

# Auditing Microbiology and Sterility Testing Laboratory

## Goals

**When you have completed this module, you should be able to:**

- Understand what the GMP requirements are for microbiological and sterility testing laboratories
- Identify which GMP regulations govern microbiological and sterility testing laboratories
- Use a range of information tools, from the contents of this training module to the Intranet, in support of microbiological and sterility testing
- Recognize compliance or non-compliance of a microbiological and sterility testing laboratories

## Definitions

**Bioburden:** The normal microbes found in raw materials, on drug product components, and other materials prior to sterilization or other forms of processing.

**Endotoxins:** Toxic molecules consisting of lipopolysaccharide originating from the outer cell wall of Gram-negative bacteria. Endotoxins may cause fever reactions in humans.

**Inoculum:** A small quantity of a microbiological organism transferred into a large volume of culture media to grow large quantities of the microbiological organism.

**Objectionable organism:** A microorganism that has been shown to cause harm to humans. Depending on the route of administration and the type of drug product, these organisms will be different for different products.

**Microbial limits testing:** A test used to determine the quantity of organisms in a pharmaceutical raw material, in process sample, or finished sample and indicator organisms for a particular drug product.

**Media:** A substrate for growing microorganisms. If the tests are compendial tests, the type of growth media (e.g. trypticase soybean casein digest, blood, etc.) will be suggested. Different media are used to characterize different microorganisms based on nutritional requirements/preferences.

**Microbial cultures:** The growing of microorganisms, tissue cells, or other living matter in a specially prepared nutrient media.

**Out of specification (OOS) result:** A laboratory test that is outside its regulatory or compendial limits. In some cases, there may be additional tests and/or limits that are used to assess the quality of a material, but are not included in registrations or compendia. In these cases, the general principles described here are useful, but more latitude is allowable in the disposition of the material as long as it meets its legal requirements.

**Out of trend (OOT) result:** A test result that lies outside of a current trend analysis for a particular product. The test result itself may be within the acceptance criteria for the test.

## **Auditing Microbiology and Sterility Testing Laboratory**

SOPs and equipment manuals should be available for all instrumentation. If equipment maintenance logbooks are used, they should be up-to-date with complete entries and controlled.

SOPs for laboratory equipment should include:

- Maintenance frequency and maintenance activities
- Calibration (if appropriate)
  
- Cleaning, and operation
- Method of cleaning/disinfection, cleaning agents, and frequency of cleaning
- Operation of equipment including monitoring frequency and documentation
- Emergency procedures in the case of a power outage or temperature deviation

### **Qualification of Equipment**

Critical instruments and equipment should be qualified and calibrated and included in a routine calibration program.

Equipment used to provide a controlled temperature should be set at the appropriate temperature for its intended use. Qualification should include temperature mapping where appropriate as for walk-in incubators and ovens.

Autoclave load patterns should be established, assigned to cycles, and tested. Tests should include heat penetration studies and biological challenge tests. If media is sterilized in house it should take into account the heat sensitivity of some media and the risk of ‘oversterilising’ the media. Autoclave re-qualification should occur periodically, according to established change control procedures and with associated test protocols to verify that the autoclave has remained in a validated state.

### **Stock cultures**

Microbial cultures are pure strains of one particular microorganism. Stock cultures are used as inoculum for testing or reference samples. The cultures can be either isolated from an environmental sample or purchased commercially as a pure strain.

Microbial cultures may be kept almost indefinitely if care is taken during the transfer process. Microbial cultures should only be transferred or “passed” five successive times if they will be used as positive controls or in assays. The passages and dates of transfer should be documented. Stability and maintenance of cultures should be documented.

Once an agar slant containing a culture is removed from storage, it should not be used again.

Storage conditions for cultures should be monitored and documented. Cultures may be frozen onto sterile glass beads and stored at -20 C. They may also be stored under ° nitrogen in a mixture of glycerol, to prevent cell breakage upon thawing. Cultures may also be lyophilized (freeze dried) and kept in a powder state. The laboratory should have an SOP that includes storage conditions for microbial cultures used in the laboratory.

Microbial cultures and test plates with growth should be inactivated before being sent for disposal. The procedures for inactivation should be in compliance with the site’s waste disposal policy and procedure.

### **Culture media**

Culture media may be prepared by the individual laboratory or may be purchased from an

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## **Auditing Microbiology and Sterility Testing Laboratory**

Care should be taken when collecting a water sample. The port should be sanitized, flushed for a specified number of minutes or volume equal to normal use, and the sample collected aseptically (i.e., hose not touching the container) in sterile container. Samples should be refrigerated and tested within 24 hours.

### **Antimicrobial Effectiveness Test**

The Antimicrobial Effectiveness Test, a test described in the pharmacopoeias, demonstrates the effectiveness of the preservative system in a product. A product is tested against a controlled quantity of different types of microorganisms. The test then compares the level of microorganisms found in a control sample versus the test sample over a period of 28 days.

This testing is performed as part of a stability study. It is necessary to determine if a

preservative system will continue to be effective over the product's shelf life and if the preservative system is compatible with the formulation of the product. If a formulation changes or a significant product or packaging change occurs, it is necessary to retest the effectiveness of the preservative system.

### **Growth Promotion Testing**

This test is performed to indicate that the selected test media is capable of supporting microbial growth. Environmental isolates may be used in addition to indicator organisms

to test growth promotion. Organisms should be incubated at their optimum temperature so that erratic results are not generated.

### **Identity Testing of Microorganisms**

Microbial organisms found through testing may need to be identified. The degree of identification needed and when should be specified in an SOP. The site should have an approved SOP that contains the test method in use. Identification may be performed through a series of testing with selected growth media or through a rapid microbial identification system. Rapid microbial systems use electronic instruments designed specifically for microbial ID. The equipment should be validated and there should be validated written procedures for operation and maintenance. Cultures of known microorganisms should be used as positive controls to verify that the test is working properly. There should be an SOP in place describing the test parameters and the operating conditions.

### **Environmental Monitoring**

This testing may include personnel, surface, water, air and other specialized testing. Agar test samples from the air, personnel, and surfaces are incubated appropriately to grow both fungal and bacterial contaminants. The temperature ranges used for incubation may be based upon the relevant compendia, regulatory guidance or validated conditions.

If growth appears, the laboratory should isolate the particular organisms, and characterize them as appropriate. When to characterize and to what level should be described in SOP. All isolates and characterizations should be documented as to date, and type of sample.

# Auditing Microbiology and Sterility Testing Laboratory

## Key Parameters in Auditing a Microbiological and Sterility Testing Quality Laboratory

### Prior to the audit

- Find out which products are tested in the laboratory
- Find out which methods/specifications should be used
- Request a list of laboratory SOPs.
- Request a list of laboratory equipment.
- Request a list of laboratory deviations, out of specifications and/or out of trend investigations from the previous 12 months.
- Review previous audits to determine if there are pending actions.

### During the audit

- Conduct a walkthrough of the laboratory.
  - Verify that there are designated areas for performing various testing functions.
  - Verify that the laboratory is maintained in a clean and orderly fashion.
  - Verify that the laboratory is in good repair, (i.e. no chipped paint on walls, no loose ceiling tiles, etc.).
  - Verify that there is physical and dress discipline segregation from other laboratories.
  - Verify that personnel are following the dress code for the area.
  - Verify that all reagents and chemicals are labeled with expiration date and have not expired.
  - Verify that equipment has been calibrated.
  - Verify that incubators, freezers and refrigerators are temperature monitored.
  - Verify that incubators have been subject to temperature mapping studies.
- Ensure that documentation is in place and approved.
  - Verify that the laboratory has approved SOPs on the following general topics:
    - Cleaning of laboratory and equipment.
    - Maintenance and disposal of laboratory cultures.
    - Operation, maintenance, and cleaning of incubators, ovens, refrigerators/cold vaults, hoods, and water baths.
    - Transfer methods, maintenance and storage of stock cultures.
    - Operation, maintenance, and cleaning of autoclaves.
    - Managing a microbiological laboratory out of specification result.
    - Managing a laboratory spill.
    - Preparation and storage of stock solutions, reagents and culture media.
  - Verify that the laboratory has a system for collecting and maintaining data. If the system is computer based, it must be validated and comply with applicable ERES requirements. Review data and verify that the chosen product is tested as required.
  - Ensure that the test method has been qualified for the product.
- Ensure that a procedure is followed for sampling and handling samples.
  - Verify that samples are labeled properly and uniquely identified.
  - Verify that there is a system in place that assures samples are stored under correct conditions.
  - Verify that the sample is signed in using a well documented and established procedure.
  - Verify that there is a documented procedure for logging samples out of the lab.