

Auditing Microbiology and Sterility Testing Laboratory

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Audit Training Manual: 014

**Auditing Microbiology and
Sterility Testing Laboratory**

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Pyrogens: Fever producing substances.

Rapid Microbial Identity Test: A commercially prepared test that is able to identify microorganisms through interactions with chemicals within a matter of hours instead of days.

Stock cultures: Pure microbial cultures that are kept for use in the lab. Sources of these cultures may be the American Type Tissue Collection or microorganisms isolated from the production environment.

Explanation of Topic

Introduction

The purpose of this training module is to provide information on what to include in a microbiological and sterility testing laboratory audit. Microbiological testing is used to release products, determine if production environments are satisfactory and determine if results of in-process testing are satisfactory.

Laboratory Administration, Organization and Personnel

It is important to understand how the microbiological laboratory functions. When auditing the lab, review the current organization chart. Determine what tests the lab performs. Determine the distribution of personnel across shifts.

Discuss with the lab manager or responsible person any corrective actions from previous audits. Determine the status of these corrective actions and if they were corrected within the agreed time period.

Personnel

Personnel working in a microbiological laboratory should follow general laboratory rules such as that lunches or other non-microbiological items should not be stored in the same refrigerator used for storage of laboratory cultures or tests. There should also be an approved SOP regarding dress code, covering the need for physical and dress discipline segregation from other laboratories and production. These rules apply to employees and visitors/contractors to the lab.

All personnel working in the laboratory should be trained in both GMP and the technical skills required for their job function. GMP training should be given at intervals specified in a site SOP. Personnel should also be formally trained and qualified, according to site procedure on the operation of specific instruments, and the use of specific methods and techniques. Personnel working in the microbiological laboratories should be trained in contamination control including aseptic techniques. This competency and all training should be documented. Personnel should have an extensive understanding of sources of contamination.

The site should have an SOP on training to include qualification practices/programs in the microbiology laboratory.

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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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significant regarding system control and potential impact on product. Consideration should be given to timely results to initiate corrective action vs. slower recovery methods that give higher counts.

Microbial Limit testing

This test is performed to determine the quantity of microorganisms in pharmaceutical ingredients and non-sterile medicinal products. The microbial limits test includes a standard plate count for total aerobic bacteria and molds and yeasts as well as several designated microbial species such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella species* and *Escherichia coli*.

Microbial Limit testing is performed on API materials, raw materials, other pharmaceutical ingredients and non-sterile medicinal products.

Bioburden Testing

Components, raw materials, and equipment are tested to determine the quantity and type of microorganisms they contain. The same techniques as for microbial limit testing are applied. This testing is usually performed on materials undergoing sterilization, e.g., components and solutions, as part of aseptic processing. The results of the bioburden testing can be used to set sterilization parameters.

Pyrogen/Bacterial Endotoxin Test

Sterile products and their components are usually tested for pyrogens and endotoxins. The current accepted test is the Bacterial Endotoxin Testing, also known as the Limulus Amoebocyte Lysate (LAL) test. This pharmacopoeial test is performed to determine if there is any endotoxin in the product or component. Methods used are the gel clot method, turbidimetric and chromatogenic methods. The gel clot method is sensitive to vibrations and equipment needs to be placed in a stable position.

The site should have an SOP that defines what should be tested and how it should be tested. The SOP should also include established endotoxin limits and maximum valid dilution for each test item.

Standard Test Methods-Water

Water used in pharmaceutical production and the test area should be tested for the number of organisms and type of microorganisms. Depending on the type of water used in the manufacturing process there will be different pharmacopoeial requirements. Examples are shown below.

Type of water	Total aerobic count	Microbiological characteristics
Drinking water	≤500 cfu/ml	No coliform organisms present
Purified water	≤100 cfu/ml	Absence of objectional organisms
Water for Injection (WFI)	≤0.1 cfu/ml	Essentially sterile

Purified water is typically used in the final steps of active pharmaceutical ingredients (API) processing and in the manufacturing of solid dosage forms, e.g., formulation and cleaning. WFI is used for sterile injectables. Total aerobic counts should be tracked and trended.

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Media fills

Filled items will be incubated and inspected for growth (turbidity). If growth appears, the laboratory should isolate the particular organisms, and characterize them as appropriate.

Sterility testing

Since sterility testing is a test for release of sterile product, it should be conducted under the same conditions as production. The cleanliness level of the testing facility should be equivalent to the product facility. Analysts should be qualified and highly trained in aseptic techniques. The material flow and handling of test samples should be similar to how product is handled. Microbial environmental monitoring should be conducted.

Sterility testing is a pharmacopoeial test. Testing should be performed using known to be sterile negative controls. Material should be handled in the same way as material is handled in production and the facility should be part of the site's environmental monitoring program.

Sterility testing may be carried out in a LAF unit placed in an environment suitable for aseptic work or in an isolator. Isolators are freestanding and sealed from the outside

environment. The analyst tests samples by either using glove ports or donning a half-body suit built into the isolator. If the sample is tested within an isolator, the isolator should have controlled HEPA filters. The isolators are decontaminated typically with hydrogen peroxide (H₂ O₂), though there are other agents used. Materials usually enter through a port that is connected to an autoclave.

Because of the nature of the closures and the barriers, the cleaning and disinfection process should be validated. The validation should also include the effectiveness of the disinfection against the organisms that will be used in the isolator. There should be an

SOP that includes when the isolator should be disinfected, the frequency, and the duration of contact with the chemical. The interface with the autoclave and the isolator needs also to be validated.

When sterility tests are performed in a clean room environment, the following must be taken into consideration:

- Certification of pre-sterilized materials
- Media preparation, storage and certification
- Maintenance of control cultures
- Bacteriostasis and fungistasis testing
- Laboratory operations
- Environmental conditions

The site should have an approved procedure in place to manage positive sterility tests e.g. growth detected. If there is a positive sterility test, an investigation needs to be conducted. The organism needs to be identified. Laboratory test failures over time need to be reviewed. The organism isolated from the positive test should be compared to any organism isolated from environmental monitoring tests to determine if the environment is the source. Environmental monitoring results for production should be reviewed. The isolated organism from the positive sterility test should also be compared with any organisms isolated from product pre-sterilization bioburden. Production records should be reviewed for events or conditions that would affect product sterility.

The pharmacopoeia only allows retesting if certain criteria are met. Retesting should be rare.

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the laboratory should have a system in place for speedy processing of the sample. If the sample is unable to be processed, it should be held under temperatures and conditions that will slow any deterioration of the sample.

Out of Specification or Out of Trend Investigations

Specifications should be based on sound scientific reasoning and established during product development. They should also be specific for the drug product or sample being tested. Sometimes the laboratory results will not be within specifications e.g. they are considered out of specification.

Some results may also be considered out of trend. This is determined through monitoring and comparing data for a specific test. Over the course of time a trend may be noted that the assay is approaching the limit of its acceptance criteria. The site should also be monitoring laboratory results of test samples to determine if there is an out of trend result. Trends may indicate a process drift and should be investigated and the cause determined. An approved SOP for defining what constitutes a trend and how to manage out of trend results should be in place.

Investigations should be conducted for those test results that are outside the acceptance criteria of the test performed. The site should have an approved SOP or testing protocol that includes details of the out of specification and out of trend elements to be investigated. If there are automated systems for documenting tracking and trending they should be validated.

Change Control Program

All laboratory equipment, methods and procedures should be part of a site change control program. This program should be outlined in an approved SOP.

All changes to both equipment, including software and hardware changes, and analytical methods, need to be:

- ✓ Documented with a description of the work performed
- ✓ Evaluated for impact on qualification,
- ✓ Approved prior to implementation.

Computer equipment changes, including software and hardware changes need to be documented, evaluated for impact on qualification, and approved prior to implementation.

Changes to analytical methods need to be properly documented, evaluated for impact on method validation and approved prior to implementation. Change control procedures should also include directions for incorporating changes based on pharmacopoeia requirements and require that the incorporation is timely.

Summary

Microbial testing is necessary for product testing. Microbiological testing can prevent microbially contaminated product from reaching the market. It also, through in-process testing, ensures that our process is under control from a microbiological perspective. This assurance is achieved through personnel highly trained in aseptic techniques, validated/qualified testing methods, qualified equipment, and extensive environmental monitoring.

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- Verify that site requirements for sample handling and storage are included in a laboratory SOP.
- Verify that samples are handled and stored based on their storage and handling instructions.
- Ensure that laboratory results and data are secure and accurate.
 - Verify that data is checked by a second person.
 - Verify that there is an approved SOP on the topic of second person review.
 - Verify that data is secure with only authorized personnel able to access it.
 - Verify that raw data is recorded in a notebook or on controlled sheets of paper, not on loose paper.
 - Verify that data is reported correctly according to approved procedure, i.e. each data point is recorded, as specified in the SOP, versus an average taken.
 - Verify that any data reported includes its units of measurement.
 - If loose sheets of paper or computer printouts are used to record additional data, verify that each page includes the number of the notebook and notebook page it should be attached to.
 - Verify that requirements are the same for automatic raw data capture with computerized systems.
- Ensure that out of specification and out of trend results are investigated.
 - Verify that the site has an SOP in place.
 - Verify that the site is following the SOP through review of out of specification and out of trend investigations.
 - Verify that once an out of specification or out of trend is discovered, the rationale for the subsequent steps is based on sound scientific reasoning.
- Ensure that there is a change control program that includes the microbiological laboratory.
 - Verify that methods, equipment, software, and instrumentation are part of the change control program.
 - Verify that appropriate approvals and levels of approval are in place.
 - Verify that testing after the modification or change is performed.
 - Verify the results are within the acceptance criteria.
- Ensure that personnel are trained and follow laboratory procedures.
 - Verify that new employees, experienced employees and supervisors are fully trained/qualified.
 - Verify that any contract employees (lab analysts and calibration and PM personnel) are fully trained and qualified to perform the assigned tasks.
 - Ensure that training requirements are defined in an approved SOP.
 - Ensure that lab personnel are receiving GMP and job skills training with an emphasis on aseptic technique and that it is documented.
 - Verify that all laboratory personnel follow the dress code and general laboratory rules established by the site.
- Ensure that laboratory equipment and instrumentation are maintained and operated according to approved SOPs.
 - Verify that equipment and instrumentation (including software) are qualified.
 - Verify that equipment and instrumentation (including software) is part of a preventive maintenance schedule.
 - Verify that equipment is current with calibration and/or preventive