

Microbiology Best Laboratory Practices



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USP<1117> Microbiological Best Laboratory Practices *USP 32 page 616*

- Introduction:

good laboratory practices in a microbiology laboratory consist of activities that depend on several principles: aseptic technique, control of media, control of test strains, control of equipment, diligent recording and evaluation of data, and training of the laboratory staff.

Major areas of audit in the microbiological laboratory

1. Reagents and Media
2. Reference Standards
3. Equipment and Instruments
4. Method Validation
5. Laboratory Controls
6. Recordkeeping and Documentation
7. Employee Training
8. Sample Control

Media, Reagents Preparation, Storage, Qualification

- **It's important to choose the correct media or components in making media based on the use of accepted sources or references for formulas.**

Media Preparation, Testing & Storage Audit Questions

- **How do you preserve your product?**
- **How do you validate that the media you use is the correct one for your product? (Show me your procedure)**
- **Do you have an objectionable microorganism list? Do you include environmental isolates in your sample validation.**

Media, Reagents Preparation, Storage, Qualification

- **Preparation of Media**

Media Preparation, Testing & Storage Audit Questions

- **SOPs of media preparation**
- **How do you control the quality of dehydrated media and water**
- **Reagent must prepared as validated**
- **PM and calibration of balances, graduate cylinders**
- **Glassware wash validations<1051>**
- **Sterilization associated records**

Media, Reagents Preparation, Storage, Qualification

- Preparation of Media - Tractability

Media Preparation, Testing & Storage Audit Questions

- Dates
- Type and lot of material used
- Ingredient quantities
- Preparer's identification
- Sterilization cycle #
- Expiration date
- SOPs

Media, Reagents Preparation, Storage, Qualification

- **Preparation of Media - Storage**

Media Preparation, Testing & Storage Audit Questions

- **Segregation and labeling - FIFO**
- **Follow manufacture's instructions**
- **Do not freeze prepared media**
- **Storage duration and re-melting need to be validated**
- **Refrigerator PM and calibration records**

Media, Reagents Preparation, Storage, Qualification

- **Preparation of Media – Qualification**
 - **How often? –Routinely, (validated vs. None validated)**
 - **What to do**
 - **pH**
 - **Growth promotion**
 - **Sterility check**

Media, Reagents Preparation, Storage, Qualification

Preparation of Media – Qualification

- pH



Media, Reagents Preparation, Storage, Qualification

Preparation of Media – Qualification

- Growth Promotion – What Organisms
 - USP
 - EM isolate
 - Objectionable organisms
 - The capability of the media to promote the growth of organisms may be affected by the media preparation process, sterilization (overheating), and storage
- Sterility Check

Media, Reagents Preparation, Storage, Qualification

The LAL reagent is critical in Endotoxin testing, and must be well controlled.

When the LAL reagent (lot) changes, the Micro Lab must confirm the labeled LAL reagent sensitivity λ (EU/ml). This is to re-calculate the Maximum Valid Dilution (MVD), which is the maximum allowable dilution of a sample to determine the endotoxin limit.

Id reagents need to be qualified routinely

Reference Standards

- **Microbial cultures are delicate standards. Procedures should specify careful handling instructions.**
- **Preparation and resuscitation of cultures should follow the instructions of the supplier or a validated, established method.**
- **USP recommends using the "Seed-Lot" technique for storage of stock cultures, i.e., using working cultures and never returning unused passages back to original stock.**
- **In addition, there should be an established maximum number of passages (5 or less), and maximum storage time for working cultures.**

Reference Standards

- **Cultures for use in compendial tests should be acquired from a national culture collection, in frozen, freeze-dried, on slants, or in ready-to-use forms.**
- **Confirmation of the purity and the identity should be performed prior to its use in quality control testing.**
- **Ready-to-use cultures may require additional confirmation of inoculum size.**

Purchased Cell Cultures

- 1. When purchasing microorganisms from a national culture collection, what incoming QC tests are run for identity and purity? Is the ID done via genotypic analysis?**
- 2. How are the # of passages of working cultures tracked, and what is the maximum # permitted (PIC/S says NMT 5 passages for cultures used in pos. ctls of sterility tests)?**
- 3. Ask to see the records of subculturing a purchased organism.**
- 4. How long can a working culture be used (≤ 1 wk)?**

Equipment and Instruments

- **Equipment**
 - Incubators
 - Refrigerators
 - Water bathes
 - Autoclave
- **Instrument**
 - KQCL machine
 - pH meter
 - Balance
 - Spectrophotometers
 - Air sampler (viable, none-viable)

Equipment and Instruments

- **Validation**
 - IQ
 - OQ
 - PQ
- **Calibration (PM)**
- **Verification**

Equipment and Instruments

- **Validation**
 - **Approved Protocols – Equipment should be qualified with intended application**
 - **Execution**
 - **Report**

Method Validation

- **Microbiology method**
 - **Antimicrobial Effectiveness Testing <51>**
 - **Biological Indicators <55>**
 - **Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests (Bioburden) <61>**
 - **Microbiological Examination of Nonsterile Products : Tests for Specified Microorganisms (Microbial Limits)<62>**
 - **Sterility<71>**
 - **Endotoxin<85>**

Method Validation

- **Method Validation**
 - **21CFR 211.194a cGMP**
 - **USP <Compendial>**
 - **CDER May 2001 guidance for Industry on Bioanalytical Method Validation”**
 - **ICH guideline [Q2A,Q2B and Q6B].**

Method Validation

- **Method Validation**
 - **Procedure (SOP)**
 - **Accuracy**
 - **Precision**
 - **Repeatability**
 - **Selectivity (specificity)**
 - **LOD/LOQ**
 - **Linearity**
 - **Range**
 - **Robustness**
 - **Method Qualification**
 - **Method Validation**

Method Validation

- **Sample Validation** (Test methods capture unique sample requirements, e.g., validated preparatory steps prior to moving into the common steps)
 - **Bacteriostasis/Fungistasis (sterility, bioburden)**
 - **Inhibition/Enhancement (endotoxin)**
 - **Prep. Test (microbial limit)**
 - **Neutralization (PE)**
- **SOP**
 - **How often**
 - **Acceptance Criteria**

Laboratory Controls

- Laboratory Operations/Housekeeping
 - Safety first. The Micro Lab should practice aseptic techniques during testing in general, to avoid microbial contamination and false positives
 - In the Micro Lab, areas where EM, water, or product samples are handled/incubated must be adequately separated from areas where there are tests that involve live cultures or subculturing, microbial ID, or investigations
 - BSC

Laboratory Controls

- Laboratory Operations/Housekeeping
 - Housekeeping must be properly maintained to prevent use of expired or contaminated testing mat's.
 - Verify cleanliness of work stations, cleared of extraneous or previous test mat'ls, prompt removal of refuse, and clean utensils and equipment
 - Know your auditors

Recordkeeping and Documentation

- Documentation

- What to document

- Microbiologist training and verification of proficiency
- Equipment validation, calibration, and maintenance
- Equipment performance during test
- Media preparation, sterility checks, and growth-promotion and selectivity capabilities
- Media inventory and control testing
- Critical components of test conducted as specified by a procedure
- Data and calculations verified
- Reports reviewed by QAU or a qualified responsible manager
- Investigation of data deviations

Recordkeeping and Documentation

- Documentation

- How to document

- User friendly

- Microbiology laboratory includes procedures and test methods, work instructions (i.e., calibration and maintenance), protocols, guidelines, manuals, etc. Furthermore, assurance must exist that testing histories are accurate and complete by having a defined system for issuance, monitoring, and reconciliation of worksheets printed/used
- SOP must reflect actual practices and test methods and must be in conformance with application commitments and/or compendium requirements.

- Who to write, to review and to approve. (QAU)

- If used for the operation of the lab and are critical for validity of results, they must be approved by the Quality Unit.

Recordkeeping and Documentation

- Proper recordkeeping is critical for the Micro Lab. A test should be performed as per SOP, and the laboratory notebook should provide a record of all critical details needed to confirm the integrity of the data. At a minimum, the laboratory write-up should include the following:
 - Date
 - Material tested
 - Microbiologist's name
 - Procedure number
 - Document test results
 - Deviations (if any)
 - Documented parameters (equipment used, microbial stock culture nos. used, media lot nos. used)
 - Management/Second review signature

TIMELY

Training of Personnel

- Company
- Department
- General Microbiological
- On the job (function)
 - Demonstrated proficiency
 - Maintain the proficiency

Sample Control

- Procedure
- Traceability

Sample Control Audit Questions

1. Ask how Micro samples are logged in and stored.
2. Does the sample log book (or other record) provide spaces for who delivered the sample and who then took it for testing (chain of custody)?
3. What type of samples might be temporarily stored while awaiting testing?
4. What method validation or compendial ref supports the sample storage conditions (e.g., water)?
5. What site SOP governs what happens when a water or an EM sample time point is missed? (should be a deviation).
6. If LIMS is used for tracking all samples and activities, check if pen raw data precedes computer and whether the former is properly retained.

Data Interpretation

- What does numbers mean?
- What does +/- mean?
- What does ID mean?

Sterility Test Audit Questions

Sterility suite is designed like a mf'g Grade A clean room, e.g.:

- a) aseptic gowning area/airlock w/ step-over bench division, full-length wall mirror, gowning instructions, hand washing, drying, and antiseptic application;**
- b) annual cleanroom certification per ISO stds;**
- c) NLT 10-15 Pa press. differential with adjacent rooms, the reading taken at least prior to entering suite;**
- d) flush-mounting of power outlets, light fixtures, hands-free intercom, etc., and no extraneous equipment;**
- e) outer surfaces of samples & equipment entering test suite is treated w/ sterile sanitizer (in EU, latter must be monitored);**
- f) environmental monitoring similar to Aseptic Core of Mf'g.**

Sterility Test Audit Questions (cont'd)

Additionally:

- a) janitorial/cleaning supplies must be sterilized before use;**
- b) ultraviolet lights, if used, are kept on at all times except when testing is in progress or when viable particle EM is occurring;**
- c) UV lights must be on an intensity measuring schedule;**
- d) where there is more than one parallel UV tube, they should be shielded from each other;**
- e) the LAF hood must be on at least 30 min prior to any use;**
- f) LAF hood is annually certified (i.e., magnehelic gauge, calibration, HEPA filter scan for leaks and an Emory challenge.**

Sterility Test Isolators Audit Questions

- 1. Ask for isolator validation & compare to USP <1208> criteria:**
 - a) Set point of overpressure of interior can be maintained and controlled during operation? Is there a press. hold test?**
 - b) a computational fluid dynamics analysis (CFD) and/or smoke studies performed to determine the worst case airflow locations in the isolator?**
 - c) a six-log sterilization kill is confirmed in 3 consecutive validation studies, & is BI resistance to the sterilization process estimated?**
 - d) containers, media, filter sets, tubing, and other supplies kept inside the isolator are known or proved unaffected by sterilant penetration?**
 - e) frequency of re-sterilization justified with data. This includes proof of a maintained aseptic environment throughout a defined operational period.**

Sterility Test Isolators Audit Questions (cont'd)

- 2. Ask for isolator use SOP and any gowning requirements (e.g., no rings, watches or other sharp objects, including long nails).**
- 3. Is the isolator directly in the flow path of an air supply grille (latter could cool sections of isolator's walls to cause condensation during vapor sterilization).**
- 4. Ask to see isolator envir. monitoring SOP & the (viable and non-viable) specs or action/alert levels for air, surfaces, and gloves.**
- 5. If any isolator EM plates have shown growth, were the organisms identified? Ask to see these records.**
- 6. Does PM SOP include a glove (and half-suit) integrity test, preemptive replacement, and possibly submersion testing of gloves in a 0.1% peptone water followed by filtration of diluent and plating? How about transfer system gaskets and seals?**
- 7. How is the identity and composition of the gassing agent (sterilant) assured (e.g., incoming inspection or supplier certification)?**
- 8. Is the gas generator in the PM program?**

(Water) Sample Control Audit Questions

- 1. Ask how water samples are drawn; compare to site SOP.**
- 2. Is the chemistry sample collected before the Micro (i.e., if a spray disinfectant is used on the sample port)?**
- 3. If any sampling sites are also points of use (POU) for Mf'g, how comparable are the Micro sampling and Mf'g cleaning/rinsing/use of the same POU's?**
- 4. Does the sample record contain time sample taken, sampler's name, sample site, time sample delivered to Lab?**
- 5. Are water-sampling bottles reused with a validated sterilization cycle and a proper storage/protection in between?**
- 6. Is the expiration date on those stored water sampling bottles supported by a study (or any data)?**
- 7. If there is a maximum number of hours that may elapse between sampling and bacterial enumeration testing, is the actual time or hour of sampling recorded along with the date?**

(Water) Sample Control Audit Questions

- 8. Ask for the most recent enumeration data of a specific critical water POU (should contain or refer to date/time sample taken, date/time plated and put in incubator & date/time out of incubator + incubator ID+ lot # of media used).**
- 9. What media is used to test water for counts? (If that water goes into a European-marketed product, then R2A Media).**
- 10. Ask to see growth promotion testing of the media (some companies require a water system isolate to be included).**
- 11. When is a colony identified? Ask for the SOP covering this decision (should be obtainable from within the Lab itself—workers need proximity to their SOPs).**
- 12. Ask to see total viable count & the organisms identified in the WFI loop (for all POU's) for past 18 mos.**

Analyst Training Audit Questions



1. **Ask to see the Lab's org. chart.**
2. **Has cross-training created sufficient designated back-ups for critical activities in the Lab (or even for reviewing analysts' results)?**
3. **Ask to see the training curricula for each unique Lab position.**
4. **Determine the most recent effective date for a change to a Micro SOP, then examine training records for updated training on the new revision.**
5. **Ask to see the Microbiology OOS procedure.**
6. **Ask to see the OOSs in Micro for the past 18 mos.**
7. **Can patterns be seen in tests or analysts associated w/ OOSs, and if so, what CAPAs have followed?**

Micro Contract Services Audit Q's



1. What external service providers (ESPs) does the site use for micro-related work?
2. What documented qualification data (e.g., audit reports, CVs, completed questionnaires) can be shown for these ESPs?
3. What indicates that an ESP is site-quality-unit-approved?
4. Ask to see quality/technical agreements between the site and the respective ESPs, especially when the ESP performs routine micro tests w/ product release implications.
5. Do reports provided by ESPs contain a review signature by an appropriate manager or supervisor within the site?
6. Can the site request and receive copies (or review at the ESP) test result or other raw data residing at the ESP?
7. Do changes to any procedures or specs of contract tests require prior approval by the site? Stated in quality/tech agreement?
8. How does site track & reassess an ESP's continuing performance?



Biological Indicator Audit Questions

1. What BI's are purchased by the site?
2. Ask to see the incoming QC test SOPs for all of them.
3. Is the bacterial population and purity confirmed for every supplier lot of BI?
4. Is the D-value ≥ 1.5 min for 121°C steam sterilization? (required by AAMI, ISO, and USP <1035>)
5. Has the D-value claimed by the supplier ever been verified by a qualified lab with a BIER (biological indicator evaluator resistometer) Vessel per ANSI/AAMI ST44 or USP <55>? If “no”, ask how the site knows D ≥ 1.5 min

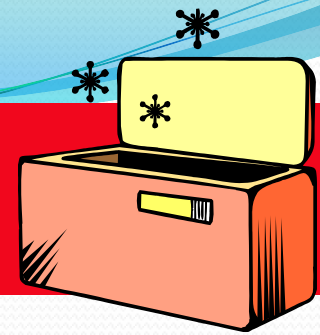
Biological Indicator Audit Questions (cont'd)

6. If accepted on certificate of analysis, what BI supplier quality assurance has been established? Ask to see file. Supplier should be “approved.”
7. Read the BI vendor’s use instructions, then look for data on elapsed time between cycle exposure and incubation.

Environmentally-Controlled Chambers/Rooms Audit Questions

1. Show how refrigerators, freezers, & liquid N2 tanks are monitored.
2. Do these have a logbook or form for contents and is it accurate and timely updated?
3. What, if any, disaster recovery plan exists for some of these environmentally-controlled rooms/chambers?
4. What actions would be taken if a large walk-in refrigerator or freezer failed?
5. What procedure exists to capture an extended temperature excursion and to assess impact on contents of affected chamber? Does that procedure allow for a 10 min (or similar) temp. excursion due to door opening?
6. Ask to see the most recent calibration record and SOP of the temperature probe for a refrigerator (does it have an equipment #. If not, why not?).
7. Ask to see the last PM report and its SOP (check if there's a test for the alarm) for the refrigerator, freezer, incubators, stability chamber.

Env-Controlled Chambers/Rooms Audit Questions



8. Are multi-shelved incubators temp.-mapped?
9. Ask to see temp.-mapping studies for one or some of the above chambers. If no mapping ever done, are there temp. probes for monitoring multiple locations?
10. Ask to see the site SOP(s) for qualifying and monitoring environmentally-controlled chambers.
11. For one of the chambers or incubators that has a chart recorder, ask for last month of archived charts.
12. Do the charts have initials/signature and date for its installation and for its removal and review?
13. If the monitoring and alarm system for all or some of the chambers is computerized, ask to see its computerized system validation protocol and executed protocol.
14. Is any cell culture or sensitive mat'l stored in a frost-free freezer (latter have warming cycles of up to 3 hr/day up to -3C)?

Sanitizer Preparation & Use



1. What sanitizers are used in the Micro Lab? Ask to see Micro Lab sanitization SOP and sanitizer preparation SOP.
2. Is each lot of sanitizer released by the site QC?
3. Does sanitization SOP specify: a) type & temp. of water used to prepare sanitizing solution; b) the method of applying sanitizer; c) contact time; d) post-contact drying method & time?
4. Are all Micro Lab sanitizers purchased sterile or rendered sterile (if to be used in Grade A or B areas)?
5. What qualification (i.e., sanitizer effectiveness testing) exists for each Micro Lab sanitizer and do they result in prescribed application quantity & contact time?
6. What records show that specified contact time is achieved?
7. What data supports exp period of the sanitizer at its use dilution?
8. What evidence shows that a non-sporocidal sanitizer working solution, or even hand antiseptic sprays and wipes, remain sterile through expiration, and are they monitored?

Environmental Monitoring (EM)

1. Ask to see the mf'g suite active air and passive air viable monitoring SOPs (either classified, unclassified, or both). Is the description of where settle plates or hand-held active air samplers sufficiently specific (e.g., supplemented w/ exactly mapped sampling locations inside the suite(s)).
2. If it's possible to observe a surface EM sampling,
 - a) see when a disinfectant spray is applied to sampling location (should only be done immediately afterward).
 - b) check what disinfectant or cleaner was used, whether it's within expiry and whether it was ever validated (mainly for a disinfectant).
3. For settle plate monitoring, check the procedure's exposure time versus actual practice. Also, If a laminar flow hood is monitored on an EM program, does Micro use a side-by-side pos. control plate or did it validate exposure time (to guarantee against agar desiccation)?

Microbiological Isolates



1. Explain how local manufacturing area air, non-product-contact surface, gown/glove, and water microbiological isolates are obtained and cultured?
2. Ask to see the “library” list of environmental isolates.
3. Is there an SOP on the culturing, subculturing, and “librarying” of local environmental isolates? Ask to see.
4. Does the procedure explain when the “library” is updated or revised with new organisms (especially for growth promotion tests)?
5. When sampling from non-product-contact surfaces, are inactivators such as Tween or lecithin used/ formulated within certain media (because of residual disinfectant)?
6. What data can demonstrate recovery from a non-product-contact surface of a spiked test organism, using the above-mentioned inactivator in the media?

Unknown Organism ID Testing

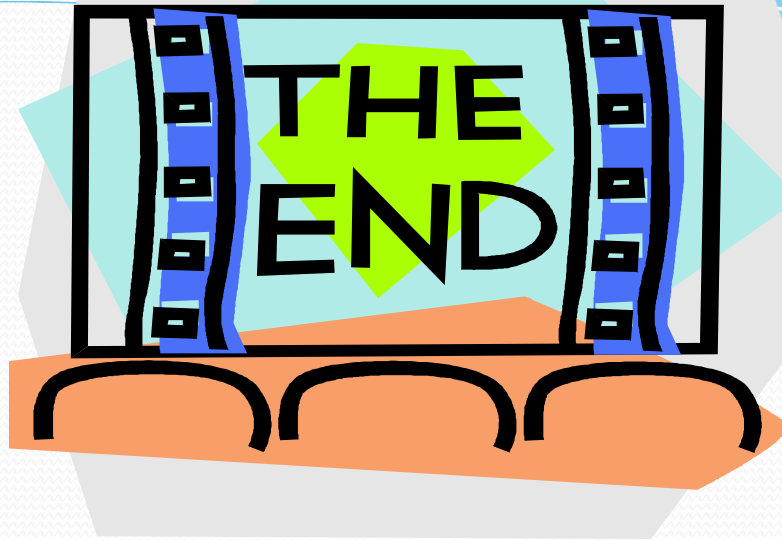


1. What method(s) are used for microbiological isolate ID?
2. Does Micro use genotype (i.e., DNA) ID for sterility test failures?
3. Have the ID methods been developed/validated here or tech transferred to this Micro Lab from another company site?
4. If fatty acid analysis via gas chromatography is used, describe the procedures for GC system suitability and calibration.
5. Is a database used for recording and trending organism ID results? If yes, ask to see this demonstrated.
6. Is the database validated plus Part 11- compliant?
7. What viable particle EM is performed in the Micro Labs?



Unknown Organism ID Testing (cont'd)

8. Ask to see the trends of identified organisms in the air and on the surfaces of Micro Lab & inside sterility test suite or isolator.
9. How is trending performed. For example, are all points in a room averaged to get a result?
10. When do water testing counts require ID and when is a water microorganism objectionable and/or requiring full speciation?
See Attachment 3, WGD 10,509 "Evaluation of Microbial Isolates Found in Water or Steam Systems"



Please ask any questions

