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1. Purpose

FDA regulatory microbiologists examine products under the purview of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for adulteration, conduct method development research, respond to outbreaks and other medical product emergencies, and participate in team establishment inspections.

Analyses range from the relatively simple to the most complex. In recent years, microbiologists have transitioned from sole use of conventional methods to a coupling of state-of-the-art rapid methods with traditional methods.

The overall purpose of the training program is to:

A. Train the analyst to think as a regulatory microbiologist.

B. Introduce typical analytical procedures a regulatory microbiologist should know and understand.

C. Show where and how the work performed fits into the regulatory framework.

This regulatory framework includes:

1. The reasons for sample collection.
2. The procedures of inspection and sample collection.
3. The sample analysis procedures.
4. Regulatory follow up actions and relationship of items to the FD&C Act.

2. Scope

The FDA microbiologist is well termed a regulatory microbiologist because everything he or she does is related to the regulation of products and manufacturers under the jurisdiction of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and Related Acts. Thus, results of his or her work impact directly on those products regulated, and therefore on the consumer. The FDA microbiologist can also be called a public health microbiologist as removing

contaminated medical products from the market directly impacts the health and welfare of consumers.

3. Responsibility

A. Trainer

1. Coordinates training with Supervisor(s) and Trainee.
2. Works with trainees to ensure completion of all training needed to meet their regulatory responsibilities.

B. Trainee

1. Completes required training within specified timeframes.
2. Reports training received and submits documentation for training to supervisor.
3. Reads and complies with standards, regulations, policies, procedures and work instructions.

C. Supervisor

1. Implements and reviews training records.

D. Quality Management

1. Maintains employee competencies matrix records for staff.

4. Background

This training program is divided into different modules. If all modules are completed, the microbiologist will be competent in many procedures in FDA microbiology.

During the first year, there will be several basic training courses offered by Office of Training, Education and Development (OTED). Training will also be supplemented by computer-based modules provided online by ORA U. However, most instructions will come from the laboratory.

Microbiology methodology is an ever-improving science. Training will be a continuing process throughout the microbiologist's career. Future training will reinforce and amplify what the trainee has learned.

5. References

A. Pharmaceutical Microbiology Manual. (current ed.) Retrieve through QMiS (ORA.007).

B. U.S. Pharmacopeia/ National Formulary (current ed.).

C. Difco manual of dehydrated culture media and reagents for microbiology (current ed.). Detroit, MI: Difco Laboratories, Inc.

D. FDA Staff Manual Guide, Vol III- General Administration Safety and Occupational Health Programs Hazardous Biological Agents and Toxins

E. 21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceuticals

F. ICH Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients

G. Drug Quality Assurance CPG 7356.002

H. Sterile Drug Process Inspections. Retrieve from Compliance Program Manual.

I. Active Pharmaceutical Inspections. Retrieve from Compliance Program Manual.

J. Sterile Drug Products Produced by Aseptic Processing.

K. Analytical Procedures and Method Validation for Drugs and Biologics.

L. CPG 7382.845: Inspection of medical device manufacturers

M. CPG 7383.001: Medical Device PMA Preapproval and PMA Post-market Inspections

N. Guide to inspection of Quality Systems (QSIT)

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6. Training Modules

6.1. Basic Microbiology Techniques

A. Objective

1. Introductory exercises demonstrating the trainee is proficient in basic microbiology techniques

B. Assignment

1. The trainee will discuss and demonstrate basic microbiology skills such as the following:

a. Gram staining
b. Aseptic techniques
c. Serial dilutions
d. Quadrant streak
e. Personal protective equipment (PPE)

6.2. Media, Reagent, Supply Preparation and Applicable Quality Release Testing

A. Objectives

1. To introduce the trainee to applicable procedures to be followed for preparation of media, reagents and supplies that will be used in medical product testing. These procedures will include aseptic technique necessary when handling sterilized media.
2. To introduce the trainee to applicable quality assurance procedures used for verifying that the media and reagents meet their required specifications.
3. The trainee will learn growth promotion testing in accordance with USP and applicable procedures for various media and reagents used in medical product testing. This will include the differences between liquid growth media and the testing of plated media and specific requirements associated with inhibitory testing and indicative testing.
4. To familiarize the trainee with safety concerns, such as use of the autoclave(s), hotplate(s) and weighing of powders.

B. Assignment

1. The trainer will discuss and demonstrate the equipment used in media preparation such as balances, hotplates, dispensers, pH meters, pipettors and autoclave(s).
2. The trainer will discuss the function of the components that comprise commonly used media such as soybean casein digest broth, fluid thioglycolate broth, Sabouraud dextrose agar and frequently prepared selective agars.
3. The trainer will discuss and demonstrate the growth promotion activities, as well as the process for release testing of media.
4. The trainee will prepare several kinds of media (enrichment broths, nonselective plated media and selective plates) and buffer solutions. The trainee will perform growth promotion testing on the prepared media and buffers.

5. The trainee will prepare and sterilize supplies including empty bottles, tubes and utensils as dictated by individual lab use.
6. The trainer will discuss storage and shelf life of prepared media.

C. Questions

1. Which media should not be steam sterilized and why?
2. Where is agar derived from and what are the special properties of agar that make it well suited as a solidifying agent in culture media?
3. Once agar is melted, what temperature should agar be kept at to prevent it from solidifying?
4. What is the purpose of conducting growth promotion testing on media used in microbiological testing?
5. In which media and buffer preparations is volume particularly critical?
6. Why is pH important in media and buffer preparation?
7. What are some safety concerns when autoclaving?

6.3. Safety and Hazardous/Infectious Waste

A. Objectives

1. To introduce the trainee to laboratory safety practices.
2. To introduce the trainee to the hazards involved in working with pathogens and/or their toxins such as *Bacillus cereus* toxin.
3. To develop the trainee's awareness of procedures for the proper segregation and disposal of laboratory waste products.
4. To identify resources which can assist the employee with the risk assessment process.

B. Assignment

1. Read the following:
   a. ORA Lab Manual, Volume III, Section 2 for safety issues
      i. Laboratory Chemical Hygiene Plan
      ii. Laboratory Hazardous Waste Plan
      iii. Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition
      iv. Laboratory Biosafety Plan and applicable SOPs for local site

v. Safety Data Sheets for each chemical used in the analytical procedure

C. Questions

1. What are the items of personal protective equipment (PPE), minimally needed, in a Biological Safety Level (BSL)-2 biological laboratory?
2. What work practices are to be in place when working in a Biological Safety Level (BSL)-2 laboratory?
3. Describe and give examples of opportunistic microorganisms that cause infections, disease, or other health hazards and identify their biosafety levels.
4. Describe proper procedures for disposal of used media. Describe how to use and operate an autoclave safely.
5. What is a SDS (Safety Data Sheet)? Where are the SDSs located? What information can be found on a chemical reagent hazard label?
6. Does the state regulate medical waste? What laboratory wastes are permitted to enter the sewer? What laboratory wastes are incinerated?
7. What are the guidelines for handling microorganisms (mostly bacteria or their toxins) in the BMBL?
8. What are engineering controls? Describe the proper use of these engineering controls in a medical product microbiology laboratory.
9. What are administrative controls? Describe administrative controls designed to minimize the risks of hazardous agent exposure to those personnel who are not directly involved with their manipulation. List those administrative controls that assist in the maintenance of quality control.
10. What safety equipment is normally found within a microbiological laboratory?
11. What types of laboratory procedures have the potential to generate aerosols? How can these procedures be contained? How can the generation of aerosols be minimized?
12. What decontamination procedures are in place and when are they performed?
13. Describe how spills are handled. Are the cleanups following a spill documented and the cleanup verified?

14. What kind of signage is to be in place in a microbiological BSL-2 laboratory?
15. What are the potential routes of exposure when working with infectious organisms?
16. Why is it necessary for a minimum of two people to be working in the laboratory at any given time?
17. Has enough training been received to perform assigned tasks and has this training been documented?
18. Is the facility designed to prevent infectious organisms from being accidentally released to other areas in the building?
19. Is there a sharps safety program in place to reduce hazards when handling syringes or pipettes or other sharps?

D. Exercises

1. Discuss with trainer or supervisor how etiologic isolates are shipped.
2. Discuss with trainer, supervisor, and/or industrial hygienist how hazardous waste is managed at the local site.

6.4. Quality Assurance and Data Integrity

A. Objectives

1. To present concepts of a Quality Management System to include:
   a. QMiS
   b. Accreditation
   c. Proficiency Testing
   d. Internal Audits
2. To present quality control concepts such as:
   a. Method Modifications
   b. Contemporaneous documentation
   c. Appropriate testing controls
   d. Qualified Suppliers, etc.
3. To present concepts of data integrity to include:
   a. Accountability
   b. Media/equipment
   c. Photography

d. Labeling
e. FACTS
f. Analytical worksheets
g. Electronic records

B. Assignment

1. Read the following:
   a. Laboratory Manual of Quality Policies for ORA Regulatory Laboratories (Vol. 1 of the ORA Lab Manual) and ISO/IEC 17025:2017 Sections 6.3, 6.4, 6.5 and 7.1 – 7.8
   b. ORA Lab Manual Volume II, Section 2,
   c. ORA Lab Manual Volume III, Section 3, Recording of Results – Analyst's Worksheet
   d. SOP-000288 Microbiological Controls for Medical Product Sample Analysis
2. Read local Standard Operating Procedures (SOPs) regarding corrective actions, preventative actions, nonconforming work, method verification and validation and record and data management.
3. Trainer will discuss with trainee the various activities to support quality assurance/control such as aseptic technique, quality controls, environmental and personnel monitoring, media growth promotion testing, suitability testing, proficiency testing, analyst competency, correct media volume, pH and formulation, equipment controls, etc.
4. Trainer will introduce the trainee to the location and content of harmonized worksheets.
5. Trainer will discuss with the trainee the principles of data integrity such as accurate calculations, review of controls, real-time recording of observations, where to find appropriate acceptance criteria, how to review analytical worksheets, how to complete relevant Quality Assurance checklists and how to report, evaluate and respond to data deviations/nonconformances.

C. Questions

1. What are culture controls and why are they required in analyses?
2. With whom does Data Integrity begin?
3. Why are Quality Assurance and Data Integrity so important?

4. What is the purpose of environmental and personnel monitoring?
5. Why are temperatures recorded for incubators, water baths, etc.?
6. Why are worksheets reviewed and signed by an analyst/Supervisor/Team Lead who has not worked on the sample?
7. Why are validated methods used?
8. What is QMiS?

6.5. Working within an ISO 5 Controlled Environment

6.5.1. Clean Rooms

A. Objectives

1. Introduce the characteristics of a cleanroom to include the types of cleanrooms, the current ISO (International Organization for Standards) 5 specification, the importance of maintaining appropriate relative humidity control and temperature control and pressure differentials.
2. Introduce clean room disinfection procedures.
3. Introduce safety practices, Do's and Don'ts while working in the clean room.
4. Introduce proper sample preparation needed before taking sample into the cleanroom.
5. Introduce ways to control contamination in a cleanroom:
    a. Control air flow
    b. Operator gowning
    c. Operator follows strict aseptic procedures
    d. Decontamination of all media and equipment used within the cleanroom
    e. Follow environmental and personal monitoring procedures
    f. Follow effective cleanroom cleaning and testing procedures

B. Assignment

1. The trainer will provide appropriate training materials about cleanroom, sterility analysis and hands on training:
    a. Disinfection Procedures
    b. Media, equipment and sample prep

c. Gowning

d. Aseptic Technique

e. Components of quality control

f. Personal monitoring procedures

2. The trainee will work with the trainer to become proficient working in a cleanroom environment.
3. The trainee will need to pass gowning qualification and perform satisfactory proficiency testing.

C. Questions

1. What environmental characteristics need to be controlled in a cleanroom?
2. Should a cleanroom be under positive or negative filtered air pressure and why?
3. Why should a relative humidity in a cleanroom be controlled?
4. What ISO Equivalent Class is equivalent to Class 100 room?
5. Name 3 acceptable agents used to disinfect the cleanroom?
6. Describe the 2-bucket cleanroom disinfection method?
7. Why should you work “wet” while disinfecting the cleanroom?
8. Describe all gowning material (PPE) used for working in the cleanroom?
9. What are the advantages of working as a team “buddy system” within the clean room?
10. What are some of the safety practices that should be followed while working in the cleanroom?

6.5.2. Isolator Technology

A. Objectives

1. Introduce the key components of what constitutes an isolator.
2. To introduce the principles of vaporized hydrogen peroxide and discuss how the sterilant is used within isolators.
3. To introduce the necessary components of quality control for isolators such as aseptic technique, environmental monitoring requirements, personal protective equipment, glove integrity checks and decontamination cycle development.

B. Assignment

1. The trainer will provide relevant training materials regarding the necessary components of an isolator and provide hands on training for the operation of the sterility testing isolator in laboratories that are equipped with isolators. These activities will be performed by referencing local procedures for the operation and maintenance of the sterility testing isolator. The trainer will provide one or more training samples for the analyst to process into the isolator, conduct sterility testing on and then remove from the isolator.
2. Using a validated cycle, the analyst will assist with loading a medical product sterility sample into the isolator, executing a decontamination cycle and performing sterility testing on the sample within the isolator environment with a qualified trainer. If the laboratory does not have access to an isolator this assignment can be performed via a discussion with the qualified trainer. During the discussion the trainer should discuss the importance of proper loading of an isolator and provide details on the types of supplies, reagents and test equipment that are needed for a sterility test within an isolator.
3. Using a training cycle or a validated cycle the analyst will independently properly clean, load and run a decontamination cycle for the sterility testing isolator in preparation for a sample analysis. The trainer will provide the analyst with suitable supplies to be used during this training for loading of the isolator. If the laboratory does not have access to an isolator this assignment can be performed via a discussion with the qualified trainer. During the discussion the trainee should describe the proper preparation procedures for readying and the isolator for a decontamination cycle and provide details on the types of supplies, reagents and test equipment that are needed for a sterility test within an isolator.
4. Analyze a medical product sample within the isolator environment. The trainer will spike one or two units of a sample with aerobic and/or anaerobic microorganisms. If the laboratory does not have access to an isolator this assignment can be performed via a discussion with a qualified trainer. The trainee should provide details on the actions that would be taken to analyze a sample within an isolator.

C. Questions

1. What are two key components of an isolator?

2. What does VHP stand for and what is its purpose in an isolator?
3. Why is aseptic technique still necessary within a sterility testing isolator?
4. What types of environmental monitoring can be performed within a sterility testing isolator?
5. What is the importance of checking the integrity of isolator gloves?
6. Why is a validated loading profile important?

6.5.3. Biological Safety Cabinets and Laminar Flow Hoods

A. Objectives

1. Introduce the key components of what constitutes a biological safety cabinet and laminar flow hood.
2. Introduce the principles of the purpose and function of a biological safety cabinet and laminar flow hood.
3. Introduce the necessary quality control related to the operation of biological safety cabinets and laminar flow hoods, such as aseptic technique, environmental monitoring, personal protective equipment, and decontamination.

B. Assignment

1. The trainer will provide relevant training materials associated with the maintenance and disinfection of biological safety cabinets and laminar flow hoods. The trainer will also provide the supplies and equipment necessary to perform required maintenance and disinfection. Utilizing local procedures, the analyst will perform maintenance and disinfection on both a biological safety cabinet and laminar flow hood.
2. The trainer will provide relevant training materials associated with operation of biological safety cabinets and a laminar flow hoods and provide hands on training related to the operation of the hoods. These activities will be performed by referencing local procedures for the operation of biological safety cabinets and laminar flow hoods. During the training instruction the trainers should discuss the importance of proper placement of items within each hood type and cautions related to the disruption of airflow within the hood. The trainer will provide training samples for the analyst to process in each hood type.

3. The trainer will provide relevant training materials associated with environmental sampling of biological safety cabinets and laminar flow hoods. The trainer will supply the necessary supplies needed to perform environmental sampling of both types of hoods. Utilizing local procedures, environmental sampling activities will be conducted utilizing the applicable personal protective equipment to simulate environmental sampling that would be required during an aseptic testing event.

C. Questions

1. What are the two main differences between a biological safety cabinet and a laminar flow hood?
2. Why is personal protective equipment required when working in either a biological safety cabinet or laminar flow hood?
3. Name three ways to prevent an interruption in air flow in a biological safety cabinet and laminar flow hood.
4. How many classes of biological safety cabinets are there?
5. What is an aseptic technique related caution associated with working in an open an open-faced laminar flow hood?
6. Describe the placement of environmental monitoring plates in a biological safety cabinet and in a laminar flow hood.
7. How often do biological safety cabinets and laminar flow hoods require certification by a qualified vendor?
8. Are UV lights in a biological safety cabinet or laminar flow hood an effective means of decontamination?
9. Why is the sample and not the user protected when utilizing a laminar flow hood?
10. What type of filters are used in a biological safety cabinet and laminar flow hood?

6.6. Methodology

6.6.1. Direct Staining and Visual Screening Method

A. Objectives

1. To introduce a direct staining method to include photography for rapid determination of the presence of microorganisms in a sterile product.

2. To introduce methods and practices for the visual examination of product container/closure defects for sterile medical products. Defects may include cracks, punctures or damage that results in a non-intact container/closure system.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to direct staining. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a series of training samples.

1. Read the following:
   a. Pharmaceutical Microbiology Manual (PMM), Chapter 10.
   b. Screening Protocol for Direct Staining on Products with Appearance of Visible Contamination (ORA-LAB.015).
2. To analyze two different samples for visual contamination of the container/content.
3. To perform Gram staining of aliquots on visually contaminated samples.

C. Questions

1. What would be some visual clues of microbial contamination?
2. Describe various types of cell morphology.
3. What are fungal hyphae?
4. What are spores?
5. Describe the difference between gram-negative and gram-positive organisms.

6.6.2. Microbiological Examination of Non-Sterile Products

A. Objective

1. Introduction to the determination of the quantitative enumeration under the conditions described in USP <61>.
2. Introduction to testing for the absence of specified microorganisms under the conditions described in the USP <62>.
3. Introduction of the concept of method suitability testing as described in the USP <61> and <62>.

4. Introduction to USP <61> and <62> concept of overcoming inhibition using neutralization and inactivation agents.

B. Assignment

It is the trainer's responsibility to transfer knowledge both practical and in theory related to sample analysis as described in USP <61>, <62>, and the PMM. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee is can independently perform the analysis, the trainee will be issued training samples to demonstrate proficiency.

1. Read the following:
    a. USP <61> (current edition). Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests
    b. USP <62> (current edition). Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms.
    c. USP <1111> (current edition). Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use.
    d. Pharmaceutical Microbiology Manual, Chapter 2.
    e. SOP Microbiological Controls for Medical Product Sample Analysis (SOP-000288).
2. Perform method suitability testing for two or more samples, such as a solid, fluid, water-immiscible fluid (waxes, ointment, creams), or fluid specimen in aerosol form for Total Aerobic Microbial Count and Total Yeast and Mold Count.
3. Analyze two or more samples such as a solid, fluid, water-immiscible fluid (waxes, ointment, creams), or fluid specimen in aerosol form for Total Aerobic Microbial Count and Total Yeast and Mold Count.

C. Questions

1. How does the analyst determine which controls to utilize for determination of the quantitative enumeration and the absence of specified microorganisms' analysis?
2. What are some agents that can be used for neutralization of interfering substances?
3. Why is the Growth Promotion Test performed?
4. What three tests are performed as part of the Growth Promotion Test?

5. Why is Method Suitability Testing required prior to performing analysis for determination of the quantitative enumeration and the absence of specified microorganisms?
6. According to USP <61>, what three methods can be used to enumerate?
7. What is the purpose of USP <1111>?

6.6.3. Special Assignments/Non-typical

6.6.3.1. Non-tuberculosis Mycobacteria (NTM)

A. Objectives

1. To present techniques for the cultivation and identification of NTMs.
2. To present techniques for the recovery of NTMs from non-sterile medical products.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a series of training samples.

1. Read the following:
   a. SOP-000059: Procedure for the Isolation and Identification of Nontuberculous Mycobacteria from Non-Sterile Medical Products
   b. USP <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests
   c. USP <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms.
   d. ORA.007 Pharmaceutical Microbiology Manual, Chapter 2
   e. ORA-LAB.018 Biosystems MicroSEQ Microbial ID System

      LIB #4569 - Isolation and Identification of Nontuberculous Mycobacteria Associated with Tattoo-related Outbreaks
2. Culture and identify the control organisms as described in the method.
3. Analyze a non-sterile medical product for the presence of NTMs as described in the method.

C. Questions

1. What are Rapid Growing Mycobacteria and Slow Growing Mycobacteria? Give some examples.
2. What media are used for the cultivation of NTMs?
3. What are acid-fast bacteria?
4. What DNA targets are used in the identification of mycobacteria?

6.6.3.2. *Burkholderia cepacia* complex (BCC)

A. Objectives

1. Introduce how special assignments may be received by the laboratory from CDER in response to surveillance data or adverse events.
2. Introduce the method for *Burkholderia cepacia* complex testing as it relates to non-sterile pharmaceutical products.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a training sample.

1. Read USP <60> Microbiological Examination of Nonsterile Products-Tests for *Burkholderia cepacia* complex
2. The trainee should have previously completed or be concurrently undergoing training for nonsterile pharmaceutical testing (USP <61> and USP <62>) in accordance with this Laboratory Manual Chapter.
3. Working with a trainer observe and assist on testing of non-sterile drug products for *Burkholderia cepacia* complex. Additional training may be provided depending on the trainee's level of understanding of the method.
4. Working independently the trainee will analyze a sample provided by the trainer which will consist of multiple units that have been spiked with *Burkholderia cepacia*.

C. Questions

1. What does the phrase *Burkholderia cepacia* complex mean?

2. What is the primary selective agar used in the method to detect organisms within the *Burkholderia cepacia* complex?
3. What is the typical morphology of *Burkholderia cepacia* colonies on BCSA?
4. Why isn't Cetrimide agar used as the sole agar for the detection of *Burkholderia cepacia* complex?
5. The *Burkholderia cepacia* complex is comprised of numerous *Burkholderia* species. What characteristics do these species have in common?
6. Describe at least two situations in which the detection of *Burkholderia cepacia* complex may be added to a non-sterile analysis.

6.6.4. Sterility Testing of Medical Products

A. Objectives

1. To analyze medical products that are labeled "sterile" for sterility using analytical techniques, such as membrane filtration and direct inoculation.
2. To introduce the following concepts: aseptic technique, method suitability testing, and visual examination of particulates.
3. To introduce sample preparation methods for sterility analysis.
4. To present gowning procedures necessary for work in clean rooms and/or isolators.
5. To read and discuss USP sections and supplemental information found in the Pharmaceutical Microbiology Manual.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a series of training samples.

1. Read the following:
   a. USP <71> Sterility Tests (current edition).
   b. Pharmaceutical Microbiology Manual, Chapter 3.

c. Local Standard Operating Procedures

2. If applicable, analyze two different drug samples for sterility using USP methodology; one tested by direct inoculation and one tested by membrane filtration. Trainer should spike one or two units with aerobic and anaerobic organisms and discuss the differences between large volume parenteral (LVP) and a small volume parenteral (SVP).
3. If applicable, analyze two different medical device samples for sterility. Trainer should spike a few units with aerobic and anaerobic organisms.

C. Questions

1. What is the purpose of method suitability test?
2. What is the inoculation level in CFUs to be used to inoculate product in media?
3. Describe what is done to ensure the work area is acceptable for sterility testing?
4. What should an analyst do if the growth in air sample plates exceeds the laboratory's alert/action levels?
5. When would the incubation time be extended beyond typical 14-day incubation period?
6. What determines if a product is a SVP or LVP?
7. What is the purpose of using two different media?
8. How does Fluid Thioglycollate maintain anaerobic conditions?
9. What is the function of the red indicator in Fluid Thioglycollate?
10. How is a sterility test by membrane filtration different from a sterility test by direct transfer?
11. What is the difference between Fluid A, D, and K.? When would a microbiologist use one over the other?
12. What is the incubation temperature for Fluid Thioglycollate and Soybean Casein Digest Medium?
13. What is the purpose of the Pharmaceutical Microbiology Manual (PMM), Chapter 3?
14. During the disinfection procedure prior to entering the cleanroom which type of products may be soaked in a disinfectant?

15. What are the six strains of microorganisms used for Growth Promotion Test and Method Suitability Test?
16. What can you do to eliminate product inhibition in Suitability Testing?

6.6.5. Investigating USP Sterility Testing Failures

A. Objectives

1. To identify equipment, materials, media, etc. used during testing and evaluate for malfunctions.
2. To identify any deviations from approved methodology.
3. To evaluate analyst qualifications.
4. To identify and evaluate environmental conditions during testing.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sterility failure investigations. This may be accomplished through completion of a sterility failure checklist. Once the trainer is confident that the trainee can successfully and independently perform the investigation, the trainee will be issued a sterility failure analytical package for investigation.

1. Read the following:
    a. ORA Laboratory Manual, Control of Nonconforming Work (ORA-LAB.4.9)
    b. Local Nonconformance procedures
    c. Pharmaceutical Microbiology Manual Chapter 4.
    d. Sterility Failure Checklist (FORM-000115).
    e. Complete sterility failure checklist.

C. Questions

1. What laboratory classification of samples would require a sterility failure investigation?
2. What actions should be taken if a negative control is contaminated?
3. What records should be reviewed to determine analyst qualifications?

6.6.6. Bacterial Endotoxin Testing

A. Objectives

1. Introduce principles related to endotoxin testing to include what endotoxin is, the purpose of endotoxin testing and the types of products that undergo the testing.
2. Introduce the methodology for USP <85> and USP <161>, as applicable.
3. Introduce the three techniques for the Bacterial Endotoxins Test (BET).
    a. Gel Cot Technique
    b. Turbidimetric technique
        i. Endpoint-Turbidimetric Assay
        ii. Kinetic-Turbidimetric Assay
    c. Chromogenic Technique
        i. Endpoint-Chromogenic Assay
        ii. Kinetic-Chromogenic Assay
4. Introduce the following concepts: Endotoxin Limit, Maximum Valid Dilution (MVD), Test for Interfering Factors, creation of test dilutions, Control Standard Endotoxin (CSE), Reference Standard Endotoxin (RSE), LAL Reagent and lysate sensitivity.
5. Introduce and work with the trainee to complete calculations for endotoxin limit, Maximum Valid Dilution and potential test dilution series.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a training sample.

1. The trainee is to read the following methods and guidance documents:
    a. USP <85> Bacterial Endotoxins Test
    b. USP <161> Medical Devices-Bacterial Endotoxin and Pyrogen Tests

c. Pharmaceutical Microbiology Manual Chapter 5: Bacterial Endotoxin Testing

2. The trainee will work with a trainer to complete the following assignments related to the Gel Clot Assay:
   a. The trainee will observe and assist on at least two gel clot assays.
   b. The trainee will work independently to complete the analyst qualification for the gel clot assay as required by your laboratory.
   c. The trainee will work independently to analyze a sample, using the gel clot assay, provided by the trainer which will consist of multiple units with varying concentrations of endotoxin.
3. The trainee will work with a trainer to complete the following assignments related to the Kinetic Assay:
   a. The trainee will observe and assist on at least two kinetic endotoxin tests.
   b. The trainee will work independently complete the analyst qualification for the kinetic assay as required by your laboratory.
   c. The trainee will work independently to analyze a sample, using the kinetic method, provided by the trainer which will consist of multiple units with varying concentrations of endotoxin.

C. Questions

1. What is endotoxin?
2. What is the difference between endotoxin and pyrogens?
3. Is the USP <85> capable of accurately detecting pyrogens that may be in a product? Explain why or why not.
4. What is the assay temperature for Endotoxin Testing?
5. What are some factors that may cause interference in endotoxin testing?
6. What are some ways to overcome interference in the endotoxin assay?
7. If a product's endotoxin limit is 0.5 EU/mg, the product's concentration is 200 mg/ml and the lysate's sensitivity is 0.05 EU/ml. What is the maximum valid dilution for this product?
8. When calculating the endotoxin limit for a product with the formula, K/M what value is used for K for an intrathecal route of

administration? What value is used for K for routes other than intrathecal?

9. What is the Gel-Clot technique/method based on?
10. What is the duration of the Endotoxin Testing via Gel-Clot?
11. What tests are performed to ensure the precision and validity of the Gel Clot assay?
12. What are acceptable results for system control, negative water control, positive product control when running endotoxin testing via Gel-Clot?
13. What are the two types of kinetic methods found in USP <85>. Briefly describe each method.
14. According to USP<85>, to perform the confirmation of the lysate sensitivity, using the kinetic assay, what is the minimum number of endotoxin concentrations that are required for a valid standard curve?
15. For a kinetic assay to be valid per the USP what are the required criteria?

6.6.7. Testing Environmental Monitoring Samples

A. Objectives

1. Introduction to the qualitative and quantitative assessment of environmental monitoring samples collected during inspections of drug manufacturers/compounding pharmacies.
2. Overview of the concept of aseptic handling and personal protective equipment (PPE) practices associated with environmental monitoring.
3. Introduction to sample, environment, and personnel monitoring.

B. Assignment

It is the trainer's responsibility to transfer knowledge both practical and in theory related to sample analysis as described in the Pharmaceutical Microbiology Manual. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can independently perform the analysis, the trainee will be issued training samples to demonstrate proficiency.

1. Read the following:

a. Pharmaceutical Microbiology Manual, Chapter 9 Environmental Monitoring

b. USP <1113> Microbial Characterization, Identification, and Strain Typing

2. SOP-000288 Microbiological Controls for Medical Product Sample Analysis
3. Demonstrate the proper donning of PPE and the various aspects of the analysis associated with aseptic technique while working in a biological safety cabinet or laminar flow hood.
4. Demonstrate proper sample, environment, and personnel monitoring to be performed during the environmental sample analysis.
5. Perform environmental monitoring analysis for two or more samples.

C. Questions

1. What are the five typical types of environmental samples that could be submitted for analysis?
2. What are some of the advantages and disadvantages of the quantitative method of environmental monitoring?
3. What are some of the advantages and disadvantages of the qualitative method of environmental monitoring?
4. What are some of the key elements that an analyst should check the environmental monitoring sample for upon the receipt of the sample?
5. What types of air monitoring plates could be utilized in the biological safety cabinet or laminar flow hood during sample analysis?
6. What organism controls are included as part of the analysis?
7. What is the incubation timeframe and temperature for the environmental monitoring sponge or swab samples?
8. What is the incubation timeframe and temperature for the environmental monitoring RODAC Plates and Hycheck™ surface samplers?
9. What are the designated time frames for the observation for growth during the environmental monitoring sample analysis?
10. When growth is observed during an environmental monitoring test what media is used to isolate the growth?

6.6.8. Particulate Matter

A. Objectives

1. To understand the basic principles of particulate matter testing.
2. To review the two methods for determination of particulate matter:
   a. Light Obscuration Particle Count Test
   b. Microscopic Particle Count Test
3. To understand the principles, applications and operation of the HIAC 9703+ Liquid Particle Analyzer
4. To familiarize the trainee with the sample preparation procedure, proper equipment uses and proper PPE use.
5. To familiarize the trainee with operation of the HIAC 9703+ Liquid Particle Analyzer.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a training sample.

1. The trainee is to read the following:
   a. USP <788> Particulate Matter in Injections
   b. USP <789> Particulate Matter in Ophthalmic Solutions
   c. USP <1788> Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions
   d. USP <771> Ophthalmic Products – Quality Tests
   e. Pharmaceutical Microbiology Manual (PMM) Chapter 6: Particulate Matter
   f. ORA-LAB.019 HIAC 9703+ Liquid Particle Analyzer
   g. Any local SOP's in reference to specific analyzer requirements
2. Working with a trainer, observe and assist on testing a particulate sample using HIAC and/or Microscope.

3. Working independently, the trainee will analyze a particulate sample using HIAC and microscope. The sample will be provided by the trainer.

C. Questions

1. What is a particulate matter?
2. What are the two methods for the determination of particulate matter?
3. What is the HIAC 9703+ Liquid Particle Analyzer flushed with and at what volume?
4. What troubleshooting techniques can be implemented if a large particle reduces or stops the liquid flow?

6.6.9. Antibiotic Potency Testing

A. Objectives

1. Introduce the purpose of performing potency testing using a microbial assay.
2. Introduce the two methods for potency determination: Cylinder Plate Method and Turbidimetric Method.
3. Introduce the use of USP monographs for the preparation of the antibiotics involved in USP <81> testing.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a training sample.

1. Read the following methods and guidance:
   a. USP <81>, Antibiotics- Microbial Assays
   b. Pharmaceutical Microbiology Manual, Chapter 7
2. Working with a trainer observe and assist on suitability testing of an antibiotic to determine the appropriate inoculum of the specified microorganism needed to achieve a zone of inhibition of 14-16mm.
3. Working with a trainer observe and assist on testing an antibiotic using the cylinder plate method or the turbidimetric method.

Additional training may be provided depending on the trainee's level of understanding of the method.

4. Working independently the trainee will analyze an antibiotic for potency. The antibiotic will be provided by the trainer.

C. Questions

1. How do you determine the preparation steps necessary to test neomycin ointment for potency?
2. What is the purpose of the standard curve and how many standards must be included on the standard curve?
3. What is the significance of the Zones of Inhibition?
4. Why is it important to know if the bench you are working on for the preparation of plates for the Cylinder Plate method is level?
5. What are some indicators, that can be observed on the agar plates used in the Cylinder Plate method, that the bench was not level?
6. How many times must the analysis be independently conducted for a given sample and why?

6.6.10. Antimicrobial Effectiveness Testing

A. Objectives

1. Introduce principles of the Antimicrobial Effectiveness Test to include its purpose, the types of products that undergo the testing and the calculations that are performed to report results.
2. Introduce the analytical techniques necessary to complete the Antimicrobial Effectiveness test in accordance with USP <51>.
3. Introduce the following concepts: preservatives found in pharmaceutical products, inactivating agents and enumeration techniques.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a training sample.

1. Complete necessary training for USP <61> analysis.

2. Read the following methods and guidance:
    a. USP <51>, Antimicrobial Effectiveness Testing.
    b. USP <1227>, Validation of Microbial Recovery from Pharmaceutical Articles
    c. Pharmaceutical Microbiology Manual Chapter 1
3. Working with a trainer observe and assist in the method suitability testing and the product testing. Due to the length of the antimicrobial effectiveness testing the timepoints found within USP <51> may be shortened to complete the training samples in a timely manner.
4. Working independently analyze a sample beginning with method suitability and then product testing. As with the trainer led samples, due to the length of the antimicrobial effectiveness testing the timepoints found within USP <51> may be shortened to complete the training sample in a timely manner.

C. Questions

1. How many categories of products does the USP <51> list? Briefly describe each category.
2. Describe the types of containers the test can be performed in?
3. What is the standardized volume of product FDA laboratories should try to test for each microorganism?
4. What microorganisms are required by the USP <51> method and in what circumstances might additional microorganisms be added?
5. At what time points are aliquots removed and plated or filtered?
6. With regard to the Criteria for Tested Microorganisms in USP <51>, how is the term “No increase” defined?
7. At what point in the method should the analyst refer to USP <1227> to determine what additional steps may be needed?

6.6.11. Biochemical Identification

A. Objectives

1. To understand the basic principles and applications of biochemical identification utilizing VITEK® 2.
2. To understand the basic principles and applications of biochemical identification using supplemental tests identified by VITEK® 2.

3. To familiarize the trainee with the techniques, procedures, and equipment used in biochemical identification utilizing VITEK® 2.
4. To familiarize the trainee with the techniques, procedures, and equipment used in biochemical identification using supplemental tests identified by VITEK® 2.
5. To identify bacteria recovered from medical products utilizing biochemical identification utilizing VITEK® 2.
6. To identify bacteria recovered from medical products utilizing biochemical identification using supplemental testing identified by VITEK® 2.

B. Assignment

It is the trainer's responsibility to transfer knowledge both practical and in theory related to sample analysis as described in the Pharmaceutical Microbiology Manual. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee is can independently perform the analysis, the trainee will be issued training samples to demonstrate proficiency.

1. Review the following:
   a. Any applicable site SOPs
   b. USP <1113> Microbial Characterization, Identification, and Strain Typing
   c. VITEK® 2 Manuals
2. The trainer will demonstrate the preparation of isolates for biochemical analysis on the VITEK® 2. The trainee will be required to demonstrate proper isolate preparation.
3. The trainer will demonstrate how to perform a Gram stain by a manual method and/or automated method. The trainee will be required to demonstrate Gram stain proficiency for the various Gram types, such as Gram positive, and Gram negative.
4. The trainer will provide an overview of the VITEK® card selection for biochemical analysis. The trainee will be required to demonstrate the understanding of how to select which VITEK® card is required for biochemical analysis via the VITEK® 2.
5. Trainer will demonstrate the proper use of the DensiCHEK™. The trainee will be required to demonstrate the ability to achieve the

appropriate optical density of a microorganism suspension utilizing the DensiCHEK™.

6. The trainee will be required to perform biochemical analysis via VITEK® 2 on the several different isolates provided by the trainer.
7. Trainer will demonstrate how to determine when supplemental testing is required based on the identification report generated by the VITEK® 2. The trainer will also instruct the trainee on how to perform the supplemental identification tests.
8. The trainer will demonstrate how to interpret the results generated by the VITEK® with and without supplemental tests. The trainee wee be required to properly interpret the results of their isolate identifications.

C. Questions

1. Why is it important to have a quality Gram stain prior to starting the identification process?
2. How is the acceptable optical density range of the microorganism suspension determined?
3. How does the analyst know if a supplemental test is required to complete the biochemical identification?
4. When preparing the microorganism suspension to load into the VITEK® 2 cassette, what is the maximum hold time?
5. When preparing to use the VITEK® 2 cassette what are some of the factors that must be considered?

6.6.12. Polymerase Chain Reaction (PCR)

A. Objectives

1. To understand the basic principles and applications of the polymerase chain reaction.
2. To familiarize the trainee with the techniques, procedures, and equipment used in PCR analysis.
3. To amplify DNA regions commonly used for microbial identification. The amplified regions will serve a template for DNA sequencing.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once

the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a series of training samples.

1. Review the following:
   a. ORA-LAB.017 Fungal Identification by DNA Sequencing
   b. ORA-LAB.018 Applied Biosystems (AB) MicroSEQ® Microbial Identification System
2. Amplify a specific region of a microbial gene using PCR.
3. Confirm the presence of the amplicon (e.g. gel electrophoresis, DNA sequencing, etc.)
4. Practice using a micropipette to dispense small liquid quantities.
5. Practice using proper molecular biology techniques while opening and closing small reaction tubes and dispensing reagents.
6. Learn how to calculate correct reagent quantities needed for master mix preparation.
7. Trainer will demonstrate how to use a thermal cycler.
8. Trainer will discuss which organisms can be analyzed using PCR.

C. Questions

1. What are the three major steps (processes) involved in the PCR cycle?
2. What components are needed in the PCR reaction mixture and what function does each element serve?
3. Why is selection of the primers so important to the success of the reaction?
4. Why are there forward and reverse primers?
5. Why does a microbiologist need an excess quantity of primers in the reaction mixture?
6. Why always run a reagent control?
7. List three ways to prevent contamination when performing PCR.
8. In what ways can PCR be inhibited?

6.6.13. DNA Sequencing

A. Objectives

1. To understand the basic principles and applications of DNA sequencing
2. To familiarize the trainee with the techniques, procedures, and equipment used in DNA sequencing
3. To identify bacteria and fungi recovered from medical products utilizing DNA sequencing

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a series of training samples.

1. Review the following:
   a. ORA-LAB.017 Fungal Identification by DNA Sequencing
   b. ORA-LAB.018 Applied Biosystems (AB) MicroSEQ® Microbial Identification System
2. Practice using a micropipette to dispense small liquid quantities.
3. Learn how to calculate correct reagent quantities needed for master mix preparation.
4. Trainer will demonstrate the proper use of equipment necessary for DNA sequencing methods such as: Centrifuge, thermal cycler, a sequencer, etc.
5. Trainer will demonstrate how to analyze sequencing data using MicroSeqID® and Geneious®.
6. Trainer will demonstrate how to search sequences on the NCBI and alternative databases.
7. Identify a bacterial and a fungal isolate using DNA sequencing

C. Questions

1. What regions are targeted for bacterial and fungal identification by DNA sequencing?
2. What are the major steps (processes) involved in DNA sequencing?
3. Why is the amplicon PCR cleaned up using prior to cycle sequencing?

4. How is cycle sequencing different from conventional PCR?
5. What libraries are used for identification of bacterial and fungal sequences generated?

6.6.14. Pulsed Field Gel Electrophoresis (PFGE)

A. Objectives

1. Prepare agarose plugs containing bacterial cultures for PFGE.
2. Set up and run PFGE to generate DNA fingerprints.
3. Record DNA fingerprints by photographic and digital imaging methods.

B. Assignment

It is the trainer's primary responsibility to transfer knowledge both practical and in theory related to sample analysis. This may be accomplished through a series of designated training samples. Once the trainer is confident that the trainee can successfully and independently perform the analysis, the trainee will be issued a series of training samples.

1. Review the following:
   a. CDC Standard Operation Procedure for PulseNet PFGE of Escherichia coli O157:H7, Escherichia coli Non-O157 (STEC), Salmonella Serotypes, Shigella sonnei and Shigella flexneri (current version).
   b. CDC CEM.TE.C.0002 Modified Pulse-Net Procedure for Pulsed-field Gel
2. Electrophoresis of Select Gram Negative Bacilli (current version)
3. Utilize agar cultures to prepare PFGE agarose plugs.
4. Lyse bacterial cells contained in agarose plugs, wash plugs, and perform restriction digestions.
5. Prepare PFGE gel, load plugs, set up and run electrophoresis equipment.
6. Perform staining and documentation of PFGE gel.

C. Questions

1. Describe briefly the preparation of agarose plugs.
2. What are the conditions for plug lysis?

3. Describe the steps needed for plug washing.
4. What restriction enzymes are used for Burkholderia cepacia complex and Salmonella?
5. What are the two methods for loading plugs into the gel wells?
6. Briefly describe equipment set up for running a PFGE gel.
7. What are the chemical agents used to stain PFGE gels, and what safety precautions are needed to handle these agents?

6.7. Developing/Establishing a Rapid Method for Implementation into Regulatory Testing

A. Objectives

1. To familiarize the trainee with the advantages of using rapid methods.
2. To familiarize the trainee with platforms designed to rapidly identify any positive growth.
3. To educate the trainee on the increased sensitivity of rapid method for sterility testing.

B. Assignment

1. Read the following:
   a. USP <1225> Validation of Compendial Procedures
   b. USP <1223> Validation of Alternative Microbiological Methods
   c. USP <71> Sterility Testing
   d. USP <1071>
   e. PDA Tech Report No. 33
   f. On line vendor-specific rapid screening methodology reference materials
   g. ORA-LAB.016 Medical Products and Tobacco Program Review and Clearance
2. The trainer will provide theoretical knowledge related to rapid sterility screening methods, through reference materials available through specific vendors, on line references and/or methods published in the literature (PubMed).

Rapid and alternative sterility test methods have progressed significantly, but no single method has emerged as dominant and/or suitable for every product class.

The primary technological platforms for Rapid Micro Methods (RMM) fall mainly into four categories:

a. Growth-based methods: e.g., carbon assimilation, enzymatic reactions, carbon dioxide generation or ATP bioluminescence, (Instrument BacT/Alert®, BioMerieux)

b. Cellular component methods, e.g., fatty acid analysis using gas chromatography, specific portions of the microbial cell, cellular ATP, membrane filter incubation as part of cell enrichment, (Instrument Milliflex™ Rapid, Millipore)

c. Nucleic acid-based methods: e.g., PCR and automated Southern blotting

d. Viability-based methods: e.g., fluorescent labelling methods, such as flow fluorescence cytometry; immunofluorescence and fluorescent nucleic acid stains used as a viability marker (ScanRDI® Instrument, BioMerieux).

Each technique has its advantages and limitations and selection of the most appropriate instrument platform/methodology that fits a product type, must be considered.

3. Learn how to request a USP-NF account and maneuver through specific chapters.
4. The trainer will inform the trainee on the processes involved to develop a new method, prior to its implementation as a regulatory method in the lab.
    a. Determine the processes/guidelines for development of a new method.
    b. Determine validation criteria/requirements for method qualification.
    c. Determine laboratory competency.
5. The trainer will inform the trainee of the process for review and approval of research projects to include following local processes, entry of the project into CARTS and approval from the Office of Research Coordination and Evaluation (ORCE).

6. The trainer will provide the trainee with information on how to navigate the CARTS tracking system to enter a research project for approval, using the following instructions:
    a. Go to the ORA Applications intranet page and select the CARTS link
    b. CARTS Intranet Page
    c. CARTS 'help' functionality

C. Questions

1. What are some of the advantages of rapid methods?
2. How does the rapid sterility screening method differ from the current USP compendial method?
3. As per the guidance outlined in USP chapter <1223> what are the two general categories used to evaluate an alternative method?
4. As per USP chapter 1223 what are the validation requirements used to evaluate a qualitative method?
5. What does ORCE stand for?

6.8. Inspectional Guidance

Microbiologists are sometimes requested to accompany investigators on inspection of establishments that produce medical products. The trainee will accompany an investigator and an experienced microbiologist, on an initial inspection when possible. The investigator has the basic responsibility for an establishment inspection and the microbiologist is the subject matter expert regarding microbiology, but will participate fully in the inspection, covering applicable sections of the compliance program for a given inspection and any topics assigned by the lead investigator. The microbiologist will have the responsibility of documenting the areas covered for the inspection report and drafting FDA Form 483 observations made by the microbiologist and participating in the final discussion with management. The investigator oversees the inspection.

The trainee should talk with the investigator prior to the inspection and work closely with the senior microbiologist, when possible, to prepare for the inspection which will include review of the following, when applicable: previous establishment inspection reports (EIR), the inspectional assignment, the compliance program and relevant sections of the CFR. It may be necessary to prepare sample collection materials or obtain proper protective clothing for the establishment.

A. Objective

1. To introduce the policies and procedures involved in the inspection of medical product manufacturers.
2. To provide instruction and practice in the preparation of an Establishment Inspection Report (EIR)
3. To provide instruction and guidance to the trainee in drafting FDA Form 483 observations to include determining the appropriate CFR cite for an observation.

B. Assignment

1. Analyst must have valid FDA credentials. Request or renew them as needed prior to the inspection.
2. If possible, meet with the investigator (Consumer Safety Officer (CSO)) and other team members before beginning the inspection. Discuss the goals of the inspection and what your specific role will be.
3. Read the following:
    a. eNSpect assignment for the upcoming inspection.
    b. EIR from the last inspection performed at the firm.
    c. Compliance Program(s) pertinent to the inspection.
    d. Investigations Operations Manual (IOM), Chapter 1 Administration, Subchapter 1.6 - Public Relations, Ethics and Conduct; Chapter 4 Sampling, and Chapter 5 Establishment Inspections.
    e. Pharmaceutical Microbiological Manual, Chapter 11 Inspectional Guidance.
    f. Guide to Inspections of Quality Systems, August 1999, if applicable.
4. Record what is reviewed and observed during the inspection electronically or in a bound notebook.
5. Determine if 483 observations from the previous were adequately addressed.
6. Collect exhibits to be used to describe processes you reviewed or support your observations.
7. Prepare your observations (Form FDA 483) for presentation and discussion with Firm management.

8. Draft portions of the Establishment Inspection Report as appropriate.

C. Questions

1. What are some of the different types of inspections?
2. Why is it useful to review the previous EIR?
3. What are 483 observations?
4. How does FDA classify inspection reports?

6.8.1. Drug Inspections

A. Objectives

1. Introduce the different types of pharmaceutical inspections, for example good manufacturing practice (GMP) inspections, pre-approval inspections, Active Pharmaceutical Ingredient inspections and For-Cause Assignment inspections.
2. Introduce the areas to be covered by a microbiologist during pharmaceutical inspections.
3. Introduce 21 CFR 210 and 21 CFR 211 regulations.

B. Assignment

1. The trainee will work with the trainer to draft an outline for various pharmaceutical inspection types such as GMP inspections, pre-approval inspections and API inspections. The GMP and pre-approval inspection outlines will focus on a sterile manufacturer.
2. The trainee will work with a trainer to identify potential FDA Form 483 observations from a set of mock situations. The trainee will draft their 483 observations, identify the relevant 21 CFR 210 or 211 citations and provide a list of the types of exhibits necessary to establish evidence for their observations.
3. The trainee will accompany an experienced microbiologist or investigator on inspection that requires expertise in microbiology and contribute to the inspectional report.

C. Questions

1. List two to three objectives for a pre-approval inspection.
2. Explain the importance of covering good manufacturing practices during a pre-approval inspection.

3. For a GMP inspection of a sterile manufacturer what is the main system from the Compliance Program Guidance Manual 7356.002A that the microbiologist will be expected to cover?
4. A microbiology laboratory lacks method suitability for sterility testing. What is a potential 21 CFR 211 cite that may be used to support these 483 observations?
5. If you were asked to inspect a microbiology laboratory for a sterile injectable manufacturer what three finished product release tests would the microbiology laboratory be responsible for?

6.8.2. Device Inspections

A. Objectives

1. To introduce the different types of medical device inspections.
2. To introduce the techniques used in the inspection of medical device manufacturers.
3. To discuss the areas of the inspection typically covered by a microbiologist on a team inspection.
4. To introduce the 21 CFR sections applicable to medical devices.

B. Assignment

1. The trainee will work with the trainer to develop an inspectional plan specific to medical device manufacturers.
2. The trainee will identify objectionable conditions from a set of mock situations provided by the trainer. From this set, the trainee will draft FDA Form 483 observations, identify the relevant 21 CFR citations and provide a list of the types of exhibits necessary to establish evidence for their observations.
3. The trainee will accompany an experienced microbiologist or investigator on a medical device manufacturer inspection and contribute to the inspectional report.

C. Questions

1. How are medical devices classified?
2. What is QSIT?
3. How do different types of devices obtain market approval from FDA?

6.8.3. Compounding Pharmacies

A. Objectives

1. Introduce the concept of compounding.
2. Introduce the concept of insanitary conditions.
3. Introduce the differences between 503A and 503B compounding pharmacies.
4. Introduce the concept of assignments for compounding pharmacy inspections.
5. Introduce the areas to be covered by a microbiologist during compounding inspections.

B. Assignment

1. Read the following:
   a. An example of compounding assignment focusing on micro-related activities
   b. Previous EIR
   c. 503A and 503B in the Act
   d. Compounding guidance document
2. The trainee will work with the trainer to draft an outline of mock situations.
3. The trainee will work with a trainer to identify potential FDA Form 483 observations from a set of mock situations. The trainee will draft their 483 observations, identify the relevant citations and provide a list of the types of exhibits necessary to establish evidence for their observations.
4. The trainee will accompany an experienced microbiologist or investigator on inspection that requires expertise in microbiology and contribute to the inspectional report.

C. Questions

1. What is compounding?
2. What are some areas to inspect/observe during Compounding Inspection of sterile drug products?
3. What can be a risk and cause of improperly compounding drugs?
4. Provide an example of a serious compounding related outbreak.
5. Under 503B in Food, Drug, and Cosmetic Act (FDCA) if compounder becomes an outsourcing facility what must they comply with?

6. What effect did the Compounding Quality Act of the Drug Quality and Security Act have on compounding facilities?
7. If a compounder is not registered as outsourcing facility what conditions must they meet?
8. How does FDA, CDER implement the compounding provisions of the FD&C Act and conduct oversight to protect public health?

6.8.4. EM Sample Collections

A. Objectives

1. Introduce the rationale for conducting an EM collection.
2. Introduce the steps taken to prepare for an EM collection.
3. Introduce the process at the firm.
4. Introduce the process of preparing and delivering the samples to the servicing laboratory.

B. Assignment

1. The trainee will work with the trainer to prepare a mock: EM request, EM sampling plan and EM supply request.
2. The trainee will work with the trainer to review all the steps of the EM collection checklist.
3. The trainee will work with the trainer to go over aseptic techniques, gloving techniques, gowning procedures.
4. The trainee will work with the trainer to go over sampling methods and location priorities.
5. Analyst going on an EM collection must have valid FDA credentials. Request or renew them as needed prior to the inspection.
6. The trainee will accompany an experienced microbiologist to perform an EM collection.

C. Questions

1. In what location order do you perform an Environmental Monitoring (EM) collection?
2. Why is Day/Engley (D/E) Neutralizing Broth suitable for Environmental Monitoring (EM) collections?
3. What are the advantages of using sponges/swabs (qualitative method) for EM monitoring?

4. What are the advantages and disadvantages of using RODACS/Hychecks™ (quantitative method)?

7. Answer Key

7.1. Media, Reagent, Supply Preparation and Applicable Quality Release Testing

A. Which media should not be steam sterilized and why? Examples of culture media that are not steam sterilized are Xylose Lysine Deoxycholate (XLD) and Violet Red Bile Glucose Agar (VRBG). Autoclaving as well as boiling longer than needed destroys the selectivity of the medium.

B. Where is agar derived from and what are the special properties of agar that make it well suited as a solidifying agent in culture media? Agar is the dried mucilaginous substance extracted from various species of algae. The plants are found primarily off the coasts of Japan, China, and southern California. Agar is insoluble in cold water but slowly soluble in hot water to give a viscous solution. A 1% solution melts at 100°C and sets at 35°C to 50°C to a firm gel. Since agar is used by a nutrient source by relatively few bacteria, it is the most satisfactory solidifying agent for the growth and isolation of bacterial and fungal species.

C. Once agar is melted, what temperature should agar be kept at to prevent it from solidifying? Agar should be kept at 55°C to prevent it from solidifying. Prior to performing analysis, melted agar used for pour-plating should be tempered to 45°C.

D. What is the purpose of conducting growth promotion testing on media used in microbiological testing? The purpose of the growth promotion test is to determine the suitability of culture media. The medium is challenged with a small number of microorganisms to assure the nutritive properties. The criteria must be met for each new batch of medium to be accepted for use in microbial tests such as the colony forming units (CFU) on the new batch of medium must be within a factor of two of the count on the previously approved medium.

E. In which media and buffer preparations is volume particularly critical? Volume of both buffer and media are important to maintain a proper ratio of product to media or buffer. Volume is particularly critical in preparing buffer solutions intended for use in dilutions for plate counts. Incorrect volume in the buffer blank can result in erroneous plate counts.

F. Why is pH important in media and buffer preparation? One of the selective factors in favoring growth of one organism over another is pH. For example, media for detection of *Burkholderia Cepacia* Selective Agar (BCSA), pH is used to differentiate between organisms. Phenol red is used as a pH indicator. *Burkholderia cepacia* complex colonies will be yellow and surrounded by a pink yellow zone. Colonies that absorb the crystal violet dye will appear purple to purple gray. Acid production from carbohydrate oxidation will create a yellow zone in the medium while peptone utilization will result in pink zone.

G. What are some safety concerns when autoclaving?

1. Autoclave vessels with vented closures only. Do not use crimped seals.
2. Use only Pyrex® glass vessels and other types of autoclavable materials.
3. For safety, always use the appropriate cycle for sterilization: “Gravity” for equipment and “Liquid” for liquids.
4. Follow manufacturer’s instructions for proper opening of the door and end of cycle.
5. Do not allow hot bottles to be jolted. This can cause hot bottle explosion. Do not move bottles if any boiling or bubbling is present.
6. Analysts are required to wear appropriate personal protective equipment (PPE) such as full-length face shields, water impervious aprons and rubber gloves whenever removing materials from the autoclave.

7.2. Safety and Hazardous/Infectious Waste

A. What are the items of personal protective equipment (PPE), minimally required, in a Biological Safety Level (BSL)-2 biological laboratory? Each person working in a biological laboratory is minimally required to be using safety glasses with shields (or goggles, face shield, other splatter guards), a protective lab coat, disposable gloves, and protective footwear (closed-toe shoes).

B. What work practices must be in place when working in a Biological Safety Level (BSL)-2 laboratory?

1. Laboratory access is limited or restricted. Personnel at increased risk for acquiring infection, or for whom infection would have serious consequences, are not allowed in the laboratory or animal rooms.
2. Laboratory doors are kept closed when experiments are in process.

3. Personnel wash their hands after handling microorganisms, when removing gloves, and before leaving the laboratory. Soap and disposable towels are readily found at sinks in the microbiology laboratories.
4. No eating, drinking, chewing gum, smoking, handling medications, handling contact lenses, or applying cosmetics is permitted in the laboratory. All foodstuffs are stored outside of the laboratory.
5. Mouth pipetting is prohibited.
6. There is a program for handling and disposing of sharps using special sharps' containers.
7. Work surfaces are decontaminated on completion of work and at the end of the day. This usually involves a system of various decontamination sprays, such as bleach, iodine, quaternary ammonium materials, amphyll, etc. Any splashes or spills of viable material are immediately cleaned up with disinfectants that are effective against the organism of concern. Contaminated equipment is to be decontaminated before it is sent for repair, sent for maintenance, placed in surplus, or shipped out of the lab. When placing the equipment in surplus, the equipment is also labeled in accordance with the DHHS requirements for property management to show that the equipment is clean.
8. All cultures, stocks, and other regulated wastes are autoclaved, or otherwise decontaminated, to destroy any viable organisms. All materials for autoclaving are placed in red (or otherwise equivalent) bags that are closed for transport out of the laboratory. These bags are set in leak-proof totes. Any materials to be decontaminated off-site are packaged in accordance with applicable local, state, and federal regulations beforehand.
9. When working with organisms with more hazardous risk, consideration is given to chemically decontaminating the wastes within the laboratory.
10. An insect and rodent control program is in place.
11. All procedures are conducted in a manner to minimize aerosols. Consideration is given to the degree of hazard when loops are used in flames, centrifugation, opening screw-capped bottles and wet petri dish covers, use of a syringe, needle, and septum, streaking plates, pipetting, slide agglutination, etc. Good microbiological techniques, substitution of disposable equipment, and use of primary containment devices such as biosafety cabinets reduce the risk.

12. Laboratory management establishes policies and procedures whereby personnel in the laboratory are informed of the potential hazards and meet special entry requirements, such as immunization.
13. A biohazard sign is posted at the entrance to any laboratory where etiologic agents are used. This label identifies what etiologic agents are used, the biosafety level, any required immunizations, the responsible person and their phone number, what PPE is worn in the laboratory, and any procedures for exiting the laboratory.
14. Immunizations or tests are offered to personnel for the agents handled or potentially present in the lab.
15. Baseline blood serum samples may be collected and stored, depending on what agents are handled.
16. Biosafety procedures are incorporated into individual SOPs or a Biosafety Manual is adopted or prepared for the local laboratory. Personnel are advised of special hazards and are instructed to read and follow instructions on practices and procedures.
17. Laboratory and support personnel receive training on potential hazards associated with the work performed, the precautions to avoid exposure, and exposure evaluation procedures. Training is updated annually or when the procedure or policy changes.
18. Substitution of glassware for plasticware is recommended.
19. A high degree of caution is taken with any procedure using needles and syringes, or any other sharps. Sharps should be restricted whenever possible.
20. Only needle-locking syringes or disposable syringes should be used for injections, etc. Needles should not be manipulated in any manner before disposal; they should be carefully placed into a conveniently located puncture-resistant container used for sharps disposal. Non-disposable sharps are to be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
21. Syringes that re-sheathe the needle, needleless systems, and other safety devices are used when possible.
22. Never handle broken glassware directly by hand. Use a mechanical means, such as brush and dustpan or tongs to pick up broken glass pieces. Dispose of broken glass in special containers.

23. Containers of broken glass, sharps, and contaminated needles are decontaminated prior to disposal, or are shipped off-site in accordance with local, state, or federal regulations.
24. Any cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
25. All spills and accidents that result in overt exposures to infectious materials are immediately reported to the Designated Laboratory Official. Medical evaluation, surveillance, and treatment are provided as needed and written records are maintained in accordance with 29 CFR 1910.1020.
26. Only animals involved with the work being performed are permitted in the laboratory.
27. Correct personal protective equipment is worn in the laboratory.
28. Disposable gloves are not reused, washed, or used when touching “clean” surfaces, such as the telephone, door handles, keyboards, etc.
29. Properly maintained safety equipment, including biological safety cabinets (Class II) or other containment devices are used whenever procedures with a potential for creating infectious aerosols or splashes are conducted, or high concentrations or large volumes of infectious agents are used.
30. High concentrations or large volumes of infectious material may be centrifuged in the open lab if sealed rotor heads or centrifuge safety cups are used and if these rotors or cups are only opened inside of the biosafety cabinet.
31. All surfaces inside the laboratory are readily accessible for cleaning and can easily be decontaminated. No carpets or rugs are used in the laboratory.
32. The biological safety cabinet is located where fluctuations of the room supply, and exhaust air do not cause the cabinet to operate outside its parameters for containment. The cabinet is located away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment to maintain the biological safety cabinets' air flow parameters for containment.

33. Eyewashes are readily found throughout the laboratory and are flushed weekly.
34. Outside windows are fitted with screens.
35. Illumination is provided without glare or reflections.

C. What are some opportunistic pathogens that you might encounter when analyzing samples? Identify their biosafety levels. A complete list of all regulated organisms appears in 42 CFR 72.3.

Most recalls of medical products are due to contamination by Gram negative bacteria. Some of the most commonly isolated organisms within this group are *Burkholderia cepacia*, *Pseudomonas* spp. and *Ralstonia picketti*. Yeast and mold are also commonly isolated. Some of the most commonly isolated genera include *Candida*, *Aspergillus*, *Penicillium*, *Exserohilum*, *Fusarium*, *Bipolari*s, and *Rhizopus*. Gram positive bacteria, such as *Staphylococcus* spp., *Enterococcus* spp. and *Bacillus* spp. are isolated less frequently.

Environmental monitoring samples collected from cleanroom environments typically contain organisms associated with human skin such as Gram-positive cocci, like *Micrococcus* and *Staphylococcus*. Microorganisms associated with the environment such as Gram-positive rods, like *Bacillus* spp from soil and Gram-negative rods, like *Pseudomonas* from water are also detected, although in lower numbers. Fungi such as *Penicillium, Aspergillus, Rhizopus, Thrycophyton, Cladosporium* and *Curvularia* are also commonly found. All the organisms listed above are Biosafety level (BSL) 1 and 2.

1. *Clostridium botulinum* - Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities with materials known to contain or potentially to contain the toxin. Additional primary containment and personnel precautions, such as those recommended for Biosafety Level 3, are indicated for activities with a high potential for aerosol or droplet production, and those involving production quantities of toxin. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.
2. *Escherichia coli* (Cytotoxin-producing (VTEC/SLT) organisms) - Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Animal Biosafety Level 2 facilities and practices are recommended for activities with experimentally or naturally infected animals.

3. *Salmonella* - all serotypes except typhi - Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially containing the agents. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals.
4. *Salmonella typhi* - Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials and cultures. Biosafety Level 3 practices and procedures are recommended for activities likely to generate aerosols or for activities involving production quantities of organisms.

D. Describe proper procedures for disposal of used media. Describe how to use and operate an autoclave safely. All BSL-2 cultures, stocks, and other regulated wastes are autoclaved or otherwise decontaminated, to destroy any viable organisms. All materials for autoclaving are placed in red bags that are closed for transport out of the laboratory. These bags are set in leak-proof totes. Any materials to be decontaminated off-site are packaged in accordance with applicable local, state, and federal regulations beforehand. When working with organisms with more hazardous risk, consideration is given to chemically decontaminating the wastes within the laboratory. All wastes are autoclaved. All bags autoclaved are to allow the heat and steam to effectively penetrate the wastes. Metal totes can be used to further circulate the heat for effectiveness. The autoclave is professionally serviced as scheduled by the laboratory and records are maintained of this service.

Proper PPE used with this process includes a rubber apron, heat and steam resistant gloves, and a face shield. Records are maintained of the destruction of any select toxins.

Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.

The manufacturer's instructions for safe operation of the autoclave are readily found or posted near the autoclave

E. What is an SDS (Safety Data Sheet)? Where are the SDSs located? What information can be found on a chemical reagent hazard label? Safety Data Sheets are required documents in OSHA's Hazard Communication standard prepared by manufacturers or distributors of

chemicals received by a facility to relay information about the hazards of those chemicals, etc.

SDSs are located where identified for the laboratory and on-line at the web sites of the various manufactures or distributors. Chemical labels must relate the manufacturer, his address, and phone number in addition to the major hazards of the chemical. The health hazards disclosed on the label include, whether the material is a carcinogen, a reproductive toxin, a toxic material, a corrosive, damaging to organs, a central nervous system depressant, a corrosive, an irritant or sensitizer. Any conditions that adversely affect the chemical are also relayed.

F. Does the local state regulate medical waste? What laboratory wastes are permitted to enter the sewer? What laboratory wastes are to be incinerated? EPA no longer regulates medical waste and its destruction, but many states have adapted previous EPA requirements and these requirements are still valid.

G. What are the guidelines for handling microorganisms (mostly bacteria or their toxins) in the BMBL? Organisms are mostly BSL-2 and are handled as any other BSL-2 organism described above.

H. What are engineering controls? Controls include use of biological safety cabinets, isolated work areas, use of equipment and procedures that reduce manipulation, and employment of ventilation and air filtering systems. **Describe the proper use of engineering controls in a medical products microbiology laboratory.** Properly maintained safety equipment, including biological safety cabinets (Class II) or other containment devices are used whenever procedures with a potential for creating infectious aerosols or splashes are conducted, or high concentrations or large volumes of infectious agents are used. Materials that are potentially not contained are managed in the biosafety cabinet. For example, infectious material may be centrifuged in the open lab if sealed rotor heads or centrifuge safety cups are used and if these rotors or cups are only opened inside of the biosafety cabinet. Any operation that potentially generates an aerosol is handled in the biosafety cabinet.

Plastic backed absorbent paper may be used in the cabinet to contain some of the aerosol and reduce an extensive cleanup. The cabinet is always decontaminated before and after use with an effective disinfectant. The sash is preset to 8-10 inches to minimize any air turbulence in the room that might adversely affect the cabinet's performance. Arm movements in and out of the cabinet are slow and minimal to not disrupt the performance. All supplies are placed so they

are readily found during use of the cabinet. No air grills are blocked during use.

All contaminated materials are laid in a flat container with disinfectant for later decontamination. No flames are used in the cabinet; disposable loops or electronic heaters are used for loops. Personnel are dissuaded from walking in front of the cabinet during use. A contractor certifies the cabinets annually.

I. **What are administrative controls? Describe administrative controls designed to minimize the risk of hazardous agent exposure to those personnel that are not directly involved with their manipulation. List those administrative controls that assist in the maintenance of quality control.** Cleaning crews should not be allowed in the lab except when scheduled. Only authorized personnel should be allowed in the labs and even tighter controls used for animal suites. All infectious materials that are transported should be contained. Use of plasticware is recommended to reduce the potential for breakage. All wastes are autoclaved to ensure nothing infectious enters the sewer system in the building. All materials shipped offsite are specially packaged to meet federal requirements. All equipment is disinfected before service or surplus.

J. **What safety equipment is found within a microbiological laboratory?** Eyewashes, sinks, telephones, paper towels, and liquid soap should be readily found in every laboratory. Cabinets used for spill supplies, first aid boxes, safety showers, fire pull stations, and fire extinguishers should be found in various locations throughout the microbiology area.

K. **What types of laboratory procedures have the potential to generate aerosols? How can these procedures be contained? How can the generation of aerosols be minimized?** Blending, opening containers of live organisms, using needles and syringes, splattering or spilling infectious liquids, injecting needle through septum, opening food containers under pressure, splattering droplets of infectious materials in a flame, etc. Analysts should use good technique and when needed use containment equipment such as biosafety cabinets.

L. **What decontamination procedures are in place and when are they performed?** Work surfaces are decontaminated on completion of work and at the end of the day. This usually involves a system of various decontamination sprays, such as bleach, iodine, quaternary ammonium materials, amphyll, etc. Contaminated equipment is decontaminated before it is sent for repair, sent for maintenance, placed in surplus, or

shipped out of the lab. When placing the equipment in surplus, the equipment must be also labeled in accordance with the DHHS requirements for property management to show that the equipment is clean.

M. Describe how spills are handled? Are the cleanups following a spill documented and the cleanup verified? Spills are decontaminated with an effective disinfectant and then cleaned up with absorbent pads. Any splashes or spills of viable material are immediately cleaned up with disinfectants that are effective against the organism of concern. The material is collected as chemical waste if chemicals are also present. If radioactive materials are present, the spill is then handled as radioactive waste. The area is monitored to verify no hazardous agents are present afterwards by plating swipes of the area. The decontaminated microbial spill is handled as a non-hazardous spill if no organisms are present. All areas are routinely decontaminated at the end of a project and at the end of the day in the microbiological lab. Routine settling plates and air monitoring is done to evaluate if there are any infectious issues in the lab. All spills and accidents that result in overt exposure to infectious materials should be immediately reported to laboratory management. Medical evaluation, surveillance, and treatment are provided as needed and written records are maintained in accordance with 29 CFR 1910.1020.

N. What kind of signage is to be in place in a microbiological BSL-2 laboratory? A biohazard sign should be posted at the entrance to any laboratory where etiologic agents are used. The hoods and biosafety cabinets should have dated certification stickers. It is recommended that refrigerator and freezers have signs that the units are not to be used for food, flammables, or other unsafe storage. Any restricted entrance rooms should be posted. The animal area has special requirements for posting which should be followed.

O. What are the potential routes of exposure when working with infectious organisms? The most common potential routes of exposure are inhalation, ingestion, punctures, and through the skin absorption and exposure to the mucous membrane of the eye. Any cuts in the skin are problematic when working with organisms.

P. Why is it necessary for a minimum of two people to be working in the laboratory at any given time? This is a standard policy for laboratories.

Q. Has training to perform the assigned tasks been completed and has this training been documented? All laboratory personnel receive

regularly scheduled training on a revolving annual basis on safety issues and waste management. This is also part of the training given to all new hires. FDA has started the new ORA U on-line training program with web-based training; completion of these modules is documented.

R. Is the facility designed to prevent infectious organisms from being accidentally released to other areas in the building? Laboratories have different features. Answers should be reflective of the local lab.

S. Is there a sharps safety program in place to reduce hazards when handling syringes or pipettes or other sharps? The answer for all labs should be yes and defined for that lab.

7.3. Quality Assurance and Data Integrity

A. What are culture controls and why are they required in analyses? Culture controls are specific organism strains used to verify system test environment (quality control). Testing typically employs positive and negative cultural controls. The positive cultural control assures that conditions at the time of analysis were satisfactory for the recovery of the target organism and that the media and biochemical tests give correct results. The negative cultural control demonstrates the atypical results for biochemical tests and growth characteristics and assures that differential media can give atypical reactions.

B. With whom does Data Integrity begin? Data Integrity begins with you, the analyst.

C. Why are Quality Assurance and Data Integrity so important? It ensures each test provides a complete, accurate, legible account of sample handling and analysis: Sample accountability, chain of custody, quality, methods, media/reagents/equipment, analytical data, attachments, etc. Quality assurance is needed to ensure our work products meet acceptability requirements. QA covers all the processes we run. Data integrity is needed to maintain and ensure accuracy and consistency of our data over its lifecycle.

D. What is the purpose of environmental and personnel monitoring? The purpose of environmental monitoring and personnel monitoring is to ensure laboratory conditions and personnel do not adversely impact the sample analysis or the environment.

E. Why are temperatures recorded for incubators, water baths etc.? Checks of the temperature of instruments such as incubators and water baths are needed to maintain confidence in the calibration status of the instrument. For instruments that maintain certain temperatures to encourage microbial growth, these checks are to be performed

frequently. The ALACC recommends incubators be checked twice daily (AM and PM) and water baths be checked daily.

Microorganisms only grow at certain temperature ranges, and each has an optimum temperature. If an incubator or waterbath fails to provide that temperature, the target pathogen may not be recovered. It is critical to never exceed the temperature range of the organism or it may die off.

F. Why are worksheets reviewed and signed by an analyst/Supervisor/Lead who has not worked on the sample? Worksheets are our "work product" and are used in regulatory actions by the agency. They are written by the lead analyst or electronically generated by the web application and subsequently reviewed by a check analyst, supervisor, director, compliance officer, and in some cases by our legal staff, judges, jury, etc. Not all the readers are scientists; therefore, the worksheet should be presented clearly enough for all to understand.

A poorly presented worksheet will not hold up in court, despite the elegant analytical science that may have gone into it.

It is critical that the worksheet be flawless before it leaves the laboratory. The lead analyst or additional analysts may not have detected all the mistakes on the worksheet. Therefore, the worksheet is thoroughly reviewed by a peer analyst who did not work on the sample, is more objective, and thus is less likely to overlook an error. If a peer analyst cannot decipher a worksheet, then a non-scientist has no chance. Worksheets are checked for method suitability, accuracy of calculations, accuracy of transcribed data, and completeness.

G. Why are validated methods used? 21CFR2.19 states that it is the policy of FDA to use official methods published in the AOAC, whenever possible. These methods have been validated to provide documented evidence which provides a high degree of assurance that a procedure does what it purports to do (the requirements for an intended use are fulfilled) and is reproducible by other laboratories. Use of validated methods ensures consistency within the various laboratories in the agency. Furthermore, use of validated methods ensures that our analytical work will hold up in court in defense of our regulatory actions.

The techniques of validation include using reference standards, comparison of results with other methods, interlaboratory comparisons, assessment of factors that influence the results, and assessment of uncertainty of the results. Methods are to be characterized for detection limit, selectivity, linearity, limit of repeatability and/or reproducibility, robustness, and interference from the matrix. Microbiological methods

are to achieve a low number of false positives, a low number of false negatives, high sensitivity, high specificity, and a sensitive limit of detection.

H. What is QMiS? Quality Management Info System (QMiS) is a repository for quality system documents. It also tracks quality activities such as document changes, corrective and preventive actions, etc.

7.4. Working within an ISO 5 Controlled Environment

7.4.1. Clean Rooms

A. What environmental characteristics need to be controlled in a cleanroom? Cleanrooms are enclosed areas where there is control over particulates in the air with temperature, humidity and pressure control.

B. Should a cleanroom be under positive or negative filtered air pressure and why? Cleanroom should be under positive filtered air pressure. The positive filtered air pressure should be greatest in the testing area, that is the cleanest area, lower in the non-analytical area and lower still in the gowning area. Positive pressure protects the product.

C. Why should a relative humidity in a cleanroom be controlled? Electrostatic charges at low relative humidity can develop with particulate matter and microorganisms being attracted to walls and other surfaces. High relative humidity can cause rusting of metal equipment. Microorganisms (bacteria, yeast and mold) growth can be enhanced at the higher relative humidity.

D. What ISO Equivalent Class is equivalent to Class 100 room? Class 100 room is equivalent to an ISO 5 room.

E. Name 3 acceptable agents used to disinfect the cleanroom?

These are some of the agents that can be used to disinfect the cleanroom:

1. 70% Ethanol/Isopropyl Alcohol (ETOH/IPA), 3-5% Hydrogen Peroxide (H_2O_2) (for direct contact surface disinfection)
2. Sodium Hypochlorite (1:10 – 1:100 dilution NOTE! Corrosive to stainless steel and other metals)
3. Iodophors
4. Quaternary Ammonium Compound
5. UV irradiation at 256 nanometers wavelength

6. Formaldehyde (May be toxic/carcinogenic)
7. Glutaraldehyde (May be irritating to the eyes, nasal passages upper respiratory tract and skin. Also leaves a residue.)
8. Phenol and phenol derivatives (Toxic and may cause skin sensitization.)

F. Describe the 2-bucket cleanroom disinfection method? Dedicate a separate set of buckets and mops for cleanroom disinfection of floors and walls. Buckets #1 and #2 contains the clean disinfectant solution. Saturate the mop with the cleaning solution from bucket#1. Mop the designated area. Wring the waste solution into (waste) bucket #2. Repeat until all areas are clean. When bucket #1 is dirty, replace it with another bucket with fresh disinfectant solution. Make the original bucket #1 into bucket #2 (waste bucket). Repeat process until all cleanroom surfaces are sanitized. In addition to the 2-bucket cleanroom disinfection method there is also a 3- bucket method that can be also implemented. The third bucket is a rinse bucket where the mop is rinsed after the dirty waste solution is wrung out in the second (#2) bucket and before adding solution from #1 bucket.

G. Why should you work "wet" while disinfecting the cleanroom? First clean a small portion of the floor. Step into the wet area that you just disinfected and start cleaning in the recommended pattern while always stepping into the wet area just cleaned. This will minimize the stirring up of particulate matter and microorganisms. Any microorganisms that then settle down on the wet surface will be inactivated.

H. Describe all gowning material (PPE) used for working in the cleanroom? The following gowning materials are needed for working in a cleanroom environment: shoe covers, sterile bouffant, beard cover if applicable, mask, hood, gloves, boot covers, coveralls, cuff tape, googles.

I. What are the advantages of working as a team "buddy system" within the clean room? Advantages of working as a team "buddy system" is for safety concerns, greater efficiency, assist as a third hand, each member of the team can monitor his/her partner so that they can be focused on working aseptically within the hood.

J. What are some of the safety practices that should be followed while working in the cleanroom? Some of the safety practices that should be followed while working within the cleanroom:

1. Cleanrooms are restricted areas, and access to them must be limited to authorized individuals only.
2. Protective clothing is always to be used by individuals entering and working in the cleanroom.
3. Smoking, chewing gum, and eating are forbidden in the cleanroom.
4. Avoid wearing jewelry.
5. Avoid mannerisms such as scratching, touching the head, face or body areas.
6. If possible use the “buddy system”.
7. Always utilize aseptic technique.
8. Turn off the UV light upon entering the room.
9. Watch out for spills on the floor.
10. No running or fast movements. Don’t walk around unnecessarily. Movement of cleanroom personnel should be restricted as much as possible to prevent stirring up of settled particulate matter on the cleanroom surfaces.
11. Be aware of vapors such as from alcohol, bleach or other chemicals used in the area.
12. Be careful when handling sharps, syringes, heavy equipment.

7.4.2. Isolator Technology

A. What are two key components of an isolator?

1. Closed working environment that is accessed through glove ports.
2. Interior surfaces are sterilized through a validated decontamination cycle. Commonly VHP is used.
3. Working environmental air is supplied by HEPA filters.

B. What does VHP stand for and what is its purpose in an isolator?

1. Vaporized Hydrogen Peroxide
2. VHP is a common surface sterilant that is used to decontaminate the surfaces of items placed inside an isolator as well as sterilize the interior surfaces of the isolator.

C. Why is aseptic technique still necessary within a sterility testing isolator? Although the surfaces of the interior of the isolator have undergone a decontamination cycle there may be present a low number

of microbes, in addition one or more sub samples may have microorganisms present and aseptic technique when used can prevent the contamination of sub samples that may have not otherwise contain microorganisms.

D. **What types of environmental monitoring can be performed within a sterility testing isolator?** Surface monitoring, non-viable particulate monitoring of the air and viable monitoring of the air.

E. **What is the importance of checking the integrity of isolator gloves?** All manipulations of the sample supplies and equipment within the isolator are performed by gloves. Over time these gloves may lose integrity. If a glove develops a tear or puncture it allows the uncontrolled environment outside the isolator to enter the controlled environment which may lead to product contamination and a false positive result. Consistent evaluation of the integrity of the isolator gloves after each testing session ensures that the controlled environment of the isolator is maintained.

F. **Why is a validated loading profile important?** Vaporized hydrogen peroxide is surface sterilant. If items are placed within the isolator chamber such that they occlude the surface of a nearby object it is possible that microbes may survive the decontamination cycle. If too many items that are known to absorb VHP are placed into the isolator chamber a decontamination cycle may not be as effective since the items may absorb the VHP and therefore there would not be enough the VHP present to sterilize all surfaces.

7.4.3. Biological Safety Cabinets and Laminar Flow Hoods

A. **What are the two main differences between a biological safety cabinet and a laminar flow hood?** The direction of the air flow and the protection provided to the user and the product.

B. **Why is personal protective equipment required when working in either a biological safety cabinet or laminar flow hood?** Personnel protective equipment is worn to prevent unwanted exposure and to prevent the introduction of contamination.

C. **Name three ways to prevent an interruption in air flow in a biological safety cabinet and laminar flow hood.** Some appropriate responses would be, do not rest arms or place equipment on or in front of the grill, proper closure of the sash, slow deliberate movements while working in the hood, make sure the hood is level, and don't overcrowd the hood.

D. How many classes of biological safety cabinets are there? Class I, Class II, and Class III.

E. What is an aseptic technique related caution associated with working in an open an open-faced laminar flow hood? Leaning head or body into the laminar flow hood or talking, coughing or sneezing while directed towards the face of the hood.

F. Describe the placement of environmental monitoring plates in a biological safety cabinet and in a laminar flow hood. The response would be based on the local procedure regarding placement of environmental monitoring plates in each type of hood.

G. How often do biological safety cabinets and laminar flow hoods require certification by a qualified vendor? Biological safety cabinets and laminar flow hoods require annual certification by a qualified vendor.

H. Are U.V. lights in a biological safety cabinet or laminar flow hood an effective means of decontamination? No, U.V. lights are not effective means of decontamination. U.V. lights can accumulate dust and dirt that will block the effectiveness of the light. Over time U.V. bulbs lose the necessary wattage for disinfection purposes.

I. Why is the sample and not the user protected when utilizing a laminar flow hood? Because of the directional air flow and the construction of a laminar flow hood.

J. What type of filters are used in a biological safety cabinet and laminar flow hood? High Efficiency Particulate Air (HEPA) filters are used in biological safety cabinets and laminar flow hoods.

7.5. Direct Staining and Visual Screening Method

A. What would be some visual clues of microbial contamination? Unusual color of solution, turbidity, stringy/thread-like fibers, granular or clumps either floating or settling to bottom, etc.

B. Describe various types of cell morphology. Cell morphology is an essential component in the characterization of bacteria. Once visible, a colony of cells is observed using microscopy and described based on size, structure, shape and form of the cells. Bacterial shapes include bacillus (rod-like), coccus (circular), or spiral (twisted into helices) and are identified through Gram staining practices.

C. What are fungal hyphae? Fungal hyphae are the vegetative growth of a fungus represented by long, branching filamentous strands and referred to as mycelium.

D. What are spores? Spores are cells produced by either bacteria or fungi that can give rise to new adult organisms without sexual fusion. Bacterial spores are formed in response to adverse environmental conditions and help in cell survival. They have no metabolic activity and are highly resistant to heat and disinfectants Fungal spores are microscopic biological particles that allow fungi to reproduce, serving a similar purpose to that of seeds in the plant world.

E. Describe the difference between Gram-negative and Gram-positive organisms. Organisms are differentiated by the structure of their cell walls. Gram-positive organisms contain high levels of peptidoglycan which allows for the retention of crystal violet stain. Gram-negative organisms lack a cell wall which prevents retention of crystal violet but allows for retention of the safranin counter stain.

7.6. Microbiological Examination of Non-Sterile Products

A. How does the analyst determine which controls to utilize for determination of the quantitative enumeration and the absence of specified microorganisms' analysis? The controls used for USP <61> and <62> analyses are outlined in the respective USP chapters.

B. What are some agents that can be used for neutralization of interfering substances? Glycine, Lecithin, Polysorbate, Thioglycolate, Thiosulfate, and Mg or Ca ions, among others.

C. Why is the Growth Promotion Test performed? The purpose of the Growth Promotion test is to demonstrate the suitability of the media for the intended use.

D. What three tests are performed as part of the Growth Promotion Test? Test for Growth Promoting Properties, Test for Inhibitory Properties, and Test for Indicative Properties.

E. Why is Method Suitability Testing required prior to performing analysis for determination of the quantitative enumeration and the absence of specified microorganisms? Suitability testing aides in demonstrating that the proposed test method adequately detects and enables recovery of microorganisms in the presence of a given product under a set of given conditions and meets the criteria of the USP.

F. According to USP <61>, what three methods can be used to enumerate? Membrane filtration, plate count, and most probable number.

G. What is the purpose of USP <1111>? To provide guidance regarding acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use.

7.7. Special Assignments/Non-typical

7.7.1. Non-tuberculosis Mycobacteria (NTM)

A. What are Rapid Growing Mycobacteria and Slow Growing Mycobacteria? Give some examples. NTMs are divided into two groups based on their growth rate. Rapid growers develop visible colonies within seven days. Some examples of rapid growers are *Mycobacterium abscesses*, *M. chelonae* and M. *fortuitum*. Slow growers take more than seven days to develop visible colonies. Some examples of slow growers are *Mycobacterium avium*, *M. chimera* and *M. intracellulare*.

B. What media are used for the cultivation of NTMs? Middlebrook 7H9 broth is used for sample enrichment. This is followed by isolation on Middlebrook 7H10 agar (for rapid growers) and Middlebrook 7H11 agar (for slow growers). M7H11 agar has added antibiotics to select against other bacteria and yeast during the prolonged incubation.

C. What are acid-fast bacteria? Acid fast bacteria have cell walls rich in lipid complexes, particularly in mycolic acids, which give them the 'acid fast'-specific character when colored by carbol fuchsin during the acid-fast staining procedure. Acid fast bacteria will be red, while nonacid fast bacteria will stain blue/green with the counterstain. All mycobacteria are acid fast.

D. What DNA targets are used in the identification of mycobacteria? Both 16S rRNA and rpoB gene are used. For some species 16S rRNA may provide enough species differentiation. For other species, sequencing of the rpoB gene may be necessary.

7.7.2. *Burkholderia cepacia* complex

A. What does the phrase *Burkholderia cepacia* complex mean? The phrase *Burkholderia cepacia* complex (BCC) refers to a group of over twenty species of the Genus *Burkholderia*. Within the complex, *Burkholderia cepacia* is the most common human pathogen.

B. What is the primary selective agar used in the method to detect organisms within the *Burkholderia cepacia* complex? FDA laboratories use *Burkholderia Cepacia* Selective Agar (BCSA), a selective agar for the detection of *Burkholderia cepacia* complex microorganisms. BCSA contains crystal violet, polymyxin B, gentamicin

and vancomycin as selective agents, which inhibit organisms other than BCC.

C. What is the typical morphology of *Burkholderia cepacia* colonies on BCSA? The typical colony morphology of *Burkholderia cepacia* on BCSA is growth with or without a color change of the agar from red-orange to yellow. BCC organisms oxidize the lactose and sucrose in the media. producing acid. The phenol red in the media indicates the pH change.

D. Why isn't Cetrimide agar used as the sole agar for the detection of *Burkholderia cepacia* complex? Cetrimide agar is designed to detect *Pseudomonas aeruginosa*. *Burkholderia cepacia* complex strains do not grow well on the agar and do not produce a typical morphology on the agar.

E. The *Burkholderia cepacia* complex is comprised of numerous *Burkholderia* species. What characteristics do these species have in common? Strains within the *Burkholderia cepacia* complex are Gram negative, catalase positive and do no ferment lactose.

F. Describe at least two situations in which the detection of *Burkholderia cepacia* complex may be added to a non-sterile analysis. Testing for *Burkholderia cepacia* complex may be requested or added to the testing of nonsterile medical products due to the following: an adverse event which has identified or implicated *Burkholderia cepacia* complex as the cause, the intended patient population may be immunocompromised or consist of children, historical trends observed with a product may require the analysis to be performed, inspectional findings may indicate the presence of *Burkholderia cepacia* complex in the manufacturing environment or product component or for surveillance purposes.

7.8. Sterility Testing of Medical Products

A. What is the purpose of the Method Suitability Test? The USP Method Suitability Test is a validation step for the USP Sterility test. It demonstrates that the product being tested does not exhibit inhibitory effects on the growth of microorganisms under the conditions of the test, i.e. the suitability of the test method to recover the test microorganisms in the media tested and at the level tested.

B. What is the inoculation level in CFUs to be used to inoculate product in media? The inoculum level is not more than 100 CFUs. The importance of inoculating a low-level inoculum is to show that the sterility test can detect low level microbial contamination.

C. Describe what is done to ensure the work area is acceptable for sterility testing Clean all operational surfaces within the clean room using an appropriate disinfectant and/or sporicidal solution, if needed. The area is uncluttered and exposed to the HEPA filtered air. Opened media controls and/or exposure plates are prepared in advance and used at the time of analysis.

D. What should an analyst do if the growth in air sample plates exceeds the laboratory's alert/action levels? Inform the supervisor immediately of this finding and follow local procedures and record findings.

E. When would the incubation time be extended beyond typical 14-day incubation period? The incubation time would be extended beyond 14-day incubation period in the following instances:

1. If the product was sterilized by irradiation usually medical devices such as catheters or bulk powders.
2. If the product material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination.
3. If the product contains sub lethally damaged cells may require additional growth factors because of damaged enzymes or transport systems.
4. If the organism of concern is a slow-grower requiring additional incubation time.

F. What determines if a product is an SVP or LVP? According to the USP a Small Volume Parenteral (SVP) is a product volume of 100 ml or less, A Large Volume Parenteral is a product volume greater than 100 ml.

G. What is the purpose of using two different media? The Soybean Casein Digest (SCD) broth is used to culture aerobic bacteria, yeast and mold. The Fluid Thioglycollate Medium (FTM) is used to culture anaerobic or microaerophilic bacteria.

H. How does Fluid Thioglycollate maintain anaerobic conditions? Sodium Thioglycollate lowers the oxidation-reduction potential of the media and the low percentage of agar reduces the diffusion of oxygen into the liquid media.

I. What is the function of the red indicator in Fluid Thioglycollate? The red indicator (resazurin) indicates the status of the oxidation or

aerobiosis of the broth. If red indicator is more than 1/3 of total media, the FTM may be reheated once per USP.

J. How is a sterility test by membrane filtration different from a sterility test by direct inoculation? Sterility test by membrane filtration allows the liquid drug product to pass through a membrane filter to collect the microorganisms from the product and be subsequently cultured in an enrichment medium. In Sterility testing by direct inoculation the product is added directly to the enrichment medium.

K. What is the difference between Fluid A, D, and K? When would one use one over the other? Fluid A is 1 gram of peptic digest of animal tissue in water to make 1 liter. Fluid D is the same as Fluid A except it contains 1 ml of polysorbate 80 (Tween) added to the 1 liter. Fluid K is 5 grams of peptic digest of animal tissue, 3 grams of beef extract and 10 grams of polysorbate 80 added to 1 liter of water. For the membrane filtration method, Fluid A is used to rinse aqueous solutions and soluble solids. Fluid D is used to rinse oils and oily solutions of low viscosity or devices with pathways labeled sterile. Fluid K is for rinsing viscous oils, ointments and creams.

L. What are the incubation temperatures for Fluid Thioglycollate Medium and Soybean Casein Digest Medium? Fluid Thioglycollate Medium is incubated at 32.5°C+/-2.5°C and Soybean Casein Digest Medium is incubated at 22.5°C+/-2.5°C.

M. What is the purpose of the Pharmaceutical Microbiology Manual (PMM), Chapter 3? The purpose of the 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 PMM supplements the USP for FDA analysts and provides detailed procedures for performing the sterility test.

N. If the product being tested renders the medium turbid, when should the medium be subcultured to fresh medium, according to the USP? If the product being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portion (each not less than 1 mL) of the medium to

fresh vessels of the same medium, and then incubate the original and the transfer vessel for not less than 4 days.

O. In the event of a dispute between an alternative method and the USP Sterility method, which method would prevail? The USP allows the use of alternative methods if the alternative method or technology for the specific test is suitable and validated and meets the criteria of traditional USP by being at least equivalent or better than the official compendial method. In the event of a dispute, only the result obtained using the method or procedure published in the USP is conclusive. The alternative method will not serve as a legal replacement for the official USP method which serves as the referee test.

P. During the disinfection procedure prior to entering the cleanroom which type of products may be soaked in a disinfectant? Ampoules

Q. What are the six strains of microorganisms used for growth promotion test and Method Suitability Test? Aerobic bacteria- *Staphylococcus aureus*; *Bacillus subtilis*, and *Pseudomonas aeruginosa*; Anaerobic bacterium - *Clostridium sporogenes* and Fungi - *Candida albicans* and *Aspergillus brasiliensis.*

R. What can you do to eliminate product inhibition in Suitability Testing? To eliminate product inhibition in Suitability Testing you can neutralize the activity of antimicrobial agents by dilution and or by using neutralizing agents such as Lecithin, Polysorbate, Glycine, Sodium bisulfite, Thioglycollate, Thiosulfate, Mg or Ca ions.

7.9. Investigating USP Sterility Testing Failures

A. What laboratory classification of samples would require a sterility failure investigation? Laboratory Class 3

B. What actions should be taken if a negative control is contaminated? Determine whether the organism recovered from the negative control is consistent with organisms recovered from the product or the positive control to ensure no cross-contamination occurred.

C. What records should be reviewed to determine analyst qualifications? Proficiency tests or training records.

7.10. Bacterial Endotoxin Testing

A. What is endotoxin? Endotoxin is the lipopolysaccharide component found in the outer membrane of Gram-negative bacteria.

B. What is the difference between endotoxin and pyrogens? Pyrogens are substances that cause fever when introduced into the blood stream or cerebrospinal fluid. Bacterial endotoxins are one subset of the larger category of pyrogens.

C. Is the USP <85> capable of accurately detecting pyrogens that may be in a product? Explain why or why not. No, the USP <85> is not intended to detect all pyrogens. USP <85> is a method to detect bacterial endotoxin which is only one category of pyrogens.

D. What is the assay temperature for Endotoxin Testing? 37 +/- 1°C

E. What are some factors that may cause interference in endotoxin testing? Interfering factors may be caused by product related physical inhibitors (i.e., ionic, viscosity, adsorption or sequestration of endotoxin) or chemical inhibitors (i.e., chelation, protein denaturation, pH perturbation) or environmental inhibitors (i.e., temperature, vibration) during assay in heating blocks, plastic containers (i.e., polypropylene) that adsorb endotoxin out of the product, micelle/agglutination and hydrophobic traits of lipopolysaccharides causing them to adhere to container surfaces (i.e., glass vials) or container closure system (i.e., rubber stopper).

F. What are some ways to overcome interference in the endotoxin assay? Interfering factors may be overcome via product dilution within the calculated MVD or the use of endotoxin dispersing agents, detergents, dialysis, ultrafiltration.

G. If a product's endotoxin limit is 0.5 EU/mg, the product's concentration is 200 mg/ml and the lysate's sensitivity is 0.05 EU/ml. What is the maximum valid dilution for this product?

$$MVD = \frac{\text{Endotoxin limit} \times \text{Product Concentration}}{\text{Lysate sensitivity}}$$

MVD= 2000

H. When calculating the endotoxin limit for a product with the formula, K/M what value is used for K for an intrathecal route of administration? What value is used for K for routes other than intrathecal? For an intrathecal route of administration K=0.2 USP-EU/Kg of body weight. For any other route of administration K= 5 USP-EU/kg of body weight.

I. What is the Gel-Clot technique/method based on? The test is based on clotting of the limulus blood in the presence of endotoxins. The presence of a clot indicates a positive test.

J. What is the duration of the Endotoxin Testing via Gel-Clot? 60 minutes +/- 2 minutes

K. What tests are performed to ensure the precision and validity of the Gel Clot assay? 1) Bacterial Endotoxins Test Confirmation of Labelled Reagent (Lysate Sensitivity) 2) Bacterial Endotoxins Test Product Compatibility Test for Interfering Factors, Inhibition/Enhancement Test for Gel-Clot Techniques.

L. What are acceptable results for system control, negative water control, positive product control when running endotoxin testing via Gel-Clot? When running Endotoxin Testing via Gel-Clot a negative result, indicated by the absence of a clot, is expected for both the System Control and the Negative Water Control. Duplicate sample dilution aliquots are spiked with CSE as Positive Product Controls and the spike amount is 2λ. A firm clot is formed.

M. What are the two types of kinetic methods found in USP <85>. Briefly describe each method. The two kinetic methods are the kinetic turbidimetric method and the kinetic chromogenic method. The kinetic turbidimetric method is an assay that measures the increases in turbidity of a product in the presence of endotoxin lysate. The kinetic chromogenic method measures the amount of chromophore that is released from a suitable chromogenic peptide by the reaction of endotoxin in the product and the endotoxin lysate.

N. According to USP<85>, to perform the confirmation of the lysate sensitivity what is the minimum number of endotoxin concentrations that are required for a valid standard curve? USP <85> requires a minimum of three endotoxin standard concentrations to generate a valid standard curve.

O. For the kinetic assay to be valid per the USP what are the required criteria? For a valid assay the following criteria must be met:

1. The absolute value of the correlation coefficient of the standard curve must be greater than or equal to 0.980.
2. The result for the negative water control must be less than the endotoxin detection limit of the lysate used.
3. The maximum percent for the Coefficient of Variation for Standards (%CV) must be less than 10%.

7.11. Particulate Matter

A. What is a particulate matter? Particulate matter consists of mobile, randomly-sources, extraneous substances, other than gas bubbles, that

cannot be quantitated by chemical analysis due to the small amount of material that it represents and to its heterogeneous composition.

B. What are the two methods for the determination of particulate matter?

1. Light Obscuration Particle Count Test
2. Microscopic Particle Count Test

C. What is the HIAC 9703+ Liquid Particle Analyzer flushed with and at what volume? HIAC 9703+ Liquid Particle Analyzer is flushed with ultra-pure water such as Milli-Q water, GenPure System, Nano-Pure System, double filter water or equivalent and with at least 10X the sample volume.

D. What troubleshooting techniques can be implemented if a large particle reduces or stops the liquid flow? A flush in the reverse direction can push the particle out through the sample probe by performing a backflush of the system.

7.12. Testing Environmental Monitoring Samples

A. What are the five typical types of environmental samples that could be submitted for analysis? The five typical types of environmental samples that could be submitted are sponges, sponge applicators, swabs for qualitative analysis and RODAC and Hycheck™ surface samplers for quantitative analysis.

B. What are some of the advantages and disadvantages of the quantitative method of environmental monitoring? An advantage of using the quantitative method of environmental monitoring analysis is the ability to determine the relative numbers of microorganisms within a finite area of a surface. Some disadvantages of the quantitative method of environmental monitoring analysis are that the plates or paddles cannot be used in cervices, cracks or other difficult to reach locations, colony overgrowth can make enumeration difficult, and the quantitative method only recovers a small percentage of the contact surface flora.

C. What are some of the advantages and disadvantages of the qualitative method of environmental monitoring? Some advantages of the qualitative method of environmental monitoring analysis are that a sponge or sponge applicators enable the sampling of a larger surface area, cotton tip swabs allow for sampling of cracks, crevices, and other tight areas, and sponges, sponge applicators and swabs provide the ability to apply mechanical contact pressure, which increases recovery of microorganisms. A disadvantage of the qualitative method of

environmental monitoring analysis is that microbes can become trapped in the pores of the sponge or the fibers of the cotton tipped swabs.

D. What are some of the key elements that an analyst should check the environmental monitoring sample for upon the receipt of the sample? Three key elements that the analyst needs to check for upon sample receipt is that the sample was received in the proper packaging, at the proper temperature, and within 48 hours of sample collection. Additionally, the analyst should check the sample for signs of tampering, leakage, or the potential for cross contamination.

E. What types of air monitoring plates could be used in the biological safety cabinet or laminar flow hood during sample analysis? The types of air monitoring plates are Modified Letheen Agar, Sabouraud Dextrose Agar or TSA w/5% Sheep Blood Agar.

F. What organism controls are included as part of the analysis? The five organism controls are included in the environmental monitoring analysis are *Aspergillus brasiliensis* ATCC 16404, *Candida albicans* ATCC 10231, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 6538.

G. What is the incubation timeframe and temperature for the environmental monitoring sponges or swab samples? All environmental sub samples are incubated for 14 days at 25-30°C.

H. What is the incubation timeframe and temperature for the environmental monitoring RODAC Plates and Hycheck surface samplers? RODAC Plates and Hycheck™ surface samplers are incubated at 30-35°C for two days, then incubated at 20-25°C for five days or longer when contaminants are suspected to be slow growing.

I. What are the designated time frames for the observation for growth during the environmental monitoring sample analysis? The sub samples must be observed every business day and returned to the incubator post observation. If turbidity is observed in the sub sample bag, the sub sample will be processed for isolation of the growth, and then the sub sample will be returned to incubation. On day 5, 6, or 7 and on day 14 all the sub samples must be streaked regardless of turbidity or lack of turbidity

J. When growth is observed during an environmental monitoring test what media is used to isolate the growth? Subculture all EM samples 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 medias for subculturing. A minimum of two agars should be utilized, 1 must be a nonselective agar.

7.13. Antibiotic Potency Testing

A. How do you determine the preparation steps necessary to test Neomycin Ointment for potency? The USP monograph for Neomycin Ointment should be referenced to determine the appropriate preparation of the ointment for potency testing.

B. What is the purpose of the standard curve and how many standards must be included on the standard curve? There are five standards on the standard curve. The standard curve is used to extrapolate the potency of the test sample.

C. What is the significance of the Zones of Inhibition? The Zones of Inhibition are the clearings observed on an agar for the Cylinder Plate Method. The diameter of the Zones of Inhibition is used to determine the potency of the test product.

D. Why is it important to know if the bench you are working on for the preparation of plates for the Cylinder Plate method is level? The Cylinder Plate method requires that single layer or bi-layer plates be used. The layers of agar are considered relatively thin and a level bench is necessary to ensure even agar layers and therefore consistent, round zones of inhibition.

E. What are some indicators, that can be observed on the agar plates used in the Cylinder Plate method, that the bench was not level? Some indicators that a bench which was used to pour agar plates for the Cylinder Plate method was not level include: oval zones of inhibition, pooling of the agar against one side of the petri dish and unevenness of an agar layer.

F. How many times must the analysis be independently conducted for a given sample and why? Each analysis must consist of three independent test runs with each test run consisting of triplicate plates or tubes. The analysis entails multiple variables and is dependent on multiple factors, such as inoculum levels. Therefore, three independent test runs are required to ensure accurate results.

7.14. Antimicrobial Effectiveness Testing

A. How many categories of products does the USP <51> list? Briefly describe each category. The USP <51> has four categories of products. The categories are: Category 1- Injections; other parenterals including emulsions, otic products, sterile nasal products, and

ophthalmic products made with aqueous based or vehicles. Category 2- Topically applied products made with aqueous bases or vehicle; nonsterile 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 an aqueous base.

B. Describe the types of containers the test can be performed in? When possible, the test should be carried out in the original container. If the original container cannot be used the test should be carried out in a container that is inert to the antimicrobial agent(s) present in the product. A suitable container may be sterile, large screw-cap glass test tubes.

C. What is the standardized volume of product FDA laboratories should try to test for each microorganism? The standardized volume FDA laboratories use is 20mls.

D. What microorganisms are required by the USP <51> method and in what circumstances might additional microorganisms be added? The following are the five microorganisms required by the method: *Candida albicans, Aspergillus brasiliensis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Additional microorganisms may be added to the assay by the request of the Center. Additional microorganisms might be included due to claims the labeling contains or due to an ongoing adverse event occurrence.

E. At what time points are aliquots removed and plated or filtered? At the following time points an aliquot should be removed for assay: time 0, 7 days, 14 day and 28 days. There may be additional time points added or the USP <51> time points adjusted based on recommendations from the Center.

F. How does the USP <51> define "No increase"? "No increase" is defined as not more than 0.5 log10 unit more than the value to which it is compared.

G. At what point in the method should the analyst refer to USP <1227> to determine what additional steps may be needed? USP <1227> provides useful information and guidance that would be helpful during the design of the test particularly during the suitability portion of the test method. USP <1227> provides guidance on how to demonstrate that any neutralizers used during the assay are shown to not inhibit the growth of the microorganisms.

7.15. Biochemical Identification

A. Why is it important to have a quality Gram stain prior to starting the identification process? A Gram stain is performed to determine which VITEK® card to select for biochemical identification. If the Gram stain is not prepared correctly the wrong VITEK® card could be chosen and an incorrect identification obtained.

B. How is the acceptable optical density range of the microorganism suspension determined? The VITEK® 2 Systems Product Information Manual contains the designated ranges for optical density of the microorganism suspension based on the VITEK® card being used for analysis

C. How does the analyst know if a supplemental test is required to complete the biochemical identification? The VITEK® result report will indicate a low discrimination or a slash line result, and list the supplemental tests required to separate the closely related microorganisms.

D. When preparing the microorganism suspension to load into the VITEK® 2 card, what is the maximum hold time? The maximum hold time of the microorganism suspension is 30 minutes.

E. When preparing to use the VITEK® 2 card what are some of the factors that must be considered? Allow the VITEK® card to come to room temperature prior to taking it out of the package. Do not use the card if the package liner is damaged or if there is no desiccant present. Do not touch the straw when removing the card from the package.

7.16. Polymerase Chain Reaction (PCR)

A. What are the three major steps (processes) involved in the PCR cycle? Denaturation, annealing (complementary binding of primers), and extension.

B. What components are needed in the PCR reaction mixture and what function does each element serve?

1. Template: provides source of target DNA
2. Primers: bind to specific locations on the target DNA
3. Nucleotides: provide the building blocks of DNA structure
4. Polymerase: extends the DNA strands

C. Why is selection of the primers so important to the success of the reaction? The primers will bind on either side of the target DNA.

Where they bind will determine what portions of the DNA strand are amplified and how large the PCR product will be.

D. Why are there forward and reverse primers? Primers are short DNA sequences complementary to the single-stranded DNA templates from which the polymerase begins replicating. PCR uses forward and reverse primers that bind to opposite strands of DNA, flanking the region that is to be amplified. DNA synthesis at one primer is directed toward the other, resulting in the replication of the desired sequence.

E. Why does one need an excess quantity of primers in the reaction mixture? To ensure the denatured DNA strands anneal to the primers, not to each other. The quantity of primers in the reaction also partly determines how many times the DNA can be amplified.

F. Why always run a reagent control? "DNA free" reagent control should always be run to show that there was no DNA present in the reagent to contaminate the PCR reaction.

G. List three ways to prevent contamination when performing PCR.

1. Prepare PCR solutions in an area different from the amplification area.
2. Use pipettes and supplies that are dedicated for PCR use only.
3. Use gloves.
4. Aliquot stock solutions and prepared solutions into small quantities and small containers to avoid contamination of a large volume of solution.
5. Avoid spilling and splashing of reagents.
6. Use filtered pipette tips.

H. In what ways can PCR be inhibited? PCR inhibitors can interfere with the cell lysis needed for extraction of DNA, interfere by nucleic acid degradation or capture, and can inhibit polymerase activity of amplification of target DNA. Potential PCR inhibitors include: components of body fluids (e.g. hemoglobin, urea, and heparin), environmental compounds (phenolic compounds, humic acids, and heavy metals), constituents of bacterial or fungal cells, non-target DNA, pollen, glove powder, laboratory plastic ware, and cellulose.

7.17. DNA Sequencing

A. What regions are targeted for bacterial and fungal identification by DNA sequencing? For bacterial identification we target the first 500

base pairs of the 16S ribosomal RNA (16S rDNA) bacterial gene. For fungi we target the internal transcribed spacer (ITS) regions and the D1D2 regions of the eukaryotic rRNA gene complex. These gene regions are commonly used for identification as they contain sequence stretches ranging from highly conserved to variable.

B. What are the major steps (processes) involved in DNA sequencing? Amplicon PCR, amplicon PCR clean up, cycle sequencing, cycle sequencing clean-up and capillary electrophoresis

C. Why is the amplicon PCR cleaned up using prior to cycle sequencing? To produce amplicon free of extra dNTPs and excess primers that might interfere with the sequencing reaction. ExoSAP-IT™ utilizes two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase. Exonuclease I removes residual single-stranded primers and any extraneous single-stranded DNA produced in the PCR. Shrimp Alkaline Phosphatase removes the remaining dNTPs from the PCR mixture.

D. How is cycle sequencing different from conventional PCR? Cycle sequencing takes advantage of the ability of DNA polymerase to incorporate 2′,3′-dideoxynucleotides (ddNTPs). These are deoxynucleotide (dNTP) base analogs that lack the 3′-hydroxyl group essential in phosphodiester bond formation. The cycle sequencing reaction requires DNA template, a sequencing primer, DNA polymerase, dNTPs, ddNTPs, and buffer. Each type of ddNTP (ddA, ddC, ddG, or ddT) is labelled with a different fluorescent dye. As the denaturation, annealing, and extension cycles are performed, DNA polymerase adds a dNTP or the corresponding ddNTP at each step of chain extension. When a dNTP is added to the 3′ end, chain extension can continue. However, when a ddNTP is added to the 3′ end, chain extension terminates. Cycle sequencing results in the formation of extension products of various lengths terminated with fluorescently labelled ddNTPs at the 3′ end.

E. What libraries are used for identification of bacterial and fungal sequences generated? For bacteria we use the MicroSEQ® Microbial Identification System proprietary libraries. For fungi we use two publicly available databases, NCBI and Mycobank.

7.18. Pulsed Field Gel Electrophoresis (PFGE)

A. Describe briefly the preparation of agarose plugs. Prepare cell suspensions of each isolate, utilizing Cell Suspension Buffer and reaching the correct turbidity. Make sure that the plug agarose is completely melted and tempered to the correct temperature before

using. Mix correct amounts of cell suspension, Proteinase K, and plug agarose quickly. Dispense into labeled plug wells without the introduction of air bubbles.

B. What are the conditions for plug lysis? Shaking water bath, 54°C, vigorous shaking, at least two hours shaking.

C. Describe the steps needed for plug washing.

1. Preheat water and TE buffer to 50°C. Warm shaking water bath to 50°C.
2. Two water washes, 15 minutes, vigorous shaking, 50°C.
3. Four TE washes, 15 minutes, vigorous shaking. 50°C.

D. What restriction enzymes are used for *Burkholderia cepacia* complex (BCC) and *Salmonella*? Salmonella utilizes Xba I. BCC utilizes SpeI.

E. What are the two methods for loading plugs into the gel wells? Plugs can be loaded onto the gel comb before pouring the gel, or they can be inserted into the cast wells of the solidified gel.

F. Briefly describe equipment set up for running a PFGE gel. Running buffer: 2.2 liters of 0.5X TBE, pre-cooled to 14°C and circulating in leveled gel box at a setting of 70. Gel on base plate setting in gel frame in gel box with wells toward top of gel box. Electrophoresis unit parameters set for correct organism.

G. What are the chemical agents used to stain PFGE gels, and what safety precautions are to be used to handle these agents? Staining can be done using ethidium bromide or GelRed®. Ethidium bromide is toxic and mutagenic. It should always be handled with care, wearing gloves. All waste should be disposed of as hazardous, including gels. GelRed® is non-toxic and non-mutagenic. The stain solution and gels can be disposed of down the drain or thrown away in the regular trash.

7.19. Developing/Establishing a Rapid Method for Implementation into Regulatory Testing

A. What are some of the advantages of rapid methods? They provide quicker results, are easy to use, may provide increased accuracy and sensitivity and are amenable to automation.

B. How does the rapid sterility method differ from the current USP compendial method? The rapid method has a fast turnaround time with less operator handling and provides automated results making this

less prone to human error. The compendial method is operator driven, requires daily monitoring and takes more than 14 days to complete.

C. As per the guidance outlined in USP chapter <1223> what are the two general categories used to evaluate an alternative method? The two general categories described are:

1. Qualitative Methods which include assays to demonstrate the presence or absence of microorganisms in a test article.
2. Quantitative methods yield a numerical result related to the microbial content in the test article.

D. As per USP chapter 1223 what are the validation requirements used to evaluate a qualitative method? The validation requirements for a qualitative test method includes:

1. Limit of detection
2. Specificity
3. Robustness
4. Repeatability
5. Ruggedness
6. Equivalency to the reference method

E. What does ORCE stand for? The Office of Research Coordination and Evaluation (ORCE) is the research and review arm of the Office of Regulatory Science (ORS). It supports collaborative scientific activities that help advance regulatory science.

7.20. Inspectional Guidance

A. What are some of the different types of inspections? Surveillance inspections are conducted as routine assignments to evaluate the firm's compliance with quality regulations. They are conducted every 2 years for Class II and Class III devices. Compliance inspections are conducted to verify the adequate correction of previous violations, to document continuing violations, or to support future regulatory action. For-cause inspections are carried out in response to specific information such as consumer complaints, recalls, or laboratory results, among others. Pre-Approval Inspections are conducted after a company submits an application to FDA to market a new product. These inspections verify the data included in the application and confirm that the facility can manufacture the product.

B. Why is it useful to review the previous EIR? The previous EIR provides an overview of the establishment's operations and products as well as an understanding of their compliance history. You should examine the previous inspectional findings to determine any follow-up that would need to occur at the next inspection.

C. What are 483 observations? The FDA 483 Inspectional Observations are intended to notify the inspected establishment's top management in writing of significant objectionable conditions, relating to products and/or processes, or other violations of the FD&C Act and related Acts which were observed during the inspection.

D. How does FDA classify inspection reports? Inspections are classified as No Action Indicated (NAI). Voluntary action indicated (VAI) some deficiencies identified but not serious. Official Action Indicated (OAI) serious deficiencies identified, and FDA must act to assure correction.

7.20.1. Drug Inspections

A. List two to three objectives for a pre-approval inspection. The following are the three objectives for a pre-approval inspection: 1) Readiness for commercial manufacturing; 2) Conformance to the submitted drug application; 3) Data integrity audit.

B. Explain the importance of covering good manufacturing practices (GMPs) during a pre-approval inspection. As part of a pre-approval inspection the manufacturing practices of the firm should be evaluated to the extent possible to determine if the firm is operating in accordance with cGMPs. A firm must be in compliance with GMPs as part of the assessment of the firm's ability to manufacture and market the drug product for which they have submitted for FDA approval.

C. For a GMP inspection of a sterile manufacturer what is the main system from the Compliance Program Guidance Manual 7356.002A that the microbiologist will be expected to cover? The microbiologist would be expected to provide complete coverage of the Laboratory Control System with respect to the microbiology laboratory.

D. If a microbiology laboratory lacks method suitability for sterility testing what is the potential 21 CFR 211 cite that may be used to support an observation? Although several citations may be used the following citation, 21 CFR 211.165(e). "The [accuracy] [sensitivity] [specificity] [reproducibility] of test methods have not been [established] [documented]. Specifically, ***" addresses the specific need for the test method to be suitable and fit for use.

E. If you were asked to inspect a microbiology laboratory for a sterile injectable manufacturer what three finished product release tests would the microbiology laboratory be responsible for? The three finished product release tests what would likely be completed by the microbiology laboratory or a contract testing laboratory are: sterility testing, endotoxin testing and sub-visible particulate matter testing.

7.20.2. Device Inspections

A. How are medical devices classified? Medical devices are classified as Class I, Class II, or Class III based on the risks associated with their use. Class I devices present minimal potential for harm to the user. Examples of Class I devices include enema kits and elastic bandages. Class II devices generally present a moderate risk of harm to the user. Examples of Class II devices include powered wheelchairs and some pregnancy test kits. Class III devices are those that sustain or support life, are implanted, or present potential high risk of illness or injury. Examples of Class III devices include implantable pacemakers and breast implants.

B. What is QSIT? Medical device routine inspections are generally conducted using the Quality System Inspection Technique (QSIT). QSIT is a tool designed to streamline medical device inspections. It divides the firm's quality system into four subsystems: management controls, design controls, preventive and corrective actions and production and process controls. QSIT uses a "top down" approach that looks at procedures and ask questions first, then reviews records.

C. How do different types of devices obtain market approval from FDA? Class I devices can generally be sold without preapproval. Most Class II devices must receive prior clearance from the FDA before they can be sold in the U.S. The clearance process is known as "premarket notification" and the application is referred to as a "510(k) application". In the 510K, the manufacturer must demonstrate that the device to be marketed is at least as safe and effective as, or substantially equivalent to, a legally marketed device that is not subject to PMA. Most Class III devices must undergo a more rigorous process known as "premarket approval" (PMA) before they can be sold in the U.S. In the PMA, the manufacturer must demonstrate that the device is safe and effective for its intended purpose.

7.20.3. Compounding Inspections

A. What is compounding? Compounding is a practice in which a pharmacist or doctor combines, mixes, or alters ingredients of a drug to create a medication tailored to the needs of an individual patient.

B. What are some areas to inspect/observe during a Compounding Inspection of sterile drug products? Some of the areas to cover during a Compounding Inspection of sterile drug products are: aseptic technique, process and facility design, environmental and personnel monitoring, product inspection, quality systems, equipment, containers, and closures, etc.

C. What can be a risk and cause of improperly compounding drugs? Drugs that are compounded improperly, or that are compounded under sustained conditions, can pose serious health risks (i.e. compounded drugs that are produced under insanitary conditions can become contaminated, and errors made during compounding can result in sub or super potent products.

D. Provide an example of a serious compounding related outbreak. An outbreak of fungal meningitis in late 2012 was one of the most serious in a long history of outbreaks associated with contaminated compounded drugs. It was a multistate outbreak where over 750 patients were diagnosed with a fungal infection after receiving injections of the contaminated drugs and numerous patients died.

E. Under 503B in Food, Drug, and Cosmetic Act (FDCA) if compounder becomes an outsourcing facility what must they comply with? Under 503B a compounder who becomes an outsourcing facility must comply with current good manufacturing practices requirements, must be inspected by FDA according to a risk-based schedule, must meet certain conditions, including reporting adverse events to FDA associated with their compounded drugs, must be registered as an outsourcing facility, is engaged in the compounding of sterile drugs, complies with all of the requirements in section 503B, including labeling provisions.

F. What effect did the Compounding Quality Act of the Drug Quality and Security Act have on compounding facilities? It removed certain previsions from section 503A related to solicitation of prescriptions and clarified that section 503A is applicable to compounders nationwide. It also added new section 503B: “Outsourcing Facilities”.

G. If a compounder is not registered as outsourcing facility what conditions must they meet? Compounders who do not register as outsourcing facilities must meet the conditions under section 503A or be regulated as conventional drug manufacturer.

H. How does FDA, CDER implement the compounding provisions of the FD&C Act and conduct oversight to protect public health? Implement the compounding provisions of the FD&C Act, as amended by the Drug Quality and Security Act (DQSA), conduct oversight through compounding inspection enforcement actions where appropriate to protect public safety, such as warning letters, letters referring to inspectional findings, recall, voluntary temporary or permanent cessations of operations by compounders, civil enforcement actions in collaboration with the Department of Justice.

7.20.4. EM Collections

A. In what location order do you perform an Environmental Monitoring (EM) collection? When collecting EM sample, you always start in locations that are under the greatest control and then move to sample to lesser controlled area. Work from cleanest to dirtiest. For example, start with ISO 5 HEPA filtered laminar flow hood then move to to outside the hood, room ISO 7 area.

B. Why is Day/Engley (D/E) Neutralizing Broth suitable for Environmental Monitoring (EM) collections?

1. D/E neutralizes a broad spectrum of antiseptics and disinfectants which may be present in the surfaces being sampled such as quaternary ammonium compounds, phenolics, iodine, chlorine preparations, mercurial, formaldehyde and glutaraldehyde.
2. The media contains nutrients necessary to support microbial growth.

C. What are the advantages of using sponges/swabs (qualitative method) for EM monitoring?

1. Can monitor a larger surface area
2. Can reach tight or remote areas that are excellent conditions for microbial contamination or build up that can be difficult to clean
3. The contact pressure increases the total number of organisms recovered. The mechanical process of swabbing dislodges the microbial biofilm.

D. What are the advantages and disadvantages of using RODACS/Hychecks™ (quantitative method)?

1. Advantages
    a. Assess the relative number of microorganisms present within a certain area (usually four-square inches).
2. Disadvantages
    a. Cannot reach small areas such as crevices, cracks or other hard to reach locations. These areas may be more likely to be areas of contamination.
    b. Low efficiency or recovery of microorganisms transferred to the agar surface plate.
    c. Can only be used on flat surfaces.

8. Glossary/Definitions

A. FACTS – Field Accomplishments and Compliance Tracking System
B. FDA – United States Food and Drug Administration
C. ORA – Office of Regulatory Affairs
D. ORA U – Office of Regulatory Affairs University
E. QMiS – Quality Management information System
F. USP – United States Pharmacopeia

9. Records

A. None

10. Supporting Documents

A. ORA Laboratory Manual. (current ed.)
B. ORA-LAB.016 Medical Products and Tobacco Program Review and Clearance
C. Applicable local procedures for media preparation, growth promotion testing and the laboratory quality assurance practices.
D. SOP-000288 Microbiological Controls for Medical Product Sample Analysis

11. Document History

Revision #	Status* (D, I, R)	Date	Author Name and Title	Approving Official Name and Title
00	I	See QMiS InfoCard	LMEB	LMEB

* - D: Draft, I: Initial, R: Revision

12. Change History

Revision #	Change
00	New document