clean room (see below). Follow applicable specific procedures for the facility.

- **Proper gowning immediately prior to entry the clean room is required of all personnel without exception.**
- Remove **street** clothes when possible and wear clean clothes the day of the analysis. Non-linting clean room scrubs that cover as much skin as possible is the ideal inner-suit to wear before gowning up for an aseptic clean room.
- Remove jewelry and makeup.
- Scrub hand (and arm when possible) before gowning. It can also be helpful to take a shower the day before analysis to reduce skin shedding.
- Non--shedding sterile uniform components should be used all the time.
- Use aseptic gowning procedure to don sterile uniform components. When donning gown, follow specific gowning procedures that are

appropriate for each individual facility and best assure gowning asepsis.

- Care should be taken to choose gowning that does not expose any skin to the aseptic clean room environment.
- Sterile non powdered gloves should be used at all times. A new pair of non-powdered sterile gloves is to be put on as the last uniform component.
- 70% sterile alcohol can be used to sanitize the gloves if possible. Note that alcohol is not a sterilant.
- If possible post the gowning procedures in the gowning room or area to help individuals follow the correct order of gowning.
- Should an analyst find it necessary to leave the room, he/she should discard all gowning components and put on new ones upon re-entry.
- If an individual scheduled to enter the clean room for analysis feels sick or is sunburned, he/she should talk to his/her supervisor to postpone entry into the clean room. Analysts that have also undergone any surgeries or procedures (tattoos) that compromise the skin should also postpone entry into the clean room until they have fully healed.

2. Sample Preparation

Repeat disinfection procedure using filtered sterilized 70% alcohol immediately prior to placing product primary containers in a working certified laminar flow hood. Allow all disinfected containers to completely air dry (recommended for at least ½ hour) in the laminar flow hood prior to opening for analysis. Alternatively if conducting the testing in an isolator, place the disinfected items into the isolator and proceed with the local procedures for the proper decontamination of the interior of the isolator.

3. Room Cleaning After Analysis

- Remove inoculated tubes of media and all controls from the analytical area by putting them in the pass-thru or on a stainless steel cart used for transporting materials in and out of the clean room.
- After analysis, all sample containers, wrapping paper, used equipment and tools are to be removed from the clean room before the analyst exits.
- Sample containers used in the analysis should be replaced in original outer containers for storage as part of the reserve sample.
- An inventory sheet should be filled to account for the amount of material available for the next analysis, if required by local procedures.
- Disinfect working area before exiting the clean room.

4. Clean room disinfection and surface monitoring should be done on a routine bases. The frequency is to be determined by the local lab policy.

E. Method of Analysis

1. Filtration

Follow the current edition of the USP for the amount of sample to be tested.

2. Direct Inoculation

Follow the current edition of USP for the amount of sample and media to be used. For example: Use 200 ml of each medium when analyzing solid form products. If the

membrane filter method is unsuitable, certain liquids may be tested by direct inoculation method.

3. Devices

All devices except devices with only the pathways labeled as sterile are to be tested by direct inoculation method.

4. Incubation of Sterility Test Media

a. Incubate Fluid Thioglycollate (THIO) at 32.5 ± 2.5oC. Do not shake or swirl test media during incubation or during examination to minimize aeration of this broth.

b. Incubate Soybean-Casein Digest Broth (SCD) at 22.5 ± 2.5^{o}C. Gentle swirling, on occasion (3-5 days) is acceptable to increase aeration of media.

c. Incubation period for THIO and SCD:

i. Not less than 14 days except for products sterilized using ionizing radiation. If tubes are not read on day 14 due to holiday or weekend then record the results, even if positive, on the first available day to observe the tubes.

ii. Additional incubation time may be warranted if the analyst is made aware of sterilization processes other than heat or filtration (e.g. 30 days (at minimum) for products sterilized using ionizing radiation).

F. Analysis of Purified Cotton, Gauze, Sutures and Surgical Dressings

The USP method for analysis of purified cotton, gauze and surgical dressings does not require testing the entire unit. The USP method for the analysis of purified cotton, gauze and surgical dressings calls for portions no larger than 500 mg, from the innermost part of the unit, to be tested in each medium. The entire unit shall be tested for individually packaged single-use articles.

1. Gauze, Purified Cotton, Sutures and Surgical Dressings

a. Using media containers as large as quart jars analyze entire unit of product.

b. If unit is too large for the container, analyze as much of unit as can be placed in container and covered by the medium.

Due to the limited availability of media and glassware that occurs when a large number of samples are collected for analysis, it would be expedient to perform compositing of device samples. This would allow the samples to be completed in a timely manner.

2. Compositing of Medical Devices

a. Devices may be tested in composites (2-4 units/composite) as long as all units are completely immersed in the medium and all composite units are of the same lot number.

b. Perform the Method Suitability Test to ensure that the growth of microorganisms is not inhibited by the number of units used in a composite. Compositing cannot be performed if the sample does not pass the Method Suitability Test.

G. Control Systems

The objective of a control system is to ensure the sterility, within designated limits, of all items, media, rinsing fluids, and equipment used in a sterility test. The control systems which will accompany all sterility analyses are outlined below.

1. System Control

A "system control" is used to demonstrate maintenance of sample integrity during all analytical manipulations. Any piece of equipment that comes in contact with the product under analysis, along with any manipulations by the analysts, must be controlled. Thus, all equipment, fluids, and culture media for the "system control" must be handled in a manner which duplicates, as closely as possible, the manipulations of the actual sample being analyzed. All materials used as system controls must be sterilized by the analyzing laboratory. However, the method of sterilization need not be the same as for the product, but must render the material sterile.

The first choice for the system control is the actual product, if enough test units are available. When complex medical devices must be sacrificed in order to design a suitable sterility test, consider using them for a system control after cleaning, repacking and sterilizing.

When there are viable alternatives, a product unit should not be sacrificed for use as a system control if this will reduce the number of units available for sterility testing below USP requirements or FDA policy requirements, except as provided in the preceding paragraph. If using a product unit would reduce the subsamples examined below the number required by USP or FDA policy, the analyzing laboratory should prepare a control from other material than a unit of the sample product whenever possible.

a. Membrane Filtration: The filter funnel furthest from the vacuum source connection on each manifold used in the test is used for the system control. Alternatively if a closed canister system is used to conduct the sterility test a canister set from the same lot used during the analysis should be used for the system control.

i. Filterable Materials (liquids, soluble solids, etc.)

Use material similar to product under test. Control material must be of the same volume, and similarly packaged as test product. Filter-sterilized and autoclaved Peptone water (USP Fluid A) may be useful for this purpose in many cases.

ii. Devices with sterile Fluid Pathway

Use tubing or other containers similarly fitted with needles, valves, connectors, etc., as product under test. Use USP Fluid D to flush lumens.

b. Materials tested by direct inoculation (devices, insoluble solids, and other non-filterable materials)

Use materials similar in size, shape, and texture, and similarly packaged as product under test. Duplicate as nearly as possible pertinent, unusual features that may reflect on the credibility of the sterility test.

In designing "system controls" for sterility testing, care must be taken to duplicate the sample product for most aspects, as nearly as possible. Be novel and innovative to meet this requirement, and make the system control meaningful.

2. Equipment Controls

All items listed below will be controlled individually. One item from each autoclave lot of equipment is tested in each medium used in the test. Therefore, for a sample tested in THIO and SCD, one item from each sterilizer load (oven or autoclave) is tested in each medium giving a total of two controls for each forceps, syringe, etc., used in the test.

- Forceps
- Syringes
- Scissors
- Scalpels
- Swabs
- Pipettes
- Membranes (dry, directly from the package). If membranes are sterilized in place, this control may be omitted.
- Hemostats
- Other special items that may be required by a specific test.

3. Media and Rinse Fluid Controls

a. Uninoculated media of the autoclave load(s) as media used in the sample is controlled.

b. Portions of each rinse fluid which is used in sample test by membrane filtration are transferred to media (i.e., 10 ml into THIO and SCD).

If rinse fluids are drawn from bulk containers during sterility tests, individual controls may be omitted. Controls for these materials are accomplished as part of the "system control" for each manifold. This will also include membrane cutters, and other items that contact the product but cannot be individually controlled.

4. Environmental Controls

a. Open Media Controls
Tubes of each medium (THIO and SCD) used in the sterility analysis are exposed to the immediate environment of the test (e.g., laminar flow hood) for the duration of the test.

b. Agar Settling Plates
Glass or plastic Petri dishes containing an effective non-selective medium (based on test requirements) are exposed in the hood for a period not to exceed one hour during the analysis. If the analysis exceeds one hour, use fresh plates for each subsequent hour. Dishes of medium are sterilized by the analyzing laboratory before use.

Plates should be incubated for 48 hours at 35° C, and an additional 5 days at 25°C in order to detect mold contamination.

c. Controls within an Isolator
When conducting the sterility test within an isolator if the isolator has been designed to allow for a connection to an air sampler and particle counter this sampling may be performed for the duration of the sample analysis in lieu of the environmental samples described above. If the isolator is unable to accommodate an air sampler and/or particle counter or the instruments are unavailable the environmental controls described in section a. and b. should be used.

5. Personnel Monitoring

Personnel monitoring may be performed after analysts conclude sterility testing and prior to exiting the aseptic clean room. The analyst shall use general media touch plates to monitor the sterile condition of their clean room attire and to ensure aseptic techniques were followed.

For example, a minimum of five touch plates should be used for the following personnel gowning sites:

RH glove finger tips.
LH glove finger tips.
Chest
Left Forearm
Right Forearm

General media touch plates will be incubated for 2 days at 35ºC.

NOTE: The numerical values for personnel monitoring limits and specifications are established on the basis of a review of actual findings within the facility. All isolates are to be identified to ensure that the analyst did not contaminate the sample. Analysts should be sanitizing their gloves throughout the sterility analysis and changing gloves when needed. However, changing gloves prior to performing personnel monitoring is unacceptable.

H. Sub-culturing Primary Media

Daily observations of primary test media (THIO and SCD) containing product should be performed without unnecessary disturbance. All handling of positive tubes, streaked

plates, or subsequent inoculations of additional media will be done outside the clean room. These culture transfers are to be performed within a HEPA filtered biosafety cabinet or equivalent outside the clean room which has been cleansed with an effective anti- microbial agent. The analyst should be gowned with at least sterile gloves, sterile sleeves and a mask to minimize any possible cross contamination.

1. Record on Analyst's worksheets the day the primary isolation media, Fluid Thioglycollate Broth (THIO), or Soybean-Casein Digest Broth (SCD) is "positive," and inform supervisor. Streak tubes on the day they first appear positive and again at 14 days to determine the presence of other possible slow-growing (i.e., fungi) microorganisms.

2. Within a HEPA filtered biosafety cabinet or equivalent outside the clean room, streak positive tubes onto Modified Soybean-Casein Digest Medium [SCD broth + 1.5% agar] (Modified SCDA).

    a. Fluid Thioglycollate Broth: Streak two plates; incubate one aerobically, and one anaerobically, each at 32.5 ± 2.5oC. NOTE: It is suggested to pipet an aliquot of media from close to the bottom of the tube to maximize the recovery of strict anaerobes.

    b. Soybean-Casein Digest Broth: Streak one plate; incubate aerobically at 22.5 ± 2.5oC.

3. All streaked plates are incubated for a period at least as long as the time required for growth in original isolation media (THIO or SCD). However, incubate plates no longer than seven days.

4. Pick a single colony, representative of each colony type, as follows:*
    a. Plates Streaked with Primary Fluid Thioglycollate Broth

        i. Anaerobic Modified SCDA Plate:
        Using growth from the single colony pick, inoculate duplicate Modified SCDA slants (as in step ii below), and one SCDA slant. Incubate one aerobically and one anaerobically. Note the oxygen requirements of the pure culture isolate from this test, and preserve the anaerobic slant culture by lyophilizing or ultra-freezing. Determine the Gram stain reaction of the pure culture isolate from the USP SCDA slant incubated anaerobically.

        * If discrete colonies are not present due to overgrowth on isolation plates, pick representative colonies from such plates and re-streak to another plate. Alternatively, transfer a pick back to a new sterile tube of original isolation broth, and when growth develops, re-streak to obtain discrete colonies.

        ii. Aerobic Modified SCDA Plate:

Following the protocol above for Anaerobic Modified SCDA Plate, using growth from the single colony pick, inoculate duplicate Modified SCDA slants, and one SCDA slant. Incubate one Modified SCDA slant aerobically and one anaerobically. Note the oxygen requirements of the pure culture isolate from this test, and if indicated, preserve the culture from the aerobic modified slant. Determine the Gram reaction from the USP SCDA slant.

b. Soybean Casein Digest Broth
From the aerobic Modified SCDA plate, or from the anaerobic plate if aerobic growth fails to develop, pick a single colony representative of each type to duplicate Modified SCDA slants. Incubate one Modified SCDA slant aerobically and one anaerobically. Report the oxygen requirements of the aerobic or anaerobic slant culture. Determine the Gram stain reaction of the culture isolate from the sugar-free USP SCDA agar slant.

5. Identify each isolate as to oxygen requirement, gram reaction, presence or absence of spores, and whether the organism is a yeast or mold. If possible, the isolate can be further identified to genus and species using a rapid identification kit such as VITEK or API. Additionally DNA sequencing may be used when necessary. Follow the subculture chart in the WS section.

- strict anaerobe
- strict aerobe
- facultative anaerobe
- facultative aerobe
- gram-positive organism
- gram-negative organism
- yeast or mold
- spore forming organism

I. Preservation of Isolates

Preserve by lyophilizing (if possible) or ultra-freezing one representative culture of each identified isolate. Identify each isolate with sample number, subsample number, initials, and date. Also identify the pick number if more than one isolate was picked from a single plate.

If by observing colony morphology, Gram stain reactions, and other growth characteristics, it appears that all isolates generated from the foregoing protocol are the same organism, only one culture should be preserved from each subsample. If more than one type of organism is demonstrated from one or both primary isolation media, based on oxygen requirements, Gram reaction and colonial morphology, preserve each type by lyophilizing (if possible) or ultra-freezing.

1. Molds

Special procedures should be discussed with the supervisor for isolating molds by the preceding protocol. If filamentous fungi are suspected, streak positive tubes within a HEPA filtered biosafety hood or equivalent to minimize air-borne spreading

of conidia (i.e., spores). This will minimize contamination of work environment and risk to analyst.

2. **Subculture of original test tubes to another set of tubes containing the same medium:**
In case the product reacts with the media and shows turbidity due to the nature of the product, subculture the original tubes after 14 days of incubation. Incubate both original and subculture vessels for not less than 4 days.

J. Worksheet Notations

1. Recording Dates

a. Form FD 431, Front Page
Block 4, Date Received- Enter in this space the date the sample was received from the sample custodian. This date must be the same as that entered in the FACT system screen for sample received.

All laboratory data, observations and findings resulting from the analysis of a sample will be recorded using worksheets, continuation sheets and attachments as described in the ON LINE ORA LAB MANUAL (LPM), Vol. III, Sec. 3 (Recording of Results- Analyst Worksheets). These include;

- The analyst worksheet (FD-431)
- The general continuation sheet (FD-431a)
- All preprinted and computer generated worksheets

b. Form FD 431a, General Continuation Sheet
On modification of this form resulting in a pre-printed worksheet used for daily observations of sample tests and controls, the following information should be included:

In the space provided at the top of the Form enter the date primary test media were inoculated with product, which also indicate the date when incubation began.

Describe whether each test was a subsample or a composite.

At space provided of each column for notation of "Daily Observations," enter the date observations were made, and validate with analyst's initials.

For the days that observations were not made, the following entries will be made:

- Weekend - W
- Holiday - H
- Leave - L
- Other - O (describe)

2. Product Turbidity in Primary Test Media

When a direct inoculation protocol is used for sample products that cause turbidity of the medium upon inoculation, the following systems for recording daily observations of incubating media should be used.

a. Record "T" for any subsample which is turbid due to product-medium mixture.

b. On the daily observation page, indicate the meaning of "T" as: "T = product induced turbidity".

c. At the end of the initial 14 days of incubation, transfer portions of the medium (not less than 1 ml) to fresh vessel of the same medium and then incubate the original and transferred vessels for not less than 4 days. Note: Follow the current edition of the USP for any changes concerning subculturing and incubation of turbid samples.

d. Examine original product inoculated media and the subculture media for growth daily when possible for not less than 4 days of incubation and record the results on a new daily observation continuation sheet.

K. Preservation and Revival of Cultures

The most common methods for preserving cultures are Lyophilization and Ultra-freezing. ATCC recommends the following procedure for the rehydration of Freeze-Dried cultures. In all cases follow any specific manufacturer's instructions if provided.

1. Opening Ampoules

Score the ampoule once briskly with a sharp file about one inch from the tip. Disinfect the ampoule with alcohol-dampened gauze. Wrap gauze around ampoule and break at the scored area. Care should be taken not to have the gauze too wet or alcohol could be sucked into the culture when the vacuum is broken.

2. Opening Vials

Care should be taken so that the freeze-dried material does not aerosolize and contaminate the working area.

3. Rehydrating the freeze-dried culture

a. Rehydrate the culture using the medium and incubation temperature specified for that organism.

b. Aseptically add 0.3 to 0.4 mL of the specified medium to the freeze-dried culture with a sterile Pasteur pipette.

c. Mix well and transfer the mixture to a test tube containing 5-6 mL of the recommended broth.

d. Cultures in stoppered vials should be rehydrated with 0.5 mL of the appropriate broth, mixed and transferred to 5mL of the recommended broth.

e. Mold cultures should be rehydrated with sterile water since fungi must be soaked for at least 30 minutes before being transferred to an agar surface.

f. Incubate at the appropriate temperature.

g. Most cultures grow in a few days. However, some may exhibit a prolonged lag phase and should be given twice the normal incubation before discarding as not viable.

Chapter 4: Investigating USP Sterility Testing Failure

INTRODUCTION:

When microbial growth is detected in a pharmaceutical or medical device product, the product is considered non-sterile, pending an investigation. Because of the public health importance of a non-sterility finding, preliminary results should be reported by your laboratory management, without delay, to ORS and the appropriate Center (e.g., Office of Compliance/OCTEC). Concurrently, a laboratory review should be conducted to answer the following question: Was the result true product contamination or was there a clear laboratory error that caused contamination of the sample during the analysis? The Out-of-Specification (OOS) investigation will review and document that the test results are based on sound laboratory operation.

INVESTIGATIONS:

Whenever a sterility positive occurs, lab supervisors are responsible for starting the investigation immediately. Four factors should be evaluated in the basic investigation:

1. Equipment:

   Determine whether equipment malfunctioned or was not operated properly. If a malfunction occurred, determine whether it was likely to cause the contamination. Determine if any checklists or logs indicate that the ISO 5 device was in good state of repair at the time of the sterility test. Be aware of the most likely failure modes in the equipment (e.g., laminar flow hood, glovebox, or isolator) used.

2. Adherence to Analytical Method:

   Determine whether there were any anomalies or deviations from the analytical method. Adherence to method should be verified at the time of analysis, and any major breach of sterility test procedure should also be documented at that time. If any method breaches occurred, determine whether it was likely to cause the contamination. Be aware of any possible weaknesses in the test method (e.g., kit, manifold, etc.) used.

3. Analyst:

   Evaluate the analyst's qualifications, including proficiency, training record, and experience. Also note whether the sterility testing practice of the analyst was observed during this or a recent analysis.

4. Cleanroom and ISO 5 (Class 100) Environmental Conditions:

Determine if disinfection/decontamination of the ISO 5 device was properly done.

Determine whether there was adverse environmental data. Note that a negative control failure, on its own, is not necessarily cause for invalidating a result. If a negative control was contaminated, consider whether the microbe identified is similar to, or the same as, the sterility test isolate and also consider whether there are other adverse environmental trends.

It is advisable to summarize this review process in a standard report, and maintain a sufficient record to reflect that these areas were investigated. In addition to the four considerations listed above, overall cleanroom design is also an important consideration. There are differences in the construction, configuration, and material flow of FDA field laboratories cleanrooms. There may be differences in size, number of rooms, shape, air handling system, pass through autoclaves, gowning room accommodations (sink, HEPA filtration, adequate space, bench, etc.). Proper practices and conditions should be assured by mitigating contamination hazards potentially presented by layout and other design provisions. These include appropriate procedures for room and material disinfection, proper cleanroom uniforms (disposable or reusable), sample preparation area, etc. These and other factors can play a major role in preventing sample contamination during product handling and testing. Such cleanroom risks can be prevented by the design of ISO 5 testing equipment. Equipment that provides barrier protection can mitigate risks of the surrounding cleanroom environment.

If an investigation finds that the conduct of the analysis included errors or events that caused the test specimens to be contaminated by the lab environment, the Sterility Test result would be invalid and the substandard laboratory practice should be corrected to prevent this problem from recurring. For more information on how to judge investigational findings to make this evaluation, see Section XI.C1.and 2 of FDA's guidance on Sterile Drug Products Produced by Aseptic Processing for principles and expectations for investigating a sterility positive.

http://www.fda.gov/downloads/Drugs/.../Guidances/ucm070342.pdf

Also see the current version of USP <71>, which provides some guidance on investigations under Interpretation of Data.

CONCLUSION:

This suggested list of areas and conditions to review should not be considered as comprehensive. Additional areas of review may need to be added based on some of the unique features or procedures employed by individual FDA ORA laboratories.

Chapter 5: Bacterial Endotoxin Testing

This chapter of the Sterility Analytical Manual is intended to supplement the methodology procedures found in the USP <85> BACTERIAL ENDOTOXINS TEST.

The scope of the initial portion of this section includes the Gel Clot Method. The organization of this chapter will follow the format of the USP chapter.

Kinetic assays are addressed in the second part of this section. Kinetic assays involve a change in color or turbidity (depending on the assay reagents used) over time. The time for change is inversely proportional to the concentration of endotoxin in the solution, i.e. more rapid change at higher endotoxin concentrations. An instrument is used to read the changes. These assays are rapid and sensitive, allowing large numbers of samples to be screened quickly. However, the gel-clot method is the reference method per USP and should be used if there are any doubts or disputes, unless otherwise indicated in the monograph for the product tested. In case of both procedures being performed, the gel clot method is considered the reference method and overrides any kinetic assay results.

New microbiologists should review the references at the end of this chapter.

A. Gel Clot Method

1. Reference Standard and Control Endotoxins

The potency of the control standard endotoxin (CSE) with respect to the reference standard endotoxin (RSE) is determined by the CSE manufacturer. This information is found in the associated package insert and need not be repeated

NOTE: Follow manufacturers' recommendations in the storage, reconstitution, and preparation of CSEs, lysates, and other LAL reagents. In case of a dispute, final decision is based on results obtained with the USP Endotoxin RS.

The field laboratories are encouraged to mix the CSE for at least 5 minutes and at least 1 minute between dilutions.

2. Preparatory testing

Run appropriate negative controls with each sample tested. This assures that the equipment and solutions used in the test contain no extraneous endotoxin. For example, a beaker full of pyrogen- free test tubes stored for an extended period should be screened with a negative control for each sample tested. Record these results on the worksheets.

When using commercially purchased pyrogen-free water for product dilutions be sure to transfer a working volume from the original stock container to an individual pyrogen-free test tube or flask in order to minimize back contamination. Run a negative control for the working volume for each sample run.

NOTE: Pyrogen free pipettes, micropipettor tips, test tubes, and other accessories are commercially available. Include appropriate negative controls to assure they do not contain extraneous endotoxin.

3. a. Test for Confirmation of Labeled LAL Reagent Sensitivity

Prior to use in the test, prepare one single dilution series. Inoculate four replicates from each tube. Multiple dilution series are not required.

The labeled LAL reagent sensitivity must be confirmed before a new LAL lot is introduced into the laboratory. Prepare control standard endotoxin having at least four concentrations equivalent to 2 λ, λ, 0.5 λ, and 0.25 λ in quadruplicates. The geometric mean of the endpoints must be within the limits of labeled claim. The acceptable variation is one half (0.5 λ) to two times (2 λ) the labeled sensitivity (λ).

3. b. Inhibition or Enhancement Test

The suitability of the test results for bacterial endotoxin require an adequate demonstration that specimens of the article or of solutions to which the test is to be applied do NOT of themselves inhibit or enhance the reaction or otherwise interfere with the test.

USP states to perform this test "on aliquots of the specimen… in which there is no detectable endotoxin". However, this characteristic of the product cannot be ascertained prior to the analysis because the specimens are unknown samples. Because of this limitation, any positive result below the 0.5 lambda level may not be an enhancement trait of the product, but instead a positive reaction due to contamination in the sample. The evidence for this conclusion should be obvious with the results of the assay tubes containing product only.

A large percent of small volume parenterals appear to be inhibitory to the LAL gel-clot method because of low pH, or some excipient / active component of the product. In order to expedite the neutralization of this interfering trait, determine the lowest product dilution overcoming the interference but still within the Maximum Valid Dilution. The detailed description of this protocol is delineated in LIB No. 2433 (July 25, 1980), "A condensed procedure for diluting product in determining compatibility with the Limulus Amebocyte Lysate test for endotoxin". In addition, the use of neutralizers such as sodium laurel sulfate or pyrospersetm has also been described (see references).

NOTE: Contact LAL manufacturers for recommendation of commercially available neutralizing buffer to be used with their LAL kits.

4. Test Procedure

The storage and mixing of samples prior to analysis may affect recovery of endotoxin contamination. Sample (product) bottles should be vigorously shaken prior to analysis, preferably on a vortex (see reference for supporting evidence for this step). A minimum of 30 seconds to 1 min on the vortex is recommended for each product unit.

5. Endotoxin Calculation

Calculate endotoxin concentration per the USP Bacterial Endotoxins test chapter. Note: Adjust the final endotoxin value taking into account the volume of the rinse solution used in the extraction procedure.

6. Compositing Samples

The Bacterial Endotoxin test <85> does not directly address the issue of combining product units (compositing). The risk of unit composites is that one unit (vial, ampoule, etc) may have bacterial endotoxin contamination at a higher level but the dilution of this one unit with endotoxin-free units of product may reduce the detectable level of endotoxin below the sensitivity of the lysate or dilute the level of endotoxin below the acceptable monograph level. Therefore, **when using a composite format for screening drug products for endotoxin it is important to adjust the MVD calculation to account for this reduced lysate sensitivity**. Secondly, **when compositing is performed for product screening, if a positive result is detected a repeat test is acceptable under the conditions stated by the Interpretation section of the USP chapter**.

It would be advisable when performing the repeat test from a composite mixture that, if remaining product is available and had been opened aseptically under controlled conditions, the repeat test be performed on the original individual units. It is strongly advised that the individual units be adequately shaken to assure that the endotoxin is re-suspended back into solution before taking the sample test aliquot. **If any of the original individual units fail the USP test at this point, the compendium does not allow any additional repeat testing unless the test can be proven not to be suitable as defined by the USP chapter**.

7. **Frequently asked questions:** (derived from the FDA Industry Guidance for Pyrogen and Bacterial Endotoxin testing)

   Question 1: Can FINISHED product units (vials, ampoules, pre-filled syringes, etc) be "Pooled" into a composite and screened for bacterial endotoxin?

   Response 1:
   Yes. With some exceptions (see below), finished drug product units may be pooled into a composite sample and assayed for bacterial endotoxins. The composite sample may be represented by the entire unit or partial aliquots (equal volumes) of finished product containers from one manufactured lot of aqueous-based pharmaceuticals. Pooling would generally be accepted for small-volume parenterals (those with volumes of 100 mL or less) as long as the MVD is adjusted to a proportional, lower value because of the potential for diluting a unit containing harmful levels of endotoxins with other units containing lower, less harmful, levels of endotoxins. This “adjusted MVD” is obtained by dividing the MVD computed for an individual sample by the total number of samples to be pooled. FDA suggests pooling no more than three units per composite in keeping with the concept of testing representative beginning, middle, and end finished product containers. If this reduction in MVD results in an inability to overcome product-related assay interference because of an insufficient dilution, then the samples should be tested individually.

   Finished medical devices may also be pooled into a composite sample and assayed for bacterial endotoxins. Testing for medical devices should be conducted using rinsing/eluting and sampling techniques as described in ISO 10993-1 and ISO 10993-12, as also used for inhibition/enhancement. Sampling can be adjusted for special situations. After a suitable eluate/extract pool is obtained from a finished

production lot, this pooled extract should be kept under conditions appropriate for stability until it is tested in duplicate.

FDA recommends that pooled samples be a composite of aseptically removed aliquots (after at least 30 seconds of vigorous mixing) from each of the product containers. In this way, the original, individual containers will be available for possible retesting in the event the pooled sample displays an OOS result.

Some product types should not be pooled. Two examples are drug products that have an initial low MVD (see discussion above of “adjusted MVD”) and products that are manufactured as a suspension, because sample aliquot homogeneity may present significant interference issues.

Question 2: Can INTERMEDIATE (IN-PROCESS) sample aliquots be "pooled" into a composite and screened for bacterial endotoxin?

Response 2:
FDA does not recommend pooling in-process samples from different in-process stages of the manufacturing process because it may be difficult to ensure the homogeneity of these materials.

Question 3: Retesting when test failure occurs:

Response 3:
When conflicting results occur within a test run, the analyst should consult USP Chapter <85>, Gel Clot Limits Test, Interpretation, for guidance on repeat testing. As specified in Chapter <85>, if the test failure occurred at less than the maximum valid dilution (MVD), the test should be repeated using a greater dilution not exceeding the MVD. A record of this failure should be included in the laboratory results. If a test is performed at the MVD and an out-of-specification (OOS) test result occurs that cannot be attributed to testing error, continue product dilution until the actual endotoxin concentration can be calculated. These results should be recorded on your worksheets.

B. Kinetic Assays

Kinetic assays are quantitative assays used for the detection of bacterial endotoxins. Kinetic assays may utilize turbidimetric or chromogenic formats.

This section provides procedural information that can be applied to the QCL chromogenic assay. The Kinetic QCL Chromogenic Assay may be purchased as a kit. A certificate of analysis is provided with each kit along with testing procedures, control standard endotoxin, Limulus Amebocyte Lysate (LAL), and pyrogen free water. Other materials such as pyrogen free pipettes, micropipettor tips, test tubes, and 96-well microplates may be purchased from various vendors.

1. Kinetic QCL Assays: The kinetic QCL software program is designed to run the following assays.

i. Initial qualification of the testing analyst

ii. RSE/CSE assesses potency of control standard endotoxin (CSE) in terms of reference standard endotoxin (RSE)
iii. Inhibition or Enhancement Test
iv. Sample Test

- The initial qualification assay verifies the proficiency of the analyst operating the Kinetic QCL program and equipment. The initial qualification assay may also be used to qualify each new lot of kinetic QCL test kits.
- The RSE/CSE assay may be used to compare the potency of the CSE with the concentration of the RSE. Normally, the RSE/CSE assay does not need to be performed, unless there is reason to believe the values in the manufacturer's certificate of analysis (COA) are not correct.
- The Inhibition or Enhancement assay must be run on all samples having positive test results, and on all sample tests that exhibit inhibition.
- The routine assay program is designed to test unknown samples for bacterial endotoxins. Samples collected for LAL analysis should be run using the routine assay program, after taking the other three programs into consideration.

2. Procedure

Perform the assay according to the instructions that enclosed with the LAL test kit. Additional instructions may be found in references 8 and 9 listed below.

C. Medical Devices

The analytical approach for testing medical devices is sparsely covered in USP. A collaborated method prepared by an FDA field laboratory is available for extraction of endotoxin from devices. Modification of testing may be necessary depending on the product configuration. Analytical validation of the final version should be conducted by the responsible laboratory. The protocol is summarized below for convenience:

1. Extraction and analysis of Endotoxin from Medical Devices:

This section applies to sterile disposable syringes and cartridges, transfusion and infusion assemblies, implants, etc.

a. Preparation of 1% SLS solution

Prepare a 1% stock solution by placing one (1) gram of sodium lauryl sulfate (SLS) into a depyrogenated glass flask and add 99 ml of pyrogen free water. Allow the SLS to fully dissolve. This should be followed by filtration through a 10, 000 MW depyrogenation membrane filter into a pyrogen free glass or plastic container.

b. Equipment needed: Ultrasonic bath with a range of 150 to 440 watts.

c. Extraction procedure

i. Dilute 2mL of 1% SLS stock solution to 20mL (0.1%) using LAL reagent water in a 20 x 150 mm screw-cap tube.

ii. Dilute 1.5mL of the 0.1% SLS solution to 15 mL (0.01%) using LAL reagent water in a 20 x 150 mm screw-cap tube.

iii. Prepare the appropriate number of tubes (one tube for each device) and one as a negative / system control. Preheat in a waterbath to 40°C.

iv. Aseptically remove the device from its packaging and cut it diagonally into pieces less than 5mm in length. Metal pieces such as needles and luer-locks should be tested whole.

v. Place all pieces into the 20 x 150 mm tube containing 15 mL of preheated (40°C) 0.01% SLS rinse solution.

vi. Vortex the tubes for 30 – 60 seconds or until all pieces of the device are immersed in the rinse solution.

vii. Sonicate the test containers for 60 minutes (wattage range 150 – 480 watts) at 40°C. Do not sonicate more tubes than can be vortexed within 15 minutes of completion of the sonication. Make sure the water in the sonicator covers the rinse solution in the 20 x 150 mm tubes. Do not allow the water in the sonicator to exceed 50°C.

viii. Vortex the tubes for 2 minutes. Remove a portion of the eluate (5 – 10 mL) for LAL testing. If the eluates are not tested immediately for endotoxin, they should be refrigerated. All eluates must be tested within 24 hours of extraction. Prior to analysis vortex at least one minute.

ix. Screen all eluates for endotoxin content using 10-fold dilutions to the 10-3 dilution. Prepare dilutions using pyrogen free water in pyrogen free tubes. Positive and negative controls must be run simultaneously with all LAL tests.

x. Quantitate the eluate by making 1:2, 1:4, 1:6, 1:8 dilutions of the last positive 10-fold dilution in step 8.

D. Endotoxin References

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Chapter 6: Particulate Matter

This chapter is intended to supplement the methodology procedures found in the USP <788> PARTICULATE MATTER IN INJECTIONS and USP <789> PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS[1]. Particulate matter consists of mobile, randomly-sourced extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis due to the small amount of material that it represents and heterogeneous composition. Injectable solutions, including solutions constituted from sterile solids intended for parenteral use, is essentially free from particulate matter observable on visual inspection. The tests described herein are physical tests performed for the purpose of enumerating sub-visible extraneous particles within specific size ranges.

All large-volume injections for single-dose infusion and those small-volume injections for which the monographs or product specifications specify such requirements are subject to the particulate matter limits set forth for the test being applied, unless otherwise specified in the individual monograph or product specification.

Not all injection formulations can be examined for particles using the light obscuration method. Any product that is not a pure solution having clarity and viscosity approximating those of water may provide erroneous data when analyzed by the light obscuration counting method. Refer to specific monographs when a question of test applicability occurs. The microscope counting method may be used to analyze such materials. In some instances, the viscosity of a material to be tested may be sufficiently high so as to preclude its analysis by either method. In this event, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

A. Light Obscuration Particle Count Test

The test applies to large-volume injections labeled as containing more than 100 mL and single-dose or multiple-dose small-volume injections labeled as containing 100 mL or less that are either in solution or in solution constituted from sterile solids, where a test for particulate matter is specified in the individual monograph or drug product specification. It counts suspended particles that are solid or liquid. Products for which the labeling in the individual monograph specifies that the product is to be used with a final filter are exempt from these requirements.

1. Test Apparatus

The apparatus is an electronic, liquid-borne particle counting system that uses a light-obscuration sensor with a suitable sample feeding device. A variety of suitable devices of this type are commercially available. It is the responsibility of those performing the test to ensure that the operating parameters of the instrument are appropriate to the required accuracy and precision of the test result and that adequate training is provided for those responsible for the technical performance of the test. It is the responsibility of the user to apply various methods of standardization applicable to the specific instrument. Critical operational criteria consist of the following:

a. Sensor Concentration Limits

Use an instrument that has a concentration limit (the maximum number of

[1] Text in portions of this document chapter was selected from the current United States Pharmacopeia (USP).

particles per ml) identified by the manufacturer that is greater than the concentration of particles in the test specimen to be counted. The vendor-certified concentration limit for a sensor is specified as that count level at which coincidence counts due to simultaneous presence of two or more particles in the sensor view volume comprise less than 10% of the counts collected for 10 micron particles.

b. Sensor Dynamic Range

The dynamic range of the instrument used (range of sizes of particles that can be accurately sized and counted) must include the smallest particle size to be enumerated in the test articles.

2. Instrument Standardization

The following discussion of instrument standardization emphasizes performance criteria rather than specific methods for calibrating or standardizing a given instrument system. This approach is particularly evident in the description of calibration, where allowance must be made for manual methods as well as those based of firmware, software, or the use of electronic testing instruments. Appropriate user validation of software and firmware systems is essential to performance of the test according to requirements. Since different brands of instruments may be used in the test, the user is responsible for ensuring that the counter used is operated according to the manufacturer's specific instructions. The principles to ensure that instruments operate within acceptable ranges are defined below.

The following information for instrument standardization helps ensure that the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution and count accuracy are appropriate to performance of the test.

a. Sample Volume Accuracy

Since the particle count from a sample aliquot varies directly with the volume of fluid sampled, the sampling accuracy must be known to be within a certain range.

i. For sample volume determination, determine the dead (tare) volume in the sample feeder with Water for Injection or distilled water that has been passed through a filter having a porosity of 1.2 microns or finer.

ii. Transfer a volume of water that is greater than the sample volume to a container and weigh.

iii. Withdraw through the sample feeding device a volume that is appropriate for the specific sampler, and again weigh the container.

iv. Determine the sample volume by subtracting the tare volume from the combined sample plus tare volumes.

v. Verify that the value obtained is within ± 5% of the appropriate sample volume for the test.

b. Sample Flow Rate

Verify that the flow rate is within the manufacturer's specifications for the sensor used. This may be accomplished by using a calibrated stop watch to measure the time required for the instrument to withdraw and count a specific sample volume (i.e. the time between beginning and ending of the count cycle as denoted by instrument indicator lights or other means). Perform the Test Procedure at the same flow rate as that selected for calibration of the instrument.

c. Calibration and Sensor Resolution

There are three methods of choice depending on the type of system used: Manual, Automated and Electronic methods. Please refer to the current USP for guidance.

i. Prepare the suspension and blank using the USP Particle Count RS.

   Set the instrument to count at 10 and 25 microns (the apparatus is calibrated using dispersions of spherical particles of known sizes between 10µm and 25µm), according to USP <788>.

ii. Mix the blank by inverting 25 times within 10 seconds and de-gas the mixture by sonicating for 30 seconds or by allowing to stand.

iii. Remove the closure from the container and gently stir the contents by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination. Stir continuously throughout the analysis. Note: Electronic Particle Analyzers have a mixing stand attached to the instrument for this purpose.

iv. Withdraw directly from the container three consecutive volumes of not less than 5 mL each.

v. Obtain particle counts and discard the data from the first portion. NOTE: Complete procedure within five minutes.

vi. Repeat procedure, using the suspension in place of the blank.

vii. From the averages of the counts resulting from the analysis of the two portions of the suspension at 10 microns and from the analysis of the two portions of the blank at 10 microns, calculate the number of particles in each ml taken as follows: Subtract the average particle count of the blank from the average particle count of the suspension, then divide the result by the average volume of the 4 portions tested. This can be summarized in the following formula:

$$(Ps - Pb)/V$$

   in which Ps is the average particle count obtained from the suspension, Pb is the average particle count obtained from the blank, and V is the average volume, in ml, of the 4 portions tested.

viii. Repeat the calculations, using the results obtained at 15 microns.

ix. Interpretation: The instrument meets the requirements for Particle Counting Accuracy if the count obtained at 10 microns and the ratio of the counts obtained at 10 microns to those obtained at 25 microns conform to the values that accompany the USP Particle RS.

x. If the instrument does not meet the requirements for Particle Counting Accuracy, recalibrate with the remaining suspension and blank.

xi. If the results of the second test are within the limits given above, the instrument meets the requirements of the Particle Counting Accuracy Test. If on the second attempt the system does not meet the requirements of the test, determine and correct the source of the failures and retest the instrument.

3. Test Environment

a. Perform the test in an environment that does not contribute any significant amount of particulate matter. Note: Glassware from Qorpack come in particle free packages which is a suitable particulate free vehicle for compositing liquid samples.

b. Specimens must be cleaned to the extent that any level of extraneous particles added has a negligible effect on the outcome of the test.

c. The test specimen, glassware, closures and other required equipment preferably are prepared in an environment protected by high efficiency particulate air (HEPA) filters.

d. It is preferable to wear non-shedding garments and powder-free gloves throughout the preparation of samples.

e. Cleanse glassware, closures and other required equipment preferably by immersing and scrubbing in warm water, nonionic detergent solution. Rinse in flowing tap water and then rinse again in flowing filtered water. Organic solvents may also be used to facilitate cleaning. Note: Particle free glassware is available which reduces the chance of contamination with environmental particles.

f. Finally, rinse the equipment in filtered water using a hand-held pressure nozzle with final filter or other appropriate water source, such as distilled water passed through a capsule filter. The filter used should have a porosity of 1.2 microns or finer.

g. To collect background counts:

i. Use a clean vessel of the type and volume representative of that to be used in the test.

ii. Place a 50 mL volume of particle free water in the vessel, and agitate the sample in the cleaned glassware by inversion or

swirling. [NOTE: A smaller volume, consistent with the article to be counted, can be used.]

iii. De-gas by sonicating for 30 seconds or by allowing to stand.

iv. Swirl the vessel containing the water sample by hand or agitate by mechanical means to suspend particles.

v. Withdraw and obtain the particle counts for five consecutive samples of not less than 5 mL each.

vi. If more than 25 particles of 10 micron size or greater size are observed in the combined 25 mL sample, the environment is not suitable for particulate analysis. The particle free water and glassware have not been properly prepared. Reexamine the procedure followed, equipment used and the environment in which the test was performed. Make any changes needed until a satisfactory preparatory test result is attained.

4. Test Procedure

For containers having volumes of less than 25 mL, test a solution pool of 10 or more units to obtain a volume of no less than 25 mL. Single units of small-volume injections may be tested individually if the individual unit volume is 25 mL or greater.

Prepare the test specimens in the following sequence:

a. Agitate the contents of the sample by inverting the container 20 times.

b. Remove outer closures, sealing bands, and any loose or shedding paper labels.

c. Rinse the exterior of containers with particle free water as described under Test Environment, and dry. Take care to protect the contents of the containers from environmental contamination.

d. Allow the container to stand for 2 minutes or sonicate in order to remove gas bubbles.

e. Withdraw the contents of the containers in the normal or customary manner of use, or as instructed in the package labeling. Containers with removable stoppers may be sampled directly by removing the closure. Optional: If test specimens are being pooled, remove the closure and empty the contents into a clean container.

5. Determination of Particle Counts

a. Method for Small Volume Parenterals

NOTE: Because of the small volume of some products, it may be necessary to agitate the solution more vigorously in order to suspend the particles completely and homogeneously.

i. Obtain and combine in a cleaned container the contents of 10 or more units to obtain a volume of not less than 25 mL.

ii. De-gas by sonicating for 30 seconds or by allowing to stand until the solution is free from air bubbles.

iii. Gently stir the contents of the container by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination.

iv. Withdraw not less than 4 aliquot portions, each not less than 5 mL in volume, into the light obscuration counter sensor.

v. Obtain the particle counts, and discard the data from the first portion.

NOTE: For some products, a pool of 15 or more units may be necessary to achieve a pool volume sufficient for three 5 mL sample aliquots. Smaller sample aliquots (i.e., less than 5 mL) can be used if the assay result obtained with the smaller aliquots is validated to give an assessment of batch suitability equivalent to that obtained with the 5 mL aliquots specified above.

b. Method for Large Volume Parenterals where the contents of each unit are 25 mL or more, Individual units are tested

i. Mix 1 unit following the same procedure as described above; Removal of closure and agitation is same as described above; Withdraw not less than 3 aliquot portions, each not less than 5 mL in volume, into the light obscuration counter sensor.

ii. Obtain particle counts, discarding the data from the first portion.

c. Dry or Lyophilized Product for Parenteral Use

i. Open the container, taking care not to contaminate the opening or cover.

ii. Constitute as directed in the product label with a suitable volume of particle free water, or with the appropriate particle free solvent if water is not suitable.

iii. Replace closure, and manually agitate the container to dissolve the drug.

iv. Allow to stand until the drug is completely dissolved.

v. Prior to analysis, gently stir the contents of the containers by hand-swirling or by mechanical means, taking care not to introduce air bubbles or contamination.

vi. Pool or test individually the appropriate number of units. Withdraw no less than 4 aliquots, each not less than 5 mL in volume, into the light obscuration counter sensor.

vii. Obtain the particle counts, and discard the data from the first aliquot.

d. Solid Drugs Packaged with Diluents

For products packaged in containers that are constructed to hold the drug product and a solvent in separate compartments, mix each unit as directed in the labeling, activating and agitating each unit so as to ensure thorough mixing of the separate components. Analyze the solutions as described under methods for large or small volume parenterals depending on container volume.

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e. Multiple-dose Containers

For products labeled Pharmacy Bulk Packages, proceed for each unit as directed under method for small volume parenterals, calculating the results on the basis of a sample volume that is equal to the maximum dose stated in the labeling. For the calculations below, consider a maximum-dose volume to be the equivalent of the contents of one full container.

6. Calculations

a. Pooled Samples (Small-volume Injections)

Average the counts from the 2 or more aliquot portions analyzed. Calculate the number of particles in each container by multiplying the average particle count obtained from the portion by the volume of the pooled sample in mL, and divide by the product of the volume of each portion analyzed and the number of containers pooled. This calculation is summarized by the formula:

$$PVt/Van$$

in which P is the average particle count obtained from the portion analyzed, Vt is the volume of pooled sample, in mL, Va is the volume, in mL of each portion analyzed, and n is the number of containers pooled.

b. Individual Samples (Small-volume Injections)

To calculate the number of particles in each container, average the counts obtained for the 5 mL or greater aliquot portions from each separate unit analyzed, multiply by the volume in mL of the unit tested, and divide this result by the volume in mL of each portion analyzed. This calculation is summarized by the formula:

$$PV/Va$$

in which P is the average particle count obtained from the portions analyzed, V is the volume, in mL, of the tested unit, and Va is the volume, in mL, of each portion analyzed.

c. Individual Unit Samples (Large Volume Injections)

To calculate the number of particles in each mL, average the counts obtained for the two or more 5 mL aliquot portions taken from the solution unit, then divide by the volume in mL of the portion taken. This calculation is summarized by the formula:

P/V

in which P is the average particle count for an individual 5 mL or greater sample volume, and V is the volume, in mL, of the portion taken.'

7. Interpretation

The injection meets the requirements of the test if the calculated number of particles present in each discrete unit tested or in each pooled sample tested does not exceed the appropriate value listed in Table 1. If the average number of particles exceeds the limit, test the article by the Microscopic Particle Count Test.

Table 1. Light Obscuration Test Particle Counts

	≥10 microns	≥25 microns
Small –volume Injections:	6000 per container	600 per container
Large-volume Injections:	25 per mL	3 per mL

8. Particulate Matter in Ophthalmic Solutions

Every ophthalmic solution for which the monograph or drug product specification includes a test for particulate matter is subject to the particulate matter limits set forth for the test being applied, unless otherwise specified in the individual monograph or specification. When higher limits are appropriate, they will be specified in the individual monograph or specification. Ophthalmic preparations that are suspensions, emulsions, or gels are exempt from these requirements, as are medical devices. Refer to the specific monograph when a question of test applicability occurs.

Light obscuration and microscopic procedures for the determination of particulate matter in ophthalmic solutions are identical to those for injections; therefore, where appropriate, USP <788> Particulate Matter in Injections is cross-referenced.

a. Light Obscuration Particle Count Test

This test applies to ophthalmic solutions, including solutions constituted from sterile solids, for which a test for Particulate matter is specified in the individual monograph. The test counts suspended particles that are solid or liquid.

i. Test Apparatus, Instrument Standardization, Test Environment, Test Procedure, and Calibrations: Proceed as directed for Light Obscuration Particle Count Test under USP <788> Particulate Matter in Injections.

ii. Interpretation: if the average number of particles exceeds the limit, test the article by the Microscope Particle Count Test.

Table 2. Light Obscuration Test Particle Count

	≥10 microns	≥25 microns
Number of particles:	50 per mL	5 per mL

NOTE: Any product that is not a pure solution having clarity and viscosity approximating those of water may provide erroneous data when analyzed by the light

obscuration counting method. Such materials may be analyzed by the microscope counting method.

B. Microscopic Particle Count Test

The microscope particulate matter test may be applied to both large-volume and small volume parenteral injections and to ophthalmic solution products as well. This test enumerates essentially solid particulate matter >/- 10 um in these products, after collection, rinsing and drying on a micro-porous membrane filter. Since a wide range of aliquots may be utilized, particle counts may be determined on a per-volume or per-container basis without dilution or extrapolation.

In the performance of the membrane microscope assay, one estimates the size of retained solids viewed at 100x magnification. Tabulating them into specific size categories. In this process, one may encounter materials on the membrane surface that do not appear solid or substantial, showing little or no surface relief such as a “stain” or discontinuity on the membrane. Chapter <788> advises not to attempt to size or enumerate such semi-solid particles, due to historical comment from LVP terminal sterilization manufacturers that encountered stain-like brown residues after heat sterilization of Dextrose solutions.

However, if not sampling a carbohydrate solution or similarly-performing formulation, recognizing the presence of such materials may be an indication that further development research is warranted to count or investigate must be based upon product formulation experience. Interpretation of microscopical enumeration may be aided by testing a sample of the solution by the LO particle count or a validated, alternate method.

The Test Apparatus is described in <788>. Additional comments are:

Use a compound binocular microscope that corrects for changes in interpupillary distance by maintaining a constant tube length.

The objective must be of 10X nominal magnification, a planar achromat or better in quality, with a minimum 0.25 numerical aperture.

The objective must be compatible with an episcopic illuminator attachment.

The eyepieces must be matched. In addition, one eyepiece must be designed to accept and focus an eyepiece grarticule. The microscope must have a mechanical stage capable of holding and traversing the entire filtration area of a 25 –mm or 47 mm membrane filter.

Two illuminators are required. Both illuminators must be of sufficient output to provide a bright and even source of illumination and may be equipped with blue daylight filters to decrease operator fatigue during use.

1. **Stage Micrometer** - Graduated in 10-um increments, utilized each day-of- use. For initial calibration, use a stage micrometer that is certified by NIST to verify the USP graticule installation. Thereafter, for daily calibration/verification, one may utilize a commercial stage micrometer graduated in 10-um increments to verify proper setup.

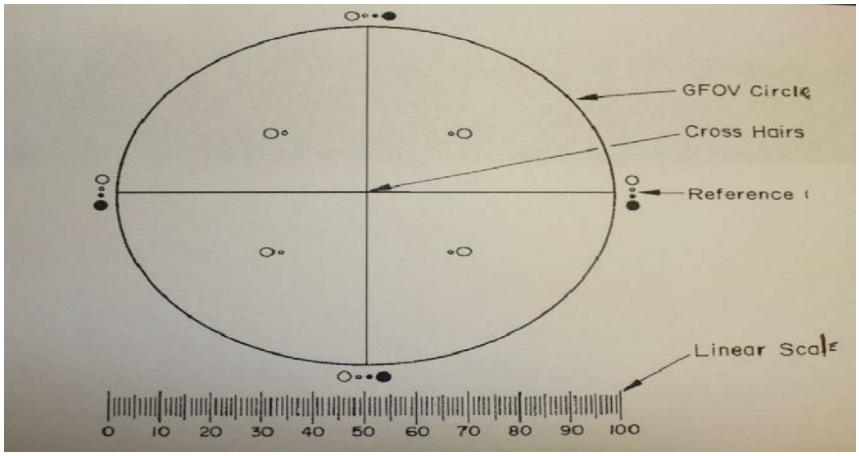
2. **Filtration Apparatus** - Use a filter funnel suitable for the volume to be tested, generally having an inner diameter of about 16 mm for 25-mm membranes or about 37 mm for 47 mm membranes. The funnel is made of plastic, glass or stainless steel. Use a filter support made of stainless steel screen or sintered glass as the filtration diffuser. A solvent dispenser capable of delivering solvents filtered through a membrane filter at a large range of pressures from 10 psi to 80 psi.

   Membranes-As describes by <788>; however, finer pore size selections will have smoother surfaces, facilitating the microscopical examination; however, may impede more viscous sample fluid during the assay.

3. **Equipment:**
   Use a suitable binocular microscope, a filter assembly for retaining particulate matter, and a membrane filter for examination.

   The microscope is adjusted to 100 +/- 10 magnifications and is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, and two suitable illuminators to provide episcopic illumination in addition to oblique illumination.

   Figure 1:



The ocular micrometer is a circular diameter graticule (Figure 1) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles 10 um and 25 um in diameter at 100 magnifications, and a linear scale graduated in 10-um increments. It is calibrated using a stage micrometer that is certified by either a domestic or international standard institution. A relative error of the linear scale of the graticule within +/- 2% is acceptable. The large circle is designated the graticule field of view (GFOV).

Figure 1: Circular diameter graticule. The large circle divided by crosshairs into quadrants is designated the graticule field of view (GFOV). Transparent and black circles having 10-um and 25-um diameters at 100X are provided as comparison scales for particle sizing.

Two illuminators are required. One is an episcopic bright field illuminator internal to the microscope; the other is an external, focusable auxiliary illuminator that can be adjusted to give reflected oblique illumination at an angle of 10-20 degrees.

The filter assembly for retaining particulate matter consists of a filter holder made of glass or other suitable material, and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark gray in color, non-gridded or gridded and 1.0 um or finer in nominal pore size.

4. General Precautions:

The test is carried out under conditions limiting particulate matter, preferably in a laminar flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution, and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside with particle free water. In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned, and that the water to be used is particle-free, the following test is carried out. Determine the particulate matter of a 50-ml volume of particle-free water according to the method described below. If more than 20 particles of 10um or larger in size or if more than five particles
25 um or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

5. Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using as jet of particle-free water, and remove the closure, avoiding any contamination of the contents.

For large volume parenterals, single units are tested. For small volume parenterals less than 25 ml in volume, the contents of 10 or more units are combined in a cleaned container; the test solution may be prepared by jixing the contents of a suitable number of vials and diluting 25 ml with particle-free water or with an appropriate particle-free solvent when particle-free water is not suitable. Small volume parenterals having a volume of 25 ml or more may be tested individually.

Powders for parenteral use are constituted with particle-free water or with an appropriate particle-free solvent when particle-free water is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment.

For large volume parenterals or for small volume parenterals having a volume of 25 ml or more, fewer than 10 units may be tested, using an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several ml of particle-free water. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply a vacuum. If needed, add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of particle-free water. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the membrane filter in a Petri dish, and allow the membrane filter to air-dry with the cover slightly ajar. After the membrane filter has been dried, place the Petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light form the illumination device, and count the number of particles that are equal to or greater than 10um and the number of particles that are equal to or greater than 25um. Alternatively, partial membrane filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by estimating the equivalent diameter of the particle in comparison with the 10um and 25 um reference circles on the graticule. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the Microscopic Particle Count Test, do no attempt to size or enumerate amorphous, semiliquid, or otherwise morphologically indistinct materials that have the appearance of a s stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases, the interpretation of enumeration may be aided by testing a sample of the solution by the Light Obscuration Particle Count Test.

6. Evaluation:

For preparations supplied in container with a nominal volume of more than 100 ml, apply the criteria for Test 2.A.

For preparations supplied in containers with a nominal volume of less than 100 ml, apply the criteria for Test 2.B

For preparations supplied in containers with a nominal volume of 100 ml, apply the criteria for Test 2.B (Note: Test 2.A is used in the Japanese Pharmacopeia).

Test 2.A: (Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 ml)-The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per/ml equal to or greater than 10 um and does not exceed 2 per/ml equal to or greater than 25 um.

Test 2.B (Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 ml)-The preparation complies with the test if the average number of particles present in the units does not exceed 3000 per container equal to or greater than 10 um and does not exceed 300 per container equal to or greater than 25 um.

Chapter 7: Antibiotic Potency Testing

A. General Information

Antibiotic potency testing is a biological assay whereby varying concentrations of antibiotic are tested against a live microorganism; the resulting biological response is then measured and evaluated against a median standard and five-point (minimum) standard curve. The biological response is referred to as antibiotic activity or potency. Antibiotic potency is dependent upon antibiotic-microorganism specificity and is physically expressed by the inability of a microorganism to grow under optimal conditions. USP <81> Antibiotics- Microbial Assays, is the primary reference for bioassay antibiotic potency testing.

Antibiotic potency testing is a multi-variable test which depends on a variety of factors: 1) Test microorganism growth requirements 2) Test antibiotic dose and 3) Preparation and use of growth media, reagent, test organism and antibiotic standards. Potency testing requires a basic knowledge of laboratory safety, analytical chemistry, microbiology and aseptic techniques. Potency testing is a manual, multi-step and multi-day process performed with common laboratory equipment. At minimum, two analysts are required for preparation and sample setup.

Improved manufacturing technology (e.g. purification methods) has evolved potency testing from a simple biological assay to different chemical assays. Most chemical assays are based upon the segregation and quantification of antibiotic components through the use of high-performance liquid chromatography (HPLC). However, chemical assays do not demonstrate biological activity and antimicrobial efficacy of a test antibiotic, particularly an antibiotic which may contain numerous active components, each exhibiting different antimicrobial activities; chemical potency does not require the use of a viable test microorganism.

Antibiotic potency testing is performed either by the plate (cylinder-plate or diffusion) or tube (turbidimetric) method. Both plate and tube methods demonstrate measurable levels of growth inhibition. Growth inhibition measurements are tabulated and integrated into a linear regression curve, resulting in extrapolated antibiotic potency values.

Potency is denoted in units (U) or µg of activity and may or may not be exact in equivalence to the µg (weight) of the active compound. The following three reasons may explain this weight-activity discrepancy: 1) Activity may be caused by the antibiotic's free base or salt form and activity is denoted in either form 2) The antibiotic may contain similar chemical components but differ in activity or 3)

The antibiotic activity is represented by a heterogeneous family of antibiotics and not a single analog.

The test antibiotic is diluted to a known value, equivalent to the median standard concentration of the five-point standard curve. The test antibiotic is analyzed in triplicate each test day for three consecutive test days. During each test day, the final calculated potency of the test antibiotic should be 80%-125% of the median standard. Additionally, USP suggests a standard curve correlation coefficient of 0.990 for each test day. Refer to USP<81> for testing parameters and acceptable data requirements.

B. Equipment

Antibiotic potency testing is a quantitative test dependent upon the precise preparation and use of growth media, reagents, test organism and antibiotic standards; therefore, Class A volumetric glassware is used to measure volumes of such solutions. Class A glassware is physically labeled either “to deliver” (TD) or “to contain” (TC). An understanding of Class A glassware prior to laboratory use is required. For example, a high viscous liquid (e.g. antibiotic ointment) cannot be delivered from a “to deliver” Class A pipette. “To deliver” Class A pipettes solely rely on gravity to assist in evacuation. In the case of the antibiotic ointment, the ointment would remain in the Class A pipette at time of evacuation.

Standard laboratory glassware such as beakers, funnels, flasks, roux and 1-2 liter bottles are also required. Sterile and disposable equipment such as test tubes, petri-plates and serological pipettes may be used so long as the use of this equipment does not affect the quantitative aspect of potency testing. Additional equipment includes a stainless steel penicylinders, cuvettes, serological pipettor, single channel micropipette, pH meter, hot plate, adjustable-temperature water bath, incubator and a manual/automatic plate reader or UV-VIS spectrophotometer.

Equipment in direct contact with the test microorganism will be clean (i.e. residue free) and sterile. Examples of such equipment include Class A pipettes used to measure and deliver the test microorganism and volumetric flasks used to prepare growth media. Residues (e.g. antibiotic or detergent) may interfere with antibiotic potency testing. Methods of equipment cleaning and dry and heat sterilization should have appropriate validation and verification checks to ensure glassware is clean and sterile. See USP <1051> Cleaning Glass Apparatus, for additional information on additional information on glassware cleanliness.

Equipment used to provide a unit of measurement should have the appropriate

validation and frequent verification checks to ensure reliable and reproducible results; examples of such equipment include a weight scale, pH meter, autoclave, manual/automatic plate reader and UV-VIS spectrophotometer.

C. Test Organism, Inoculum Preparation and Standardization

Antibiotic potency is dependent upon antibiotic-microorganism specificity. USP <81> identifies specific test microorganisms and correlating antibiotics to be used in potency testing. The test organism must be pure and robust and further portioned into a primary and working culture. From the working culture an inoculum is prepared. Inoculum preparation and standardization is required prior to antibiotic potency testing; both are multi-step, multi-day processes and require a significant amount of time to complete. Refer to USP <81> Antibiotics- Microbial Assays, for test organism and inoculum preparation.

Preparation of the inoculum requires a basic knowledge of microbiology, aseptic technique and laboratory safety. Cross contamination may result in uncharacteristic microbial growth. Therefore, frequent monitoring during inoculum preparation is recommended. Specifically, visual inspection and basic microscopy will be used evaluate growth characteristics and morphology. Additionally, AOAC approved rapid identity testing methods such as API or VITEK should be performed. Contaminated inoculum cannot be used for antibiotic testing; likewise, all antibiotic potency data generated through the use of contaminated inoculum will be invalid.

A working inoculum stock should also be prepared to prevent possible cross contamination of the primary stock. As part of good laboratory practice, primary and working cultures should be identified with the microorganism specie name, ATCC (American Type Culture Collection) number and inoculation and expiration date. Storage requirements for primary and working inoculum stock are specified in USP <81>.

Each batch of prepared inoculum must be verified before use. Verification is a preliminary test which evaluates the purity and robustness of the inoculum. Verification also tests the bioactivity of a known median standard and five-point standard curve. The inoculum is verified if the bioactivity of the known median standard exhibits final potency between 80%-125% of the expected value and the standard curve correlation coefficient is at least 0.990. Refer to USP<81> for inoculum verification, testing parameters and acceptable data requirements and Section D, Antibiotic Standard and Sample Solution Preparation.

USP <81> identifies the microorganism and inoculum volume for each antibiotic,

except Vancomycin. In the case of Vancomycin, the analyst must perform verification on varying volumes of the inoculum until standard ranges of growth inhibition are achieved. Growth inhibition may decrease over time and inoculum volume may need to be increased during verification and/or antibiotic potency testing in order to achieve standard ranges of growth inhibition as per USP <81>.

Testing requirements include analyzing the test antibiotic in triplicate each test day for three consecutive test days. During each test day, the final calculated potency of the test antibiotic should be between 80%-125% of the known median standard. Additionally, USP suggests a standard curve correlation coefficient of 0.990 for each test day. Refer to USP<81> for testing parameters and acceptable data requirements.

D. Antibiotic Standard and Sample Solution Preparation

Antibiotic potency testing requires the use of a five-point (minimum) standard curve and test sample. Reference standards must originate from a verified source such as USP. Only Class A volumetric glassware will be used to prepare the reference standards and test sample. Procedures for reference standard preparation can be found in USP <81>. Test sample preparation, storage and expiration require the dose and administration information (as per label claim) and procedures prescribed in USP <81> and the appropriate USP antibiotic monograph. The standard curve will be analyzed (at minimum), in triplicate each test day for three consecutive test days. Refer to USP <81> for tube method test procedures.

Antibiotic potency samples will vary in physical form, dose and administration; examples of differing sample types include tablets, powders, solutions or semi-solids. Regardless of these physical and chemical attributes, the test sample and reference standard must be diluted prior to testing. The target concentration of the test sample will be equivalent to the median standard of the five-point (minimum) standard curve. Diluting the sample in this manner ensures a detection limit within the linear portion of the standard curve.

As per USP <81>, a single reference standard will be used to prepare a five-point (minimum) standard curve. The five solutions increase in concentration by a ratio of 1:1.25 (e.g. 6.40, 8.00, 10.0, 12.5 and 15.6 µg/mL). Labeling accompanying the reference standard contains preparation, storage and expiration information; this information contains specific handling instructions and should be followed in order to achieve reproducible and reliable potency test data. A new standard curve must be prepared each day of inoculum verification and sample testing.

Each dilution for the test sample and reference standard must be taken into account. The dilution factor (for each dilution) and the total dilution (for multiple dilutions) are data required to calculate potency.

Along with the standard curve, the median standard will be used to perform a direct comparison of growth inhibition during inoculum verification and sample testing. For example, a five-point standard curve containing the following reference standard concentrations, 6.40, 8.00, 10.0, 12.5 and 15.6 µg/mL, the median standard concentration is 10.0 µg/mL. To perform a direct comparison between this median standard and the test sample, the test sample must also be diluted to10.0 µg/mL; any difference in growth inhibition between the test sample and median standard demonstrates the test sample is either over, under or equivalent to label claim potency.

E. Growth Media and Additional Test Solutions

In order to culture a pure and robust test organism and perform potency testing with this organism, growth media (agar or broth), buffers and diluents are required. Components or final preparations of growth media, reagents or diluents may be prepared on-site and/or purchased from outside sources. Regardless of origin, any final product made from growth media components and/or preparations must be verified for identification, sterility and/or proper function prior to use. Refer to USP <81> for preparation, use and storage of all growth media, buffers and diluents.

F. Antibiotic Potency Testing: Plate Method

The plate method uses a solid medium (growth agar) to demonstrate antibiotic activity. This method requires the use of a five-point (minimum) standard curve, median standard, verified inoculum, stainless steel penicylinders and petri-plates containing growth agar. Growth inhibition is measured by an analyst with the use of a manual or automatic plate reader. Test antibiotic will be analyzed (at minimum), in triplicate each test day for three consecutive test days. Further, USP <81> states specific standards of growth inhibition for the plate method; if these standards are not achieved each test day for three consecutive test days, the entire test is considered invalid and all data generated will be discarded. Refer to USP <81> for plate method test procedures.

Test plates containing the media growth agar are prepared and used the same test day. Specifically, one lot of growth agar is prepared and sterilized. Prior to solidification, a portion of the growth agar is poured into the base of a petri-plate and allowed to solidify; this is the base layer of the test plate. The remaining portion of growth agar is further cooled and inoculated with the verified test

organism. This inoculated agar is thoroughly mixed, poured onto the cooled base layer and allowed to solidify.

Once the entire petri-plate solidifies, stainless steel penicylinders are applied to the agar surface in an equidistant and upright fashion. Each penicylinder is dosed with two concentrations of antibiotic, the median standard and the test sample. Further, both antibiotics are dosed in equal volume into alternating penicylinders. The plates are then incubated. As the plates incubate, the antibiotics diffuse through the two layers of inoculated and un-inoculated agar, creating a zone of clearing below and around the penicylinder; this clearing is referred to as the zone of inhibition (ZOI) and demonstrates the antibiotic activity of the median standard and test sample. Any difference in growth inhibition between the test sample and median standard demonstrates the test sample is either over, under or equivalent to label claim potency. To determine final potency, the median average of all ZOIs is required.

Refer to Section H. Potency Calculations, for additional information on data requirements and final potency calculations.

G. Antibiotic Potency Testing: Tube Method

The tube method uses a liquid medium (growth broth) to demonstrate antibiotic activity. This method requires the use of a five-point (minimum) standard curve, median standard and verified inoculum. Growth inhibition is measured by an analyst with the use of a UV-VIS spectrophotometer. Test antibiotic will be analyzed (at minimum), in triplicate each test day for three consecutive test days. Refer to USP <81> for tube method test procedures.

When a microorganism is placed into a broth containing the appropriate nutrients to support growth, the microorganism flourishes and the broth becomes turbid. Turbidity is typically a simple visual indicator of microbial growth; this growth can be quantified by a UV-VIS spectrophotometer by measuring values absorbance or transmittance exhibited by the broth.

Test tubes containing growth broth, inoculum, test sample and median standard will be prepared and read the same day. Specifically, one lot of growth broth is prepared and sterilized. A portion of this broth placed into test tubes. Test sample and media standard are added to these tubes, followed by an aliquot of verified inoculum. The test tubes are then placed into an incubator or water bath. After incubation, formaldehyde is added to each test tube to inhibit additional microbial prior to reading. The absorbance or transmittance is read ad 580 nm or 530 nm.

Refer to Section H. Potency Calculations, for additional information on data requirements and final potency calculations.

H. Calculations

To calculate final potency, growth inhibition data is entered into potency calculations listed in USP <81>. Potency calculations are specific to the plate or tube method. Calculations may be performed by commercial software. In order to achieve reliable and reproducible results, the commercial software should be validated.

Chapter 8: Bioburden Estimation for Medical Devices

According to FDA Compliance program 7382.845, *Inspections of Medical Device Manufacturers, Part IV*, - "Bioburden testing is to be performed in accordance with the guidance provided in *ISO 11737-1, Sterilization of medical devices – Microbiological methods – Part I: Estimation of population of microorganisms on products.* The methodology used for estimating the bioburden is to be validated. Twenty units are to be tested." [1]

The term "bioburden" is commonly used to describe the population of microorganisms present on unsterilized material or products. The bioburden quantity and types of bioburden organisms present can impact the sterilization process of the material or product. It is important to develop procedures which provide accurate, precise, and reproducible measurement of the bioburden population associated with the material or product. There are several approaches to remove microorganisms from a medical device. Some examples of these recovery methods include: filtration followed by plating; ultrasonics/shaking followed by filtration then placed on an agar medium; Stomaching/rinsing/flushing followed by filtration and plated on an agar medium; if all else fails perform a direct swabbing or contact plate.

The bioburden estimation of a medical device generally consists of four distinct stages:

1. Collection of microorganisms from the medical device. (See Annex A and B)
2. Enumeration of the collection sample containing recovered microorganisms.
3. Bioburden characterization.
4. Application of the correction factor(s) determined during bioburden recovery studies in order to calculate the bioburden estimate from the raw presterilization count.

It is not possible to define a single microbial collection technique because of the wide variety of materials used in health care products. Furthermore, the selection of conditions for enumeration will be influenced by the types of microbial contamination which may be anticipated.

The current method *"ANSI/AAMI/ISO 11737-1:2006/(R) 2011 sterilization of health care products – Microbiological methods – Part 1: Determination of the population of microorganisms on product"* has the latest revisions and provides a great deal of information that will guide an analyst to the method needed for a particular type of product.

Annex A contains a decision tree "that addresses designing a bioburden method based on the nature of the product being tested and includes guidance for choosing such things as agitation techniques or filtration versus direct plating."[2] Annex A also addresses the procedures (repetitive recovery method, product inoculation method) available for the validation of the method for determining bioburden.

Annex B has a comprehensive list of the different removal techniques that can be employed and alternatives for samples where removal of microorganisms by elution is not used.

Annex C has a more in-depth explanation of the validation of the repetitive recovery and product inoculation methods.

References:

1. FDA Compliance Program 7382.845 Inspections of Medical Device Manufacturers, February 2, 2011.
2. ANSI/AAMI/ISO 11737-1:2006/(R) 2011, Sterilization of health care products – Microbiological methods – Part 1: Determination of the population of microorganisms on product.
3. PDA Technical Report No. 21, Bioburden Recovery Validation. 1990

Chapter 9: Environmental Monitoring

FDA field microbiologists are requested to assist CSOs during on-site inspection of pharmaceutical manufacturers of drugs made under controlled environmental conditions. Occasionally they may be required to perform environmental monitoring (EM) sampling of those facilities to assess the microbiological bioburden of critical surfaces. This chapter describes a suggested procedure for conducting this activity. This should only serve as a guide with some modifications depending on the specific facility or special instructions from FDA management. Be sure to confirm with ORS which FDA field laboratory was designated to receive the EM samples. These procedures allow for a qualitative and quantitative assessment of environmental monitoring samples for the microbial presence in critical processing or laboratory area(s) being monitored.

A. Materials/Equipment

1. Sampling Materials

a. Sterile Dacron or cotton swab with a sterile transport media solution.
b. Alternative sampling system:
c. Sterile sponge with detachable handle
d. *Hycheck surface samplers
e. *RODAC plates (with locking lid)

**Use media containing lecithin and tween neutralizers*

f. Sterile Whirl-pak® bags.
g. Sterile water for irrigation or sterile saline held in screw cap containers.
h. Dey/Engley (D/E) neutralizing broth
i. Sterile 70% alcohol spray bottle or wipes
j. Black magic marker (permanent, fine point).
k. Digital Camera

2. Testing Equipment and Materials

a. Biological Safety Cabinet (BSC) with HEPA filtration
b. Laminar Flow Hood (LFH) with HEPA filtration
c. 10% Bleach or appropriate disinfectant/sporicide
d. Sterile 70% ethanol (ETOH) or Isopropyl Alcohol (IPA)
e. Sterile Sleeves
f. Sterile Gloves
g. Hair Net
h. Lab Coat
i. Beard Cover and/or Mask
j. Incubator set at 32.5ºC ± 2.5ºC
k. Incubator set at 22.5ºC± 2.5ºC
l. Modified Letheen Broth (MLB)
m. Modified Letheen Agar (MLA)
n. Sabouraud Dextrose Broth
o. Sabouraud Dextrose Agar
p. Malt Extract Agar w/chlorotetracycline
q. TSA w/5% Sheep Blood Agar

r. MacConkey Agar
s. RODAC plates
t. Hycheck slides
u. Soybean Casein Digest Agar (TSA)
v. Soybean Casein Digest Broth (with neutralizers)
w. Neutralizers (i.e. lecithin, tween, etc...)

B. Sampling Preparation

Don appropriate personal protective equipment (PPE) as follows:
Note: *When on an inspection, it's best to use the firms PPE when/if provided. The firm's procedures/guidelines should be adhered to when entering classified areas.*

a. Sterile Disposable Lab Coat or Gown.
b. Safety Goggles
c. Hair net, mask and/or beard cover
d. Sterile Gloves
e. Shoe Covers

1. Sampling Equipment Controls

a. Aseptic Technique Control: Place one (1) sterile Dacron or cotton swab into sterile water and place back into its sterile transport media solution. Place this negative control into a sterile Whirl-pak® bag.
Note: *There is no surface contact for this control.*

b. Swab/Sponge Sterility Control: Place an intact unused swab (or sponge) unit into a sterile Whirl-pak® bag.

c. RODAC/Hycheck Sterility Control: Place an unused RODAC plate and/or Hycheck plate within a sterile Whirl-pak® bag.

d. Whirl-pak® bag Sterility Control: Include one unopened Whirl-pak® bag as a closed control.

e. Glove Sterility Control: If the sampler uses FDA sterile gloves then have an intact unit containing gloves placed into a sterile plastic bag and sent as a control.

f. Include any other sterile equipment used during EM sampling (i.e. sterile specimen cup, sterile media, etc.).

C. EM Sampling Procedure

It is recommended that the investigative team bring equipment for both qualitative and quantitative EM methods. Qualitative methods utilizing sponges/ swabs are used for hard to reach areas. RODACs or Hychecks are employed for the quantitative method to

enumerate microbes on open flat work surfaces. See suggested sampling locations listed in section E of this procedure.

1. Disinfect gloved hand with a suitable sanitizing agent (i.e. sterile 70% alcohol).
    a. Repeat this step between each EM sample.

    b. Allow gloves to air dry so no alcohol is dripping from gloves.

    c. Some swab/sponge sampling packages include a secondary set of sterile gloves. In these instances, the secondary glove can be aseptically used on top of the primary gloves to expedite the sampling process. The secondary pair of gloves will need to be disposed of aseptically after use. If the primary gloves touch the secondary gloves outer surface, then a suitable sanitizing agent must be used on the primary gloves. Allow primary gloves to air dry after sanitizing.

    d. When sampling a Class 100 scenario. For example,
        i. Verify current certification of LFH/BSC

        ii. Allow LFH/BSC to run approximately 10 minutes before initiating sampling

        iii. Wipe down all outer sampling containers with a suitable sanitizing agent before placement in the LFH/BSC

        iv. Do not open sampling materials outside the LFH/BSC

2. Qualitative Swabbing
    a. Open a sterile swab (or sponge). Dampen with wetting agent (sterile water, saline, or D/E neutralizing broth) and squeeze off excess by pressing against the inside of the container holding the wetting agent.

    b. Apply swab (or sponge with handle) to surface (or equipment) being monitored with **firm** application pressure. Be sure to emphasize that the swab contact should be firmly pressed against the surfaces being sampled.

    c. When sampling (monitoring) flat surfaces allow the swab (or sponge with handle) to firmly rub an area of approximately 24 to 30 cm^2.

    d. Apply the swab (or sponge) within this contact area in both a horizontal and vertical direction for approximately 10 seconds (count it out).

    e. Replace the swab (or sponge) back into the carrier container (if it came with one) and place into the sterile Whirl-pak® bag. (Be sure to break off the handle portion of the sponge applicator stick.)

3. Quantitative RODAC/Hycheck Sampling

a. Carefully remove the lid of RODAC plate or loosen the cap on the Hycheck slide tube. **Take care not to touch the agar surface**.

Note: *Examine agar for contamination and or dehydration*

b. Gently but firmly touch the RODAC agar surface against the area being sampled, exert moderate, even, vertical pressure and then carefully replace lid. Avoid using rubbing motions with the plate at the sample site as this may break the agar.

c. When using Hycheck press down on the spike to bend the paddle at the hinge line gently lowering the slide and press the agar to the surface with firm and even pressure. Repeat this step using the 2nd agar surface on an area adjacent to the initial test site. Replace slide in container and close tightly.

4. Place the EM sample into a sterile Whirl-pak® bag and identify the bag immediately after.

5. Assign a consecutive number to the sample (i.e.1, 2, 3, etc.), in addition include the date, location of sample site (be specific) and your initials. Record in your inspectional record book the swab number and the location of the swab site.

6. Later, place all the Whirl-pak® bags in an officially sealed plastic or brown paper bag. **Do not freeze or refrigerate**. Freezing can kill the microbes and the cold can induce a shock to vegetative cells. These microbes were surviving at room temperature (ambient). Therefore, an ambient temperature should be maintained during transportation.

7. Place into a suitable mailing container to prevent crushing or physical damage to the swabs. The container should have some insulation capacity to prevent extreme temperature (freezing or excessive heat).

8. Ensure contact with the receiving laboratory in advance regarding pending samples in order for them to have appropriate personnel and materials for sample set up within 48 hours.

D. Recommended Environmental Monitoring Sites

When on an inspection, do not allow the firm to disinfect the work area prior to sampling. The facility and the equipment should be sampled during a ready-to-use state as determined by the firm. The presence of disinfectant on the swab may reduce the microbial bioburden or increase inhibition during broth incubation. When collecting EM samples start in locations that are under the greatest control (ISO 5- HEPA filtered LFH/BSC or Isolator) and sample to lesser controlled areas (areas outside the work station but still within the room).

1. Swab the frequently utilized surfaces within the controlled work station such as:

a. Center of work surface
b. Fingertips & sleeves of Isolator gloves
c. Storage bins inside work station
d. Shelving inside work station or any other stationary items
e. Equipment control panels including on/off switches of LFH/BSC
f. Flexible plastic curtains used to separate multiple workstations

2. Swab corner crevices inside the HEPA Filtered work station.

3. Swab the handle, squeeze trigger and nozzle of any bottle kept in the clean room or work station used for spraying (i.e., 70% alcohol, disinfectant solutions, etc.).

4. Swab the underside of the chair in front of the work station. Specifically, on the front bottom rim where personnel would hold to pull up the chair.

5. Swab tables or benches within the controlled room where product container(s) or post sterilized product may be held outside of the HEPA filtered workstation.

6. Swab the air in-take grid on each of the HEPA filtered work stations. Usually located on top of the unit holding the course filters.

7. Swab the exhaust (return) grid for the room air handling system that is connected to the facility air supply where the product manufacturing or compounding occurs.

8. Swab the light switch and door knob or handles leading into and out of the clean room.

9. Swab any cardboard boxes, handles of plastic containers, tools (crimpers) or scissors, key pads on weighing scales or computers kept in the cleanrooms.

10. Swab the exterior cuffs of the used lab coats worn by personnel during manufacturing or compounding. They may be hanging in the entry (ante) gowning room.

11. Swab the bottom horizontal window sill within the clean room.

12. Swab any area under open or dislodged ceiling panels.

13. Sample areas of discoloration, stains or water and oil droplets.

14. Use your discretion to swab any other high risk surface locations.

15. Photograph surfaces or equipment that display gross signs of contamination (i.e., particulate matter, fungi, discoloration, etc).Try to include a distant picture of the targeted area along with a focused close up. Be sure to EM sample this location, as well.

E. Analysis Preparation conducted by FDA field laboratory

1. All processing of swabs must be aseptically performed within a HEPA Filtered Class II Biological Safety Cabinet (BSC) or HEPA Filtered Laminar Flow Hood (LFH). *It is not necessary to analyze environmental monitoring samples in a clean room or isolator.*

2. All surfaces within the BSC or LFH must be thoroughly disinfected with appropriate disinfectant (i.e. 10% bleach, etc.) followed by filter sterilized 70% ethanol or IPA prior to placing swabs under the hood and beginning analysis.

3. In order to assure that the BSC/LFH and media are free of microbial contamination, standard open and closed controls used for sterility testing should be performed concurrently with analysis.

4. Don appropriate PPE as follows:

    a. Freshly laundered lab coat
    b. Sterile disposable sleeves
    c. Hair net
    d. Sterile gloves
    e. Beard Cover and/or Mask

5. Sterile gloves must be decontaminated between the processing of each individual swab. Sterile gloves and sleeves can be discarded and replaced as needed.

6. Sample Preparation

    a. Examine swab containers for closure integrity to ensure tampering, leakage, or potential cross contamination has not occurred.

    b. Carefully disinfect exterior of each swab container and place into the sanitized BSC/LFH and allow to air dry.

7. Media Selection

    a. Neutralizing additives (i.e. Tween/Polysorbate, Lecithin, etc.) are utilized to neutralize inhibitory disinfectant residues transferred to the swab during sampling that might inhibit microbial growth.

    b. For a broad spectrum recovery of microorganisms, one should utilize a nutrient rich general purpose media containing neutralizers (i.e. MLB, MLA, etc.).

    c. When targeting fungal populations only, it is necessary to use an appropriate fungal media such as Sabouraud Dextrose or Malt Extract media. It is beneficial to use an antibiotic (i.e. Chlortetracycline) which will help to selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing molds.

d. TSA w/5% Sheep Blood Agar is beneficial for cultivating fastidious microorganisms.

e. MacConkey Agar is used for the isolation and differentiation of gram negative and enteric organisms.

f. RODAC plates and Hycheck slides are used for the detection and quantification of microbiological contamination.

F. Analytical Procedure

1. Approximately 100 ml of sterile media (i.e., SDB, MLB, etc.) should be aseptically added to each plastic bag containing a square sponge swab. Mix or swirl thoroughly.

2. Approximately 10 ml of sterile media (i.e., SDB, MLB, etc.) should be aseptically added to each tube containing a swab. Mix or swirl thoroughly.

3. All swabs are incubated at 25ºC- 30ºC for at least 14 days to allow for the resuscitation of potentially stressed microbes.

4. Hycheck slides and RODAC plates should be directly incubated at 20ºC- 35ºC for at least 5-7 days. Longer incubation times may be required when contaminants are suspected to be slow growing. Check plates daily for colony formation to minimize obscuring visualization of smaller colonies by over growth.

    a. Count and record the number of colony forming units for RODAC plates. All colony types should be picked and re-streaked for purity and subsequently identified.

    b. Count the number of colonies on both sides of the paddle for the Hycheck slide. Report the colony counts for each side of the paddle and all colony types should be picked and re-streaked for purity and subsequently identified.

5. Check all swabs daily for turbidity and subculture for isolation as turbidity is observed. All sub culturing must be performed under LFH or BSC.

6. Subculture all turbid swabs onto a combination of non-selective media (i.e. MLA, etc.) and selective/differential media (i.e. MacConkey agar, MEA, etc.). *It is recommended to include TSA w/5% Sheep Blood Agar as one of the differential media for subculturing.*

    a. Fungal media should be incubated at 20º to 25º for 5 to 7 days. Possibly longer but no more than 14 days

    b. All other cultural media should be incubated at 30º to 35º for 2 to 3 days.

7. Re-incubate all cultured swabs until the full incubation (14 days) timeframes are met.

8. Culture all submitted negative controls & diluents in order to confirm that the equipment or any aseptic techniques were not compromised.

9. Once isolation of the organism is achieved, perform microbial characterization and identification following USP <1113> Microbial characterization, Identification and Strain Typing, as guidance. Typically, rapid identification systems (i.e. VITEK) are employed after primary screening and characterization are performed. Other identification platforms may be beneficial if acceptable identification is not obtained through biochemical testing.

Chapter 10: Rapid Screening Methods

A. Screening Protocol for Direct Staining on Products with Appearance of Visible Contamination

1. Purpose

   To standardize and implement a protocol that can rapidly identify microbial presence in drugs intended to be sterile through direct microscopic staining.

2. Scope/Policy

   Any sample received with visible, potentially microbiological matter in a sterile drug must be given the highest priority for testing, due to the importance of quickly ascertaining whether the product poses an infection risk to patients. This procedure requires all ORA laboratories involved in microbiological testing of sterile pharmaceutical products to conduct microscopic screening of samples that appear to have visible contamination.

3. Responsibilities

   ORA Microbiology Laboratories that routinely test pharmaceutical products using the USP compendia methods must adopt a rapid screening practice to visually examine sterile pharmaceutical products that appear to have macroscopic contamination by using a direct microscopic staining protocol to determine if microbial contamination is present. It is the responsibility of the analyst to note any modifications to this procedure in the worksheet.

4. Background

   FDA analysis of marketed drugs purporting to be sterile and containing visible foreign matter has revealed microbial contamination, which has been linked in some cases to outbreaks of patient infections and fatal outcomes. Because microbiologically contaminated products pose a grave health hazard to patients and the consequences of exposure to contaminated product can be mitigated once healthcare providers and their patients are alerted, it is important for FDA to obtain analytical information as early as possible when microbial contamination is suspected.

   This requires the FDA laboratory to undertake special measures upon receipt of a sample with visible, potentially microbiological contamination. Accordingly, CDER and ORA have met and agreed on recommendations for the immediate conduct of a microscopic screening test, under specified circumstances, to rapidly identify microbiological contamination in products purporting to be sterile." The objective of this protocol is institute rapid microscopic screening in ORA laboratories to expedite provision of the analytical findings to ORS and CDER.

5. Procedure

   a. Before Sample is transferred to Class 100 Clean room or Sterility Test Isolator

      i. Remove and count the number of product units from the sample collection package bag and examine each unit to confirm the

number of units and labeling accuracy (most importantly the product lot numbers) with the collection report.

ii. Examine each unit (vial, ampoule, pre-filled syringe, IV bag, etc.) for the integrity of the container/closure system, and record in lab worksheets whether units are intact. There should be no product leakage, cracks, existing puncture in the rubber septum or damage to the unit container that may have allowed microbial ingress during storage and shipping. Include a description of any units that are defective or damaged and set them aside. However, set aside any open samples in case there is potential interest in additional analysis of these units. Seek management advice with regard to whether any subsequent analysis should be performed. If contamination of the product is observed, for example color of the solution, turbidity, pellicle, sediment, these too should be noted in your worksheets under “Product Description.”

iii. During step 2, the analyst(s) should wear sterile gloves and use sterile 70% alcohol and sterile lint free wipes to disinfect the exterior of the unit to assure that there is no exterior debris obstructing the view of the product.

iv. Using appropriate lighting, carefully inspect each unit against both a black and white background. Allow the container to stand for several minutes to allow diffuse bubbles to disperse and dissipate out of solution. Shaking of the solution during shipment and handling can cause bubbles to appear, making it difficult to screen for particulate matter and microbiological contamination.

v. Some obvious signs of microbial contamination would be: (a) general turbidity; (b) stringy or thread-like fibers;(c) granular or particulate clumps floating within the solution or settling to the bottom; (d) an oil-like layer or pellet on top of the solution; (e) a film on the interior glass that appears to lift off upon swirling the container in a gentle manner; or any other anomaly that is beyond the normal appearance of the product (e.g., crystals, unusual color tint, etc.).

vi. Set aside those units that contain the potential visible contaminants noted in step 5 and have their appearance confirmed by a second analyst or a laboratory supervisor. Photograph the unit(s) with a digital camera is the best way to observe and document the contamination. Use a ruler (or another appropriate object) for scale and be sure to follow the laboratory procedures manual for documenting this as evidence.

vii. Consult with a supervisor or subject matter expert on how many of these units to examine with the sterility test and concurrent staining procedure. Also, consult with the Center on testing needs whenever there is a potential public health hazard posed by the product.

b. Samples moved into the Class 100 Clean room or Sterility Test Isolator

i. Follow the standard disinfection procedure for transferring the suspect unit(s) into the bioclean room/sterility test glovebox used for sterility testing.

ii. When using a sterility test isolator, ensure it has been exposed to a decontamination cycle and that any materials or supplies are disinfected and enter the isolator through transfer ports.

iii. Immediately after conducting the USP Sterility test, remove an aliquot of the visually contaminated product(s) and place onto a sterile glass slide and place in a sterile container within the HEPA filtered laminar flow hood/glovebox/sterility test isolator. Make every effort to capture the suspected foreign matter for microscopic examination and characterization.

iv. Remove the sterile container containing the glass slide to outside of clean room/glovebox/sterility test isolator and fix the smear to the glass slide using the standard procedure. Perform a stain (i.e., simple stain using crystal violet, or other stain), record the results and report your finding to your supervisor. Detailed description should be provided regarding cell morphology, and other notable features (e.g., presence of fungal hyphae, presence and location of spores in cell, chain-like cocci). If possible, photograph the images from the slide with a digital camera. If microbial contamination appears to be present, communicate the findings to CDER and ORA/ORS without delay. State clearly if the findings are still preliminary. Tabulate the results in a standard table or spreadsheet (see Attachment B) and forward the results to the appropriate contacts in ORS and CDER (OCTEC and/or Office of Compliance).

v. Continue with the Sterility test as outlined in the USP and FDA standard procedures. Observe daily, and report any growth in the media as soon as possible to ORS and CDER.

6. Attachments


C:\Documents and Settings\marilyn.khan


Medical Product Adverse Event Tracki

Chapter 11: Inspectional Guidance

A. Microbiological Issues for Inspection of Pharmaceutical Laboratories

1. Finished product testing using USP or Non-compendia method

   Sterility, Bacterial endotoxin, Microbial limits test: specified microorganisms and enumeration, Antimicrobial- effectiveness test, Bioburden determination, water quality control testing; review all original test results

2. Method Suitability (Sterility), Preparatory test (Bacterial endotoxin), Validation of method used for bioburden and water analysis

3. Reagents and medium- proper storage, expiration date activity, and growth promotion

4. Equipment and Instrument- (Steritest, manifold, Automated/Molecular identification system, Vitek, etc) isolator & bio-decontamination system calibration, maintenance, validation- IQ, OQ, PQ

5. Sterility testing area design, operational procedures, monitoring, aseptic technique, gowning procedures, proper sample container disinfection, surface/air monitoring, HEPA filter certification, etc

6. Method description, modifications, and verification along with recording of sample results and appropriate review and evaluation by management

7. Qualifications, training and identification of the personnel conducting each step of the analysis

8. Qualification and training of management to critically review data and interpret its significance (Risk Assessment)

9. Microbial standards set for raw material, finished product, water bioburden, and EM for analytical areas

10. Integrity and accuracy of the laboratory information management system (LIMS) for microbiology data entry, review and approval Selection, handling, and storage of Biological Indicators (BIs)

11. Private (contract) testing laboratory quality agreements, data review, and associated problems; Have there been any changes in contract labs and why?

12. Proper use and control for In-vitro diagnostic test kits, Positive and negative controls, Interpretation and reliability of results

13. Risk assessment of microbiological results for non-sterile products

14. Request a list of the entire laboratory's Microbiological Data Deviations (OOS/OOL results) and Corrective Action Preventative Actions (CAPA) (since last FDA on-site inspection)

15. Stability Testing – sample storage conditions, missed sampling dates, etc.

B. Microbiological Issues for Inspection of Pharmaceutical Manufacturing Facilities

1. Product Sterilization or bioburden reduction stage and validation- Aseptic/Filtration, steam, ETO, radiation, and other chemical processes

2. Depyrogenation- dry heat ovens for glass containers, Wash/rinse for stoppers, adequacy of validation using spiked endotoxin, Recovery studies before depryrogenation, Filtration and column applications

3. Environmental monitoring- Types of equipment, calibration, operation and maintenance; Surface, Air, Personnel and Water; Critical work areas for aseptically filled products (class 100, isolators, etc); Process simulation (media fills) studies for process validation, growth promotion, reading turbidity, volume adequacy, surface contact, surface sanitizer neutralizing media (e.g., TSA w/ Lecithin & Polysorbate 80), sampling technique (observe), sample must represent dynamic/operational conditions, trending/CAPA, etc.

4. Disinfection and sanitization- agents used (sporicidal?), preparation problems, (over dilution); Applicator (i.e., mop, spray. Aerosol), time of exposure, areas of contact, supervision; residues, UV lights, water systems, filling equipment, work surfaces, process columns, Verification and validation

5. Room design and Equipment- accessibility for disinfection and cleaning; 'aseptic filling critical area; HEPA filter certification and maintenance , air flow patterns/smoke studies, change evaluation/re-certification (rearranging cleanroom, adding equipment, HVAC, etc.), test during dynamic/operational conditions with maximum number of personnel in place, personnel equipment traffic, room differential pressure and temperature; adequacy of primary and secondary barriers

6. Water Purification and delivery system: Vulnerability of Distillation, RO, Deionizers, cartridge filters, etc; UV lights, dead legs, biofilm; corrosion (heat exchangers); Water borne microorganisms (nanobacteria) and endotoxin production; Cold system problem, disinfection problems

7. Personnel- training procedures for aseptic technique, gowning procedures, Cleaning and maintenance personnel training for Class 100 room entry; Glove and garment monitoring and procedures

8. Product Sampling: representation of lot required minimum values per USP <71>: quantity per container & units per batch, sample storage (time and temp), sampling

port sanitization or sterilization problems; problem with skip lot testing on raw material.

9. Maintenance records- determine dates, and location of equipment failure or out-of-service that may have an impact on microbial ingress; looks for signs of roof leaks and water stains on ceiling panels, the degree of dirt and dust accumulation on supply and exhaust vents; Ask about new construction, plumbing or air handling system and the reason for change.

10. Compressed Air Systems—sterile process air, microbial particulate filtration (0.2 µm, hydrophobic), condensate causing blockage & microbial growth, routine point-of-use sampling, maintenance, filter integrity test

C. Sample Data Review – All Negative Results

What to review when all the firm's Sterility and/or Microbial Limits test results indicate no microbial growth and you need to know whether this is true:

1. Medium- Growth Promotion; pH; Low agar or broth volume in container, Incubator temp not set correctly; improper medium storage after QC (crystals from freezing, inadequate mixing prior to dispensing, agar plates dried out during incubation, etc.)

2. Method Suitability testing- Validation for Sterility; Preparatory testing (BET). May need added neutralizers, product dilution, filtration; water chemical contaminant with toxin effects in buffers, Not following method (excess of product added to broth during test but not during suitability testing, etc)

3. Improper tube or agar plate examination- check filter surface on submerged filter membrane (mold budding); surface film, light hazy growth in Thio broth, microbes settle to bottom of tube, pinpoint colonies (microaerophilic); medium not inoculated. Disinfection process adds antimicrobial residue onto/into product during sample preparation; Gas used for Isolator sterilization with medium inside chamber may penetrate into liquid broth and/or test product packaging

4. Fastidious microorganisms found in the product bioburden may require special additives to the medium- Halophilic contaminants in bicarbonate, or high salt products need medium supplements with essential salts for survival

5. Water test method- membrane filter with a 0.45 micron pore size may miss quantities of water-borne organisms. Suitability testing necessary. Note: USP <71> only requires "pore size not greater than 0.45µm".

6. Inadequate incubation time (14 days)/temp (USP required temps)

7. Possible falsification or incorrect entry onto worksheets or Laboratory LIM system. Compare LIMs database entries to the analyst's laboratory notebook; Phrase the question "When you get a positive test result…." not "If you get a positive test

result...” Inspect the laboratory refrigerator or freezer for evidence of stored sample isolates. If they lyophilize sample isolates ask to review the spread sheet data storage directly from the computer screen; hard copies could be obtained later. Review the Vitek or Micro Id isolate log book for all microorganisms identified and work backwards to the product lot number, filling rooms, equipment used, components or raw material used for that lot. This may allow you to find other lots associated with the contaminated lot.

8. Personnel- review training records and personnel qualifications and experience Observe analysts during sample collection, preparation, etc., to look for errors which may inhibit microbial recovery.

9. Visit the microbiology laboratory and look into the refrigerators, incubators, discarded plates from that day’s work or request speciation log book and determine if microbial recovery has occurred, but not recorded on official worksheets or entered into LIMs.

D. Sample Data Review – Microbial Growth Indicated

When you encounter inspectional evidence that the firm has manufactured microbiologically contaminated product, the few suggestions listed below should help you evaluate and proceed with this information.

1. Documentation- Review and obtain copies of all records for lots indicating contamination; determine if there are other lots manufactured either before or after the “bad” lot(s); Review all associated activity and equipment related to the contaminated lot. There may be common water, mixing tanks. Piping, raw material, sterilizers, filters, etc that may have been cross contaminated and transferring microbes to subsequent lots of products.

2. Review current established validation studies for product/component sterilization (disinfection for non-sterile products); has there been any equipment changed or modified; has there been a change of personnel or training; any new source material for equipment (vent filters, gaskets, filter manufacturer, etc.); any processing changes or room modifications, construction elsewhere in the facilities, etc.

3. Review of Environmental monitoring (EM) procedures and results for manufacturing and laboratory area- Would the product contaminant grow on the EM medium; was the product contamination found in the manufacturing area; Growth promotion potential of contaminant in other medium (i.e., TSB and Thio)

4. Speciation- Record and copy the method of identification (i.e. API, Vitek, etc); Determine if there were possible secondary contaminants that were not identified or recorded (check original plates, or isolates); verify accuracy into the LIM system.

5. Determine potential source- Staphylococcus (skin, insect, etc); Pseudomonas (water, plants, etc) ; yeast and mold (spores) (environmental)

6. Review investigation report- Source of contamination; Corrective action; repeat testing and release; does it include related lots and ancillary systems? Was the product rejected or released? If released ask why? Evaluate justification.

E. How to Investigate a Microbiological OOS test result(s)

1. When a firm has an end product test result that indicates a failure (USP test failure, OOS, etc) the inevitable question is –Were the results laboratory error or a true process contamination? Below are a few starter questions to help in your FDA review of the firm's investigative report.

2. During the subsequent investigation, there are two areas for the review to focus: the manufacturing site and the laboratory that determined the OOS result. The following review questions suggest possible variables that may impact the final conclusions. The investigations may run concurrently between manufacturing and the laboratory. For ease of review I listed my questions first with manufacturing and secondly with laboratory data. I divided the manufacturing review into aseptic manufacturing (High risk) and terminally sterilized products (low risk). Part two covers those questions that I would ask for a critical review of the microbiological data accumulated for the Sterility failures, Microbial limits failures, etc.

F. Laboratory Facility and Analytical Review

1. Review QC records for proper/validated sterilization of all equipment and media used during the sterility test method: manifold/ steritest; rinse fluid, culture media, canister kits, etc.

2. Review the EM data acquired during sterility testing (i.e., settling plates, RODAC), simulation system controls, etc. What are the microbial species and its determined normal habitat (i.e., water, plants, people, etc?)

3. Review training records and qualification of analysts performing the test; interview and/or observe analysts

4. Review the qualification of the bio-clean room facilities or isolator chamber used during testing. Are there any leaks in the gloves, improper sanitization of product container before placement into work station or isolator? Has the isolator been evaluated for leaks?

5. Review cleaning and sterilization requirements for reusable glassware and equipment. Poorly cleaned glassware will make sterilization of equipment more difficult and possibly shelter trapped microbes from the killing effect of the sterilant.

6. Review laboratory areas used for sub-culturing the sterility test medium onto enrichment plates. Cluttered work space or un-sanitized surfaces may cause plate contamination.

7. Check the original plates used for isolation for possible pre-existing contamination (i.e. growth in non-streaked locations on the agar surface, subsurface growth)

8. Check to see if the medium had been recalled or has had past problems with contamination during manufacturing.

9. It may be necessary to perform a genotype identification on the two isolates (product source and manufacturing area isolate) if they are the same species.

G. Manufacturing Facility Review

1. Aseptically filled pharmaceuticals

Check environmental monitoring (EM) data taken from production areas and the testing environment (i.e., S-T-A, settling plates, RODAC, etc) for microbial contamination that matches the microbe isolated from the finished product sterility test

If no microorganisms are detected, check the adequacy of the EM method used during manufacturing for proper sensitivity and applicability, for example

a. Are they using proper medium (i.e. non-selective medium)?

b. Have they performed growth promotion?

c. Did they use appropriate incubation time and temperatures?

d. Are they sampling in the appropriate room locations, during dynamic conditions, longest time between cleanings/sanitation and at frequency to assure reliability of the results?

e. If they recovered an anaerobic bacterium from the sterility test (Thioglycollate broth) do they perform EM for anaerobic bacteria?

Have they performed a filter integrity test on the membrane use for the product sterilization? Review the products pre-filtration bioburden levels to assure that the concentration of bacteria in the bulk has not exceeded the membrane filtration capacity that was determined in their validation studies. Have they changed the source or model for the membrane filter cartridge used in the process?

Has the firm manipulated or excluded some of the data used in the final QC report? Perhaps raw data was averaged to bring the bioburden count below the alert or action levels. It can be helpful to request electronic Excel sheet version of data, to allow sorting (by frequency of organism, location, etc.) and trend analysis; hard copies can be requested later, if necessary.

Review the media simulation studies. Did the microbial species recovered in past simulation studies match the microbe(s) recovered from the current product test failure?

Has there been a change or breach in the personnel barrier system to protect the product? Were there any interventions by maintenance or other staff personal during the manufacturing of the contaminated lots? Review glove/uniform monitoring results.

Review the Antimicrobial Effectiveness challenge studies for the product. Where there any changes to the container/closure component source or requirements?

Were there any changes to the disinfection procedure, reagents use, new personnel, application, equipment (mops, aerosols, etc.) etc.?

2. Terminally sterilized drug product

Check autoclave validation studies for sterilization process- cold spot, heat penetration (challenged inside dry tubing, connectors/caps/stoppers, largest liquid volume, etc.), changes in chamber load configuration, etc.)

Check maintenance records for house steam, records for autoclave repair, new plumbing

Check Biological Indicator (BI) information- improper storage of BIs; changes in the culture (inoculum level and/or BI organism species) and incubation parameters

Evaluate the heat resistance characteristics of the microbial isolate found in the product during Sterility testing and determine if it can survive during the process condition , review product container/closure integrity data and possible recent supply source changes to vials or rubber stoppers; Check possible post sterilization package integrity problem- mostly medical device issue.

H. Inspectional Elements listed in the six (6) Inspectional Systems covered by the CP 7356.002 that cover ONLY Microbiological Issues

Using the Inspectional criteria described in the FDA Compliance Program Guidance Manual Program 7356.002, I selected only key coverage elements listed in five of the six (6) Inspectional Systems that relate to **microbiological** issues and in some cases I included an example for clarification.(Labeling system not included)

1. QUALITY SYSTEM

-Discrepancy and failure investigations related to manufacturing and testing: documented; evaluated; investigated in a timely manner; includes corrective action where appropriate.

- Validation: status of required validation/revalidation (e.g., computer, manufacturing process, laboratory methods).

- Training/qualification of employees in quality control unit functions.

2. FACILITIES AND EQUIPMENT SYSTEM

a. Facilities

- Cleaning and maintenance

- Facility layout and air handling systems for prevention of cross-contamination (e.g. penicillin, beta-lactams, steroids, hormones, cytotoxics, etc.)

- Specifically designed areas for the manufacturing operations performed by the firm to prevent contamination or mix-ups

- General air handling systems

- Lighting, potable water, washing and toilet facilities, sewage and refuse disposal

- Sanitation of the building, use of rodenticides, fungicides, insecticides, cleaning and sanitizing agents

b. Equipment

- Adequacy of equipment design, size, and location

- Equipment surfaces should not be reactive, additive, or absorptive

- Appropriate use of equipment operations substances, (lubricants, coolants, refrigerants, etc.) contacting products/containers/etc.

- Cleaning procedures and cleaning validation

- Controls to prevent contamination, particularly with any pesticides or any other toxic materials, or other drug or non-drug chemicals

- Qualification, calibration and maintenance of storage equipment, such as refrigerators and freezers for ensuring that standards, raw materials, reagents, etc. are stored at the proper temperatures

3. MATERIALS SYSTEM

-Representative samples collected, tested or examined using appropriate means

- Testing or validation of supplier's test results for components, containers and closures

- Rejection of any component, container, closure not meeting acceptance requirements. Investigate fully the firm's procedures for verification of the source of components.

- Appropriate retesting/reexamination of components, containers, closures

- Water and process gas supply, design, maintenance, validation and operation

- Containers and closures should not be additive, reactive, or absorptive to the drug product

- Documented investigation into any unexpected discrepancy

4. PRODUCTION SYSTEM

Training/qualification of personnel

- Validation and verification of cleaning/sterilization/ depryrogenation of containers and closures

- Established time limits for completion of phases of production (i.e. microbial growth potential of product)

- Implementation and documentation of in-process controls, tests, and examinations (e.g., bioburden determination pH, adequacy of mix,)

- Justification and consistency of in-process specifications and drug product final specifications

- Prevention of objectionable microorganisms in non-sterile drug products

- Equipment cleaning and use logs

- Process validation, including validation and security of computerized or automated processes (i.e. simulation studies)

- documented investigation into any unexpected discrepancy

5. LABORATORY CONTROL SYSTEM

Training/qualification of personnel

- Adequacy of staffing for laboratory operations

- Adequacy of equipment and facility for intended use

- Calibration and maintenance programs for analytical instruments and equipment

- Validation and security of computerized or automated processes

- Reference standards; source, purity and assay, and tests to establish equivalency to current official reference standards as appropriate

- System suitability checks on chromatographic systems (e.g., GC or HPLC)

- Specifications, standards, and representative sampling plans

- Adherence to the written methods of analysis

- Validation/verification of analytical methods

- Control system for implementing changes in laboratory operations

- Required testing is performed on the correct samples

- Documented investigation into any unexpected discrepancy

- Complete analytical records from all tests and summaries of results

- Quality and retention of raw data (e.g., chromatograms and spectra)

- Correlation of result summaries to raw data; presence of unused data

- Adherence to an adequate Out of Specification (OOS) procedure which includes timely completion of the investigation

- Adequate reserve samples; documentation of reserve sample examination

- Stability testing program, including demonstration of stability indicating capability of the test methods (i.e. container/closure, AET)

SAMPLING

Samples of defective product constitute persuasive evidence that significant CGMP problems exist. Physical samples may be an integral part of a CGMP inspection where control deficiencies are observed. Physical samples should be correlated with observed control deficiencies. Consider consulting your servicing laboratory for guidance on quantity and type of samples (in-process or finished) to be collected. Documentary samples may be submitted when the documentation illustrates the deficiencies better than a physical sample. Districts may elect to collect, but not analyze, physical samples, or to collect documentary samples to document CGMP deficiencies. Physical sample analysis is not necessary to document CGMP deficiencies.

When a large number of products have been produced under deficient controls, collect physical and/or documentary samples of products which have the greatest therapeutic significance, narrow range of toxicity, or low dosage strength. Include samples of products of minimal therapeutic significance only when they illustrate highly significant.

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DOCUMENT HISTORY/CHANGE HISTORY:

Version 1.1, 10/06/14:

ii.- updated
Chapter 3. A. 2. a. iv. – revised
Chapter 8. D. 1st paragraph – revised
Chapter 10. H. 5. – author information updated

Version 1.2, 03/20/15

Chapter 3, D. 1. a. - revised
Chapter 7 – Antibiotic Potency Assay (inserted)

Appendix A: Literature and Resources

A Comprehensive List of Only Microbiological Regulatory and Scientific Literature Resources

The scope of this reading material will ONLY include microbiological scientific and regulatory publications or websites for conventional drugs, biologics and combinatorial products. Some references to medical device regulations will be included if relevant during an FDA investigation that covers microbiology.

I. Legal requirements and regulations-

CFR 210 & 211 “cGMPs for finished Pharmaceuticals”
(http://www.fda.gov/cder/dmpq/cgmpregs.htm)

CFR 210 & 211 amended effective Dec, 2008 (several changes that include microbiological requirements of aseptically filled products)
(http://frwebgate6.access.gpo.gov/cgi-bin/TEXTgate.cgi?WAISdocID=336828190639+0+1+0&WAISaction=retrieve)

CFR 610 General Biological Product standards” (Not covered during this review)
(http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=610)

CFR 820 “Quality Systems Regulation (Devices, not covered)
(http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=820)

CFR 314.81(b)(3)(ii)- Applications for FDA approval to market a new drug (revised April 1, 2008)
For submission of an alternate microbiological method with a comparability study
(http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm)

CFR 1271 Human cells, tissues, and cellular and tissue-based products
(http://www.fda.gov/BiologicsBloodVaccines/TissueTissueProducts/default.htm)

II. FDA Compliance program Guidance Manual for FDA Staff: Drug Manufacturing Inspections program 7356.002

(http://www.fda.gov/cder/dmpq/compliance_guide.htm)

(http://www.fda.gov/ora/cpgm/default.htm)

During an inspection this program designated six (6) critical systems for review. They include: Quality System (always covered during an FDA inspection); Facilities &Equipment; Material; Production; Packaging and labeling; and Laboratory control systems.

For ease of review, I included the Inspectional section for the

LABORATORY CONTROL SYSTEM

For each of the following, the firm should have written and approved procedures and documentation. The firm's adherence to written procedures should be verified through observation whenever possible. These areas are not limited only to finished products, but may also incorporate components and in-process materials. These areas may indicate deficiencies not only in this system but also in other systems that would warrant expansion of coverage. When this system is selected for coverage in addition to the Quality System, all areas listed below should be covered; however, the depth of coverage may vary depending upon inspectional findings.

- Training/qualification of personnel

- Adequacy of staffing for laboratory operations

- Adequacy of equipment and facility for intended use

- Calibration and maintenance programs for analytical instruments and equipment

- Validation and security of computerized or automated processes

- Reference standards; source, purity and assay, and tests to establish equivalency to current official reference standards as appropriate

- System suitability checks on USP compendial tests and/or Microbial Id. system

- Specifications, standards, and representative sampling plans

- Adherence to the written methods of analysis

- Validation/verification of analytical methods

- Control system for implementing changes in laboratory operations

- Required testing is performed on the correct samples

- Documented investigation into any unexpected discrepancy

- Complete analytical records from all tests and summaries of results

- Quality and retention of raw data (e.g., microbial identification printout)

- Correlation of result summaries to raw data; presence of unused data

- Adherence to an adequate Out of Specification (OOS) procedure which includes timely completion of the investigation

- Adequate reserve samples; documentation of reserve sample examination

- Stability testing program, including demonstration of stability indicating capability of the test methods

FDA Compliance program Guidance Manual for FDA Staff: Sterile Drug Process Inspections 7356.002A

http://www.fda.gov/downloads/ICECI/ComplianceManuals/CompliancePrograMManual/UCM125409.pdf

Read sections entitled “Inspectional” and “Analytical” and “Attachment A” Extremely helpful.

III. Compliance Policy Guide

Sec. 100.550- Status and Responsibilities of Contract Sterilizers Engaged in the Sterilization of Drugs and Devices (CPG 7150.16)(Oct 2006)
(http://www.fda.gov/ora/compliance_ref/cpg/cpggenl/cpg100-550.html)

Sec. 490.100 Process Validation Requirements for Drug Products and Active Pharmaceutical Ingredients Subject to Pre-Market Approval (CPG 7132c.08) (3/2004)
(http://www.fda.gov/ora/compliance_ref/cpg/cpgdrg/cpg490-100.html)

Manual of Policies and Procedures, CDER, MAPP 5040.1

Product Quality Microbiology Information in the Common Technical Document - Quality (CTD-Q)

(http://www.fda.gov/cder/mapp/5040.1.pdf)

Compliance Policy Guidance for FDA Staff- Sec. 280.110 Microbiological Control Requirements in Licensed Anti-Human Globulin and Blood Grouping Reagents

(http://www.fda.gov/ora/compliance_ref/cpg/cpgbio/cpg280-110.html)

IV. US Pharmacopeia (USP) Compendium

Review all relevant product monographs (not all have microbiological requirements); the following are regulatory chapter that contain enforceable microbiology requirements -

(http://inside.fda.gov/Library/ElectronicResourcesWebLERN/Alphabeticallist/index.htm) FDA access to the USP is available through this link

General Notices and Requirements (page 1-13), Chart 10- Microbiology

<1> Injections

<51> Antimicrobial Effectiveness test

<55> Biological Indicators-Resistance Performance tests

<61> Microbiological Examination of Nonsterile products: Microbial enumeration tests

<62> Microbiological Examination of Nonsterile products: Tests for Specified Microorganisms

<63> Mycoplasma

<71> Sterility Tests

<81> Antibiotics-Microbial Assays

<85> Bacterial Endotoxins Test

<151> Pyrogen Test

<161> Transfusion and Infusion Assemblies and Similar Medical Devices

<171> Vitamin B12 Activities Assay

<797> Pharmaceutical Compounding-Sterile Preparations

Dietary Supplements General Chapters Information-

<2021> Microbial Enumeration Test-Nutritional and Dietary Supplements

<2022> Microbiological Procedures for Absence of Specified Microorganisms- Nutritional and Dietary Supplements

<2023> Microbiological Attributes of Non-sterile Nutritional and Dietary Supplements

USP Informational chapters (<1000>through <1999> are not generally enforced by FDA but found to be extremely informative. These Informational chapters will help explain or expand on scientific principles established in the regulatory chapter.

<1035> Biological Indicators for Sterilization

<1072> Disinfectants and antiseptics

<1111> Microbiological Examination of Nonsterile Products: Acceptable Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use

<1112> Application of water activity Determination to Non-sterile pharmaceutical products.

<1113> Microbial Characterization, Identification, and Strain Typing

<1116> Microbiological evaluation of clean rooms and other controlled environments

<1117> Microbiological Best Laboratory Practices

<1207> Sterile Product Packaging—Integrity Evaluation

<1208> Sterility Testing –Validation of Isolator Systems

<1209> Sterilization—Chemical and Physicochemical Indicators and Integrators

<1211> Sterilization and Sterility Assurance of Compendial Articles

<1223> Validation of alternative microbiological methods

<1227>Validation of Microbial Recovery from Pharmacopeial Articles

<1237> Virology Test Methods

V. AOAC international-

Includes chapters on disinfectants evaluation (i.e., Phenol coefficient Methods; Hard surface carrier test methods; Use-Dilution Method) Online access is available through (http://inside.fda.gov/Library/ElectronicResourcesWebLERN/Alphabeticallist/index.htm)

VI. Association for the Advancement of Medical Instrumentation (AAMI)/ International Organization for Standardization (ISO).

(http://inside.fda.gov/scripts/first/stdsactivity/csam_committees.cfm?center_id=3&page=1)

There are over fifty (50+) documents available through AAMI/ISO on the topic of "Sterilization Processes and Validation". These are internationally recognized standards and procedures recognized by FDA and Industry.

AAMI/ISO Guidance documents- are available at the FDA intranet weblink given below.

VII. FDA Inspection Guidance documents-

(http://www.fda.gov/cder/guidance/index.htm)

Listed below are all the FDA guidance documents that contain only microbiological information relevant to inspection. Not all the citations have direct web links to the specific documents. In most cases these will be listed in the general website for CBER or CBER guidelines (http://www.fda.gov/cber/guidelines.htm)

Submission of Documentation in Applications for Parametric Release of Human and Veterinary Drug Products Terminally Sterilized by Moist Heat Processes (draft 8/2008) (http://www.fda.gov/cber/gdlns/moistheat.pdf)

Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products (draft guidance, 2/2008) http://www.fda.gov/cber/guidelines.htm.

Guidance for Industry- Container and Closure system Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products (2/2008)

(http://www.fda.gov/cder/guidance/index.htm)

Guidance for Industry- Quality Systems Approach to Pharmaceutical cGMP Regulations (9/2006) (http://www.fda.gov/cder/guidance/index.htm)

Draft Guidance for Industry and FDA Staff- Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens (12/2005) Docket number 2005D-0434 (http://www.fda.gov/dockets/ecomments)

Guidance for Industry- Manufacturing Biological Drug Substances, Intermediates, or Products using Spore-forming Microorganisms (2/2005) (http://www.fda.gov/cder/guidance/index.htm

Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice, 9/2004 (http://www.fda.gov/cder/guidance/5882fnl.htm)

Comparability Protocols - Chemistry, Manufacturing, and Controls Information (http://www.fda.gov/cber/gdlns/cmprprot.htm), Required for industry interested in substituting an automated/Rapid Microbiological method in place of the USP compendial method cited in their original application (2/2003)

Guidance for Industry- Sterility Requirement for Aqueous- Based Drug Products for Oral Inhalation—Small Entity Compliance Guide (11/2001) (http://www.fda.gov/cder/guidance/4774fnl.pdf)

Guide to Inspections of Quality Systems-Medical Device (8/1999)

Guidance for Industry-Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product (1/1999) (http://www.fda.gov/cber/guidelines.htm)

Guide to Inspections of Lyophilization of Parenterals (10/18/97)

(http://www.fda.gov/ora/inspect_ref/igs/lyophi.html)

Guide to Inspections of Cosmetic Product manufacturers (2/1995) (http://www.fda.gov/ora/inspect_ref/igs/cosmet.html)

Guide to Inspections of Sterile Drug Substance Manufacturers, (7/1994) (http://www.fda.gov/ora/inspect_ref/igs/subst.html)

Guide to Inspections of Topical Drug Products (7/1994) (http://www.fda.gov/ora/inspect_ref/igs/topic.html)

Guidance for Industry- Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products (11/1994) (http://www.fda.gov/cder/guidance/cmc2.pdf)

Guideline for the manufacture of In Vitro Diagnostic Products (1/1994) (http://www.fda.gov/cdrh/comp/918.pdf)

Guide to Inspections of Microbiological Pharmaceutical Quality Control Laboratories (7/1993) (http://www.fda.gov/ora/inspect_ref/igs/micro.html)

Guide to Inspections of High Purity Water Systems, (7/1993) (http://www.fda.gov/ora/inspect_ref/igs/high.html)

Guide to Inspections of Validation of Cleaning Processes (7/1993) (http://www.fda.gov/ora/inspect_ref/igs/valid.html)

FDA Biotechnology Inspection Guide, Reference materials and training aids (11/1991) (http://www.fda.gov/ora/inspect_ref/igs/biotech.html)

Guidance for Industry, Pyrogen and Endotoxins Testing: Questions and Answers (June 2012)

VIII. Inspectors technical guidance (ITG)-

There were a few ITGs that were written regarding microbiological issues. I listed all of them below with direct link for your perusal. Although they are a little dated the information is still relevant.

PYROGENS, STILL A DANGER (1/12/79 Number: 32) (http://www.fda.gov/ora/inspect_ref/itg/itg32.html)

HEAT EXCHANGERS TO AVOID CONTAMINATION (7/31/79 Number: 34) (http://www.fda.gov/ora/inspect_ref/itg/itg34.html)

REVERSE OSMOSIS (10-21-80 Number: 36) (http://www.fda.gov/ora/inspect_ref/itg/itg36.html)

BACTERIAL ENDOTOXINS/PYROGENS (3/20/85 Number: 40) (http://www.fda.gov/ora/inspect_ref/itg/itg40.html)

LYOPHILIZATION OF PARENTERALS (4/18/86 Number: 43) (http://www.fda.gov/ora/inspect_ref/itg/itg43.html)

WATER FOR PHARMACEUTICAL USE (12/31/86 Number: 46) (http://www.fda.gov/ora/inspect_ref/itg/itg46.html)

MICROBIOLOGICAL CONTAMINATION OF EQUIPMENT GASKETS WITH PRODUCT CONTACT (12/31/86 Number: 48) (http://www.fda.gov/ora/inspect_ref/itg/itg48.html)

Report No. Title- PDA Technical Reports related ONLY to Microbiological Issues Date

1	Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control	July 2007
3	Validation of Dry Heat Processes Used for Sterilization and Depyrogenation	1981

4	Design Concepts for the Validation of Water-for-Injection Systems	1983
5	Sterile Pharmaceutical Packaging: Compatibility and Stability	1984
7	Depyrogenation	1985
11	Sterilization of Parenterals by Gamma Radiation	1988
13	Fundamentals of an Environmental Monitoring Program	1990 (Revised 2001)
15	Industrial Perspective on Validation of Tangential Flow Filtration in Bio-pharmaceutical Application	1992
20	Report on Survey of Current Industry Gowning Practices	1990
21	Bioburden Recovery Validation	1990
22	Process Simulation Testing for Aseptically Filled Products	2011
23	Industry Survey on Current Sterile Filtration Practices	1996
26	Sterilizing Filtration of Liquids	2008
28	Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals	2006 (revised)
29	Points to Consider for Cleaning Validation	2012
30	Parametric Release of Pharmaceuticals Terminally Sterilized by Moist Heat	1999
33	Evaluation, Validation and Implementation of New Microbiological Testing Methods	2000
34	Design and Validation of Isolate Systems for the Manufacturing and Testing of Health Care Products	2001
35	A Proposed Training Model for the Microbiological Function in the Pharmaceutical Industry	2001
36	Current Practices in the Validation of Aseptic Processing -- 2001	2002
40	Sterilization Filtration of Gases	2005
41	Virus Filtration	2005

45	Filtration of Liquids using Cellulose-based depth filter	2008
57	Analytical Method Validation and Transfer for Biotechnology products	2012
61	Steam in place	2013

IX. Miscellaneous FDA Documents and References

This list of references may not be entirely microbiology but very important none the less if you want to be a serious FDA field investigator

FDA Inspectional Operational Manual (http://www.fda.gov/ora/inspect_ref/iom/default.htm)

FDA Warning Letters and Responses http://www.fda.gov/foi/warning.htm

FDA Bacteriological Analytical Manual (BAM) (1/2001) (http://www.cfsan.fda.gov/~ebam/bam-toc.html)

FDA Sterility Analytical Manual (2001, contact FDA Division of Field Science for a copy)

X. Important Government and International organizations:

National Institute of Health (www.nih.gov)

Center for Disease Control and Prevention (www.cdc.gov)

CDC report on environmental monitoring
(http://www.cdc.gov/ncidod/dhqp/gl_environinfection.html)

World Health Organization (www.who.org) Those of you interested in world travel and global inspections with the FDA may want to become familiar with this website. International pharmaceutical regulations along with monitoring of disease outbreaks around the globe may be important to you if you have been assigned to work in a high risk area.

XI. Industry Technical references-

Parenteral Drug Association (PDA) Technical Reports- Although the scientific recommendations in these technical reports are not enforceable by FDA they do contain industry current manufacturing practices and scientifically sound principles that support regulatory concerns. This is prime reading material for novice and experts interested in understand microbiological principles and applications.

(http://eroom.fda.gov/eRoom/CDER7/CDERPDATechnicalReports/0_227d)

XII. Books and Commercial Trade reports:

(This is not an FDA endorsement, just a potential starter list

ASM, Manual of Clinical Microbiology;

Disinfection, Sterilization, and Preservation, by S Block;

Bergey's manual systematic Bacteriology

Remington's Pharmaceutical Sciences

F-D-C Monthly Reports

Excellent summary of conferences, FDA regulation changes, Key Industry personnel and often a list of the most recent Product Recalls and regulatory actions by FDA. Need to sign up for email membership. Instructions for membership enrollment are available at FDA website below. (So easy even a Microbiologist can do it)

(http://inside.fda.gov/Library/ElectronicResourcesWebLERN/Alphabeticallist/index.htm)

The "Gold Sheet"- Pharmaceutical & Biotechnology Quality Control

The "Pink Sheet"- Prescription Pharmaceuticals and Biotechnology

The "Gray Sheet"- Medical Devices Diagnostics & Instrumentation

The "Silver Sheet"- Medical Device Quality Control reports

XIII. Free Trade publications available on line-

(Not an extensive list but a fair start)

Pharmaceutical Technology (www.pharmtech.com)

Controlled Environments (www.cemag.us)

American Pharmaceutical Review (www.americanpharmaceuticalreview.com)

International BioPharm (www.biopharminternational.com)

XIV. Professional memberships

These organizations have available searchable references. There are many other professional societies chose one you can afford and enjoy.

International Society of Pharmaceutical Engineers (www.ispe.org)

American Society for Microbiology (www.asm.org)

Parenteral Drug Association (www.pda.org)