# MICROBIOLOGICAL BEST LABORATORY PRACTICES

USP <1117>

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# MEDIA PREPARATION AND QUALITY CONTROL

- Type of Media
  - Ready prepared media
  - Ingredients  media
    - accepted sources or reference
  - Dehydrated media (Manufacturer's formula)
- Weighing
  - A calibrated balance with appropriate weight range for the ingredients should be used
  - Clean weighing containers and tools
- Glassware
  - Cleaning process removes debris  and foreign matter

- Water
  - purified
  - deionized or distilled may be appropriate in certain cases
- Dehydrated medium
  - dissolved in water before dispensing and sterilization
- Equipment should be appropriate to allow for
  - controlled heating
  - constant agitation
  - mixing of the media
- When adding required supplements to media
  - adequate mixing of the medium after adding the supplement

- Sterilization of Media: performed within parameters provided by the manufacturer or validated by the user
  - Autoclaving by moist heat
  - Boiling : avoid deterioration of heat-labile components
  - Filtration may also be appropriate for some formulations
- The effect of the sterilization method and conditions on the media should be validated by
  - sterility
  - growth promotion

- Sterilized by moist heat
  - Typically, manufacturers recommend using an autoclave cycle of 121◦C for 15 minutes
  - However, the sterilization time will be dependent on the media volume and autoclave load
  - The autoclave cycle should be validated
    - ensure the proper heat distribution for selected loads and volumes
    - balancing the need for sterile media against the tendency of the media to degrade under excessive heating

- Improper heating or sterilizing conditions may results in
  - A difference in color change, loss of clarity, altered gel strength, pH drift from the manufacturer's recommended range
  - Reduced growth promotion activity and/or selectivity
- Storage of the media in the autoclave after liquid cycle complete is not recommended after cooling, as it may damage the media

- Media Storage
  - Ready prepared media
    - Lab should store according to the manufacturer's instructions
  - Media prepared in house
    - should be stored under validated conditions
    - Do not store agar at or below 0◦C, as freezing could damage the gel structure
    - Protect stored media from exposure to light and excessive temperature
    - Before prolonged storage, agar plates should be placed into a sealed package or container to retard moisture loss

- Remelting of an original container of solid media
  - performed only once to avoid overheating or potential contamination
  - performed in a heated water bath
  - The use of microwave ovens and heating plates should be taken to avoid
    - damaging media by overheating
    - the potential injury to laboratory personnel from glass breakage and burns
- The molten agar medium should be held in a monitored water bath at 45-50◦C for NMT 8 hrs.

- Caution should be taken when pouring the media from a container immersed in a water bath. Wiping the exterior of the container dry before pouring may be advisable
- Disposal of used cultures media (as well as expired media) should follow local biological hazard safety procedures

# Quality Control Testing

- Prepared media should be checked by appropriate inspection of plates and tubes for the following
  - Crack containers or lids
  - Unequal filling of containers
  - Dehydration resulting in cracks dimpled surfaces on solid medium
  - Hemolysis
  - Excessive number of bubbles
  - Microbial contamination
  - Status of redox indicators (if appropriate)
  - Lot number and expiration date checked and recorded
  - Sterility of media
  - Cleanliness of plates (lid should not stick to dish)

- Performed on all prepared media, including media associated with swabs or media in strips and other nontraditional formats
- Tests routinely performed on in-house prepared media should include
  - pH
  - growth promotion
  - Inhibition
  - indicative properties (as appropriate)
  - periodic stability checks to confirm the expiration dating

- pH
  - should be confirmed after it has cooled to room temp.(20-25◦C) by aseptically withdrawing sample for testing
  - Refrigerated purchased media should be allowed to warm up to ambient room temp.
  - A flat pH probe is recommended for agar surfaces, and an immersion probe is recommended for liquids
- Growth promotion
  - Test organisms may be selected from the appropriate compendial test chapter.
  - In addition, microorganisms used iselected from the appropriate compendial test chaptern growth-promotion testing may be based on the manufacturer's recommendation for a particular medium

- Inhibition
- Indicative properties (as appropriate)
- Periodic stability checks to confirm the expiration date

- The length of shelf life of a batch of media

- depend on the stability of the ingredients and formulation under specified conditions, as well as the type of container and closure

- When a batch of media does not meet the requirements of growth-promotion testing, an investigation should be initiated to identify the cause.

- This investigation should include a corrective action plan to prevent the recurrence of the problem

- Diagnostic reagents purposes to help support identification e.g., Gram stain and oxidase test reagents.
  - Select the microorganisms, following the manufacturer's instructions
  - Perform the testing before unknown sample diagnostic testing
- All relevant diagnostic reagents should be subjected to incoming quality confirmation before use

# Maintenance of Microbiological Cultures

- Biological specimens
  - The most delicate standards
    - their viability and characteristics are dependent on
      - handling
      - and storage
  - Standardizing the handling and storage of cultures
    - should be done in a way that will minimize
      - the opportunity for contamination
      - alteration of growth characteristics

- Cultures for use in compendial tests should be acquired from a national culture collection or a qualified secondary supplier.
  - frozen,
  - freeze-dried,
  - slants,
  - ready-to-use forms

- Confirmation before its use in quality control testing
  - purity of the culture
  - identity of the culture
- Preparation and resuscitation of cultures should follow the instructions of the supplier or a validated, established method
- The Seed-lot technique is recommended for storage of stock cultures

- The original microorganisms from the national culture collection or a qualified secondary supplier is resuscitated and grown in an appropriate medium
- Aliquots of this stock culture (the first transfer or passage) are suspended in a cryoprotective medium, transferred to vial, and frozen at -30◦C or below, until use.
- If stored at -70◦C, or in lyophilized form, strains may be kept indefinitely
- These frozen stocks can then be used to inoculate monthly or weekly working cultures

- Once opened, do not refreeze unused cell suspensions  after culturing a working suspension
- The unused portion should be discarded to minimize the risk of loss of viability and contamination of the stock

- The number of transfers
  - working control cultures should be tracked to prevent excessive subculturing that increase the risk of phenotypic alteration or mutation
  - Allowable for specific compendial tests may be specified in that test
- One passage is defined as the transfer of organisms from viable culture to a fresh medium with growth of the microorganisms
- Any form of subculturing is considered to be transfer/passage

# Laboratory Equipment

- Most equipment : incubators, water baths, and autoclave
  - Standard validation practices
    - incoming qualification,
    - operational qualification
    - performance qualification
  - Periodic calibration : generally annually
- Instruments : pH meters and spectrophotometers
  - should be calibrated on a regular schedule and tested to verify performance on a routine basis

- The frequency of calibration and performance verification will vary based on the type of instrument and the important of that equipment to the generation of data in the laboratory
- Regular cleaning and sanitization of equipment :
  - incubators, refrigerators, and water baths
    - To minimize the potential for contamination in the laboratory
- Door seals of incubators and refrigerators should be cleaned and checked for state of repair

- Equipment that is difficult to sanitize (such as refrigerators and incubators) should be dedicated to aseptic operations (such as storage of media for testing and incubation of sterility test sample) and live culture operations to minimize the potential for inadvertent contamination of the tests

- Autoclaves are central to the operation of the laboratory and must have proper validation in place to demonstrate adequate sterilization for a variety of operations
- Autoclave resources must be available (and validated) to
  - sterilize waste media (if performed in that laboratory)
  - the media prepared in that laboratory

# Laboratory Layout and Operations

- Laboratory layout and design should carefully consider the requirements of good microbiological practices and laboratory safety
- It is essential that cross-contamination of microbial cultures be minimized to the greatest extent possible, and it is also important that microbiological samples be handled in an environment that makes contamination highly unlikely

- In general, a laboratory should be divided into clean or aseptic areas and live culture areas
- Areas in which environmental or sterile product samples are handled and incubated should be maintained completely free of live cultures, if possible

- If complete separation of live and clean culture zones cannot be accomplished
  - other barriers and aseptic practices should be employed to reduce the likelihood of accidental contamination
  - These barriers include
    - protective clothing
    - sanitization and disinfection procedures
    - biological safety cabinets designated for clean or aseptic operations only

- Procedures for handling spills or mishaps with live cultures should be in place, and all relevant technical personnel should be trained regarding these methods

- Some samples will demonstrate microbial growth and require further laboratory analysis to identify the contaminants. When growth is detected, the sample should be taken from the clean section of the laboratory to the live culture section without undue delay

- Subculturing, staining, microbial identification, or other investigational operations should be undertaken in the live culture section of the laboratory
- If possible, any sample found to contain growing colonies should not be opened in the clean zone of the laboratory
- Careful segregation of contaminated samples and materials will reduce false-positive results

- It is important to consider that
  - microbial contamination of samples, which leads to false-positive results, is always possible unless careful aseptic precautions are taken
- Facilities should be designed so that raw material and excipient sampling can be done under controlled conditions, including proper gowning and sterilized sampling equipment

- All testing in laboratories used for critical testing procedures should be performed under controlled conditions
  - sterility testing of final dosage forms, bulk product
  - seed cultures for biological production
  - cell cultures used in biological production

  - Isolator technology is also appropriate for critical, sterile microbiological testing

- Isolators have been shown to have lower levels of environmental contamination than manned clean rooms, and therefore, are generally less likely to produce false-positive results

# Sample Handling

- Viable microorganisms in most microbiology samples are sensitive to handling and storage conditions.
- Therefore, it is important to minimize the amount of time between the sampling event and the initiation of testing and to control, as much as possible

- Product mixing before sampling may need to be evaluated and applied in order to ensure microbial dispersement and representation in the sample aliquot.
- All microbiological samples should be taken using aseptic techniques.

# Microbiological Media Incubation Times

- Incubation times for microbiological tests of less than 3 days duration should be expressed in hours
- Tests longer than 72 hours duration should be expressed in days

- For incubation times expressed in hours, incubate for the minimum specified time, and exercise good microbiological judgment when exceeding the incubation time
- For incubation times expressed in days, incubation started in the morning or afternoon should generally be concluded at that same time of day

# Training of Personnel

- Each person engaged should have
  - Education : Microbiology or a closely related biological science
  - Training
  - Experience

- A coherent system of standard operating procedures (SOP) is necessary to run the microbiology laboratory
- These SOPs describe the methodology that the microbiologist will follow to obtain accurate and reproducible results, and so serve as the basis for training
- The procedures in which a particular microbiologist has demonstrated proficiency, the procedure number or title also serves to identify what training the microbiologist has received specific to his or her job function

- Training curricula should be established for each laboratory staff member specific to his or her job function
- He or she should not independently conduct a microbial test until qualified to run the test
- Training records should be current, documenting the microbiologist training in the current revision to the particular SOP

- Periodic performance assessment should provide evidence of competency in core activities of the microbiology laboratory
  - Hygiene
  - Plating
  - Aseptic technique
  - Documentation
  - Others as suggested by the microbiologist’s job function

- Laboratory supervisors and managers have a demonstrated level of competence in microbiology at least as high as those their supervise
  - to provide assistance in areas which the persons may not have adequate knowledge and understanding

# Laboratory resources

- The laboratory management is responsible for ensure that the laboratory has sufficient resources to meet the existing testing requirements
- A measurement of laboratory performance
  - The number of performed on tests conducted by the laboratory
  - The period of time between sample submission and initiation of testing
  - The period of time between end of test and report release

- Significant delays in these measures are also indications of an under-resourced laboratory staff
- Budgetary considerations related directly to the need of the laboratory for sufficient resources must be addressed to ensure reliable testing results

# Documentation

- Documentation should be sufficient to demonstrate that the testing was performed in a laboratory and by methods that were under control

# Documentation

- This includes, but is not limited to, documentation of the following
  - Microbiologist training and verification of proficiency
  - Equipment validation, calibration, and maintenance
  - Equipment performance during test (e.g., 24 hour/7 day chart recorders)
  - Media preparation, sterility checks, and growth-promotion and selectivity capabilities

# Maintenance of Laboratory records

- Proper recording of data and studies is critical to the success of the microbiology laboratory
- The over-riding principle is that the test should be performed as written in the SOP
- The SOP should be written to reflect how the test is actually performed
- The laboratory notebook should provide a record of all critical details needed to reconstruct the details of the testing and 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
  - Deviation (if any)
  - Documented parameters
    - equipment used
    - microbial stock cultures used
    - media lots used
  - Second review signature

- Every critical piece of equipment
  - Should be noted in the write-up
  - Should be on a calibration schedule documented by SOP and maintenance records
- Equipment temperature should be recorded and traceable
  - water bath
  - Incubators
  - autoclaves

- The governing SOP and revision should be clearly noted in the write-up
- Changes in the data should be crossed off with single line and initialed
- Original data should not be erased or covered over
- Test results should include the original plate counts, allowing a reviewer to recreate the calculations used to derive the final test results
- Methods for data analysis should be detailed in cited SOPs

- If charts or graphs are incorporated into laboratory notebook, they should be secured with clear tape and should not be obstructing any data on the page
- The chart or graph should be signed by the person adding the document, with the signature overlapping the chart and the notebook page

- Lab notebook should include
  - page numbers
  - a table of contents for reference
  - an intact timeline of use
- All laboratory records should be archived and protected against catastrophic loss
- A formal record retention and retrieval program should be in place

# Interpretation of Assay results

- Analytical microbiological assay results can be difficult to interpret for several important reasons
  - Microorganisms are ubiquitous in nature, and common environmental contaminants--particularly organisms associated with humans—predominate in many types of microbiological analysis
  - The analyst has the potential to introduce contaminating organisms during sample handling or processing in the laboratory

- Microorganisms may not be homogeneously distributed within a sample or an environment
- Microbiological assays are subject to considerable variability of outcome

Therefore, apparent differences from and expected outcome may not be significant

- Because of these characteristics of microbiological analysis
  - laboratory studied should be conducted with the utmost care to avoid exogenous contamination as previously discussed
- When results are observed that do not conform to a compendial monograph or other established acceptance criteria, an investigation into the microbial data deviation is required

- There are generally two distinct reasons for the observation of microbial contamination that does not comply with a target or requirement
  - Lab error or lab conditions that produced an invalid result
  - Product contains a level of contamination or specific types of contaminants outside established levels or limits
- Laboratory management and the Quality unit should be notified immediately

- All microbiological conditions or factors that could bring about the observed condition should be fully considered
- In addition, an estimate of the variability of the assay may be required in order to determine whether the finding is significant

- The laboratory environment, the protective conditions in place for sampling, historical findings concerning the material under test and the nature of the material, particularly with regard to microbial survival or proliferation in contact with the material, should be considered in the investigation

- In addition, interviews with the laboratory anaylyst (s) may provide information regarding the actual conduct of the assay that can be valuable in determining the reliability of the results and determining an appropriate course of action

- If laboratory operations are identified as the cause of the nonconforming test outcome, then a corrective action plan should be developed to address the problem(s)
- Following the approval and implementation of the corrective action plan, the situation should be carefully monitored and the adequacy of the corrective action determined

- If assay results are invalidated on the basis of the discovery of an attributable error, this action must be documented
- Laboratory also should have approved procedures for confirmatory testing (retesting), and if necessary, resampling where specific regulatory or compendial guidance does not govern the conduct of an assay investigation