Mycobacterial Culture
1. Essentials for the Mycobacteriology Laboratory: Promoting Quality Practices

1.1 Introduction

**Mycobacterial Culture**

**Presentation Topics**

1. Overview and purpose
2. Media
3. Automated systems
4. Culture process
5. Reporting
6. Contamination
7. False positives

Notes:

Welcome to the Association of Public Health Laboratories Series, Essentials for the Mycobacteriology Laboratory, Promoting Quality Practices.

This module will cover the topic of Mycobacterial Culture.
1.2 Overview and Purpose

Notes:

In the first section, we'll discuss the purpose of mycobacterial culture and describe the overview of the process.
### 1.3 Mycobacterial Culture

<table>
<thead>
<tr>
<th>Mycobacterial Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Gold standard for sensitivity and specificity</td>
</tr>
<tr>
<td>• Use of culture increases the number of TB cases found by 30-50% over smear alone</td>
</tr>
<tr>
<td>- ~10 viable bacilli/mL of sputum needed for culture compared to at least 5000 bacilli/mL of sputum for microscopy</td>
</tr>
<tr>
<td>• Culture used for species identification, drug susceptibility testing (DST), and genotyping</td>
</tr>
<tr>
<td>• Culture also used to monitor patient response to treatment</td>
</tr>
</tbody>
</table>

**Notes:**

Mycobacterial culture remains the gold standard method for identification of TB illness.

Using culture increases the number of TB cases identified by 30 to 50 percent over smear alone. You'd need only 10 organisms per a ml of sputum to identify a positive culture, compared to five thousand organisms per a ml of sputum for a positive smear.

Isolation of the organism is critical for species identification, drug susceptibility testing, genotyping, and is also used to monitor a patient's response to treatment.
1.4 Purpose of Mycobacterial Culture

**Purpose of Mycobacterial Culture**

- **Detection of *Mycobacterium tuberculosis* complex (MTBC)**
  - The most clinically significant mycobacterial species for public health
  - Isolation almost always signifies disease, except in the case of laboratory cross-contamination
  - MTBC organisms are not present in the environment

- **Detection of Non-tuberculorous Mycobacteria (NTM)**
  - Can be opportunistic pathogens in humans and may cause significant human disease
  - Clinicians ultimately responsible for determining the importance of a NTM
  - Almost all of these species can be found in environmental samples

**Notes:**

The isolation of *Mycobacterium tuberculosis* complex (MTBC) via culture is important.

TB complex is the most clinically significant species for public health. The isolation of the organism almost always indicates disease, the exception being cross contamination in the lab, which we will discuss later. MTBC is not present in the environment.

Isolation of non-tuberculalous mycobacteria (NTM) from culture can also be significant. While they are often found in the environment, species of NTM can be opportunistic pathogens in humans, and can cause significant human disease. Clinicians are ultimately responsible for determining the importance and significance of an isolated NTM.
3. Media

Notes:

In the next section, we'll talk about mycobacterial culture media.
3.2 Culture Media

Notes:

Typically, specimens are cultured on solid media which is either egg- or agar-based, and a liquid media.

Ideally, media must be able to support rapid and robust growth of small numbers of mycobacteria and must allow for preliminary differentiation of species based on pigment production and colony morphology. The ideal media will also inhibit the growth of contaminants.

Because there is no single media which will do all of these things, both a liquid and solid media are recommended for initial culture.

These may be prepared in house or purchased commercially. However, quality control is important in either case.
3.3 Solid Media: Egg-based

Lowenstein-Jensen (LJ)

- Selective; malachite green-Inhibits growth of contaminating bacteria and fungi
- Supports growth of MTBC better than NTM
- Shelf life of 6-12 months when refrigerated
- May have excess of water which should be removed before inoculation

Notes:

The most commonly used egg-based solid media is Lowenstein-Jensen or LJ. Lowenstein-Jensen is a selective agar containing malachite green to inhibit the growth of contaminating organisms.

LJ will support the growth of MTBC better than NTM.

LJ has a six month shelf life when refrigerated, but often will have an excess of water on the slant, which should be removed prior to inoculation.
3.4 Solid Media: Agar-based

Notes:

For solid agar-based media, Middlebrook 7H10 or 7H11 are used. These media are clear, allowing easier colony observation and quantification. It's easy to isolate a mycobacterial colony if the specimen becomes contaminated.

Middlebrook 7H10 and 7H11 can be selective if antimicrobial agents are added to inhibit the growth of contaminating flora.

Typically, the average time to detection of growth is less for agar-based media than for the egg-based media.

As opposed to Lowenstein-Jensen, Middlebrook 7H10 and 7H11 are available as a plate or a slant. The slant has a longer shelf life but it's easier to visualize isolated colonies growing on a plate.
### 3.5 Comparison of Solid Media

**Comparison of Solid Media**

<table>
<thead>
<tr>
<th>Conditions for Mycobacteria Growth and Recovery</th>
<th>Egg-Based Media</th>
<th>Agar-Based Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selective</td>
<td>Malachite green</td>
<td>Antibacterial and antifungal</td>
</tr>
<tr>
<td>Rate of Growth</td>
<td>Slower</td>
<td>Faster, better able to support growth of fastidious organisms</td>
</tr>
<tr>
<td>Contamination</td>
<td>Media may become discolored or liquified</td>
<td>May still be able to isolate colonies of mycobacteria in the presence of contaminants</td>
</tr>
<tr>
<td>Area of inoculum</td>
<td>Relatively small (slant)</td>
<td>Larger (if plate used)</td>
</tr>
<tr>
<td>Visual Examination</td>
<td>More difficult due to media opacity, can only visualize upper surface of colonies</td>
<td>Media is clear, allows for visualization of upper surface and underside of colonies (if plates used). Allows for easier quantification of colonies.</td>
</tr>
<tr>
<td>CO₂</td>
<td>Helpful but not required</td>
<td>Required for duration of incubation (M-48 and Man Clin Micro)</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>Long (6-12 months); Good for storing cultures</td>
<td>Short (1-3 months)</td>
</tr>
</tbody>
</table>

**Notes:**

This slide shows a side by side comparison of the egg-based and agar-based media which can be used for reference.
3.6 Liquid Media

Notes:

Liquid media is recommended as standard practice in mycobacteriology labs.

In general, there is an increased recovery of mycobacteria and a decreased time to detection when compared to solid media.

The average time to growth of slow-growing mycobacteria is 12 to 16 days.

There are some fastidious species that will only grow in liquid media.

The shelf life of liquid media is long, the tubes can be stored at room temperature, and CO2 is not required for incubation.

However, liquid media is more easily contaminated than solid media, requiring the addition of antimicrobials.
4. Automated Systems

Notes:

The next section we'll talk about automated liquid culture systems.
4.2 Automated Systems

**Notes:**

Automated liquid culture systems will continuously monitor the media for detection of growth in the culture tube for six weeks.

The most common FDA-cleared automated systems are the BacT/ALERT 3D from bioMerieux, the BD BACTEC MGIT, and Thermo Scientific VersaTREK™.

The MGIT and the VersaTREK are also FDA-cleared for drug susceptibility testing of MTBC.

We will discuss each in limited detail in the next slides. For specifics on each system, it is important to read each package insert in its entirety.
4.3 BacT/ALERT 3D

BacT/ALERT 3D

- BacT/ALERT MP media consists of:
  - Modified Middlebrook 7H9 broth
  - Reconstitution Fluid
  - Antibiotic Supplement
- Non-sterile and sterile body sites except blood
  - Blood specimens require specific bottles: BacT/Alert MB
- Detection of growth
  - Colorimetric sensor monitors changes in CO₂
  - As CO₂ is produced, sensor at the bottom of bottle changes from gray to light green or yellow
- Uses sharps for inoculation
  - May use needleless devices but must follow package insert carefully

Notes:

The BacT/ALERT system by bioMerieux, consists of a modified Middlebrook 7H9 broth, a reconstitution liquid, and an antibiotic supplement.

Both sterile and non-sterile body sites are acceptable as specimen types, however blood requires a specific bottle: the BacT/ALERT MB.

Growth in this bottle is detected through a change in the level of carbon dioxide. A colorimetric sensor responds to changes in the CO₂ level in the bottle.

This system does require the use of sharps for inoculation. There are needleless device options, but labs must be sure to follow the package insert carefully.
4.4 MGIT

**MGIT**

- **Culture system consists of:**
  - Modified Middlebrook 7H9 broth
  - Growth supplement
  - Antimicrobial agent mixture: PANTA
    - Inhibits gram-negative and gram-positive bacteria
    - Once reconstituted must be used within 5 days

- **Not approved for urine and blood specimens**

- **Detection of growth**
  - Fluorescence detected after consumption of O\(_2\) by growing organisms
  - A manual fluorescent detector is also available

**Notes:**

The Becton-Dickinson MGIT system, consists of a modified Middlebrook 7H9 broth, a growth supplement, and PANTA, which is an antimicrobial agent mixture designed to inhibit gram-negative and gram-positive bacteria.

The MGIT system is not approved for urine or blood specimens, though published studies have demonstrated its utility with urine.

Growth is detected as fluorescence which is produced as oxygen is consumed by growing organisms.

This system has the option for a manual read as well.

The MGIT system is available as the 960 or the 320, which refer to the number of bottles it can hold.

As a reminder, any off-label modifications from an FDA-approved test such as the use of urine with MGIT, will require a full validation in the laboratory.
4.5 VersaTREK

Notes:

The VersaTREK system by Thermo Scientific, consists of a modified Middlebrook 7H9 broth, a growth supplement, antibiotic supplement, and a cellulose sponge to provide an increased surface for microbial growth.

This system is acceptable for most specimen types, including urine and blood.

Growth is detected as a change in headspace pressure, due to the consumption of oxygen. This system is sensitive to changes in environmental temperatures.
### 4.6 Comparison of Automated Systems

#### Comparison of Automated Systems

<table>
<thead>
<tr>
<th></th>
<th>BacT/ALERT</th>
<th>MGIT</th>
<th>VersaTREK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Means of Inoculation</strong></td>
<td>Sharps or needleless devices</td>
<td>Disposable pipettes</td>
<td>Sharps or disposable pipettes</td>
</tr>
<tr>
<td><strong>Specimen Types</strong></td>
<td>Blood—must use MB bottles</td>
<td>Urine, blood</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Specimen Types Not Validated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Detection</strong></td>
<td>Production of CO₂—color change</td>
<td>O₂ consumption—fluorescence</td>
<td>O₂ consumption—pressure change</td>
</tr>
<tr>
<td><strong>DST (FDA Cleared)</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Antibiotic Supplement</strong></td>
<td>Amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin</td>
<td>PANTA—polymyxin B, amphotericin B, naladixic acid, trimethoprim, and azlocillin</td>
<td>PVNA—polymyxin B, vancomycin, nalidixic acid, and amphotericin B</td>
</tr>
</tbody>
</table>

#### Notes:

This slide shows a summary and a comparison of the three systems we just talked about.

As a reminder, it's important to read all package inserts carefully for specific and detailed information on each system.

The table provided on this slide goes through the means of inoculation for each system, the specimen types which can be used and those which are not validated, how detection occurs, whether or not it's FDA cleared for drug susceptibility testing, and specifics on the antibiotic supplements which must be added.
In the next section, we'll talk about the culture process itself.
5.2 The Culture Process

Notes:

This slide walks through the culture process.

A respiratory specimen is received in the laboratory and undergoes a process of digestion and decontamination to break down respiratory secretions and mucus, and to reduce the normal respiratory flora.

The resulting sediment is simultaneously set up for smear and nucleic acid amplification test and inoculated to solid and liquid media.
5.3 Inoculation

Notes:

In setting up solid media, two to three drops of the processed specimen are inoculated onto the surface of the slant or plate.

Plates are kept at room temperature until the inoculum is absorbed, then sealed in a polyethylene bag or shrink-wrapped.

For a liquid media, the specimen is added to the tube by pipetting against the side of the tube to reduce the likelihood of splashing.
5.4 Incubation

Notes:

Tubed solid media is incubated in a slanted position with loose caps for five to seven days. After seven days, the caps are tightened and tubes may be placed upright.

Plates in CO2-permeable bags are incubated media side down and stacked no more than six high.

Incubation at 35 to 37 degrees Celsius is best for recovery of MTBC. Some NTM will prefer to grow at 25 to 33 degrees Celsius.

For certain specimen types, such as non-respiratory biopsies, two sets of media may be set up and incubated at two different temperatures.

Refer to CLSI M48 for additional growth requirements for some specific NTM.
5.5 Incubation (2)

Notes:

Mycobacteria species typically grow best in five to 10 percent carbon dioxide for the first seven to 10 days of incubation.

Solid media should be held for six to eight weeks per CLSI, however recent studies have demonstrated that most MTBC is isolated within six weeks.

Labs are encouraged to review their own data to determine if incubation beyond six weeks is necessary.

If certain species of NTM are suspected, longer incubation times may be necessary.
5.6 Typical Growth Rates

**Notes:**

In liquid culture systems, MTBC will be detected in an average of 14 days. This is in comparison to Lowenstein-Jensen slants which may grow in 25 days.

Rapid growers can be detected in as low as three to seven days.

Increased growth rates can be attributed to harsh specimen processing, tightened caps during the first week of incubation, incorrect incubation temperatures, and a low bacterial load in the specimen.
5.7 Solid Media Examination Schedule

Solid Media Examination Schedule

- Examine at end of week 1 to assess growth and possible contamination
- Continue to examine weekly for 6-8 weeks
- Corresponding liquid media is likely to become positive first but continue to incubate solid media until growth is observed or end of incubation time is reached
  - Growth should be identified as soon as possible regardless of media type

Notes:

Typically, solid cultures are observed for growth or contamination after one week of incubation with weekly reads until six to eight weeks.

According to the recent study by Tyrrell, et al. published in the 2012 Journal of Clinical Microbiology, it may not be necessary to hold solid media longer than six weeks. Labs are encouraged to review their own data to make this determination.

It is likely the liquid media will become positive first. But it is important to hold the solid media until growth is observed or the end of the incubation schedule is reached. Growth should be identified as soon as it is detected.
5.8 Use of Low Power Stereoscope

Notes:

Laboratories will often use low power stereoscopes to get more information on colony morphology in young cultures.

This slide illustrates a culture growing on a plate and the same colony seen under the stereoscope.
Notes:

This is another slide illustrating a culture as viewed under a stereoscope. This is a mixed culture of MTBC with a Gordonia species mixed in.
5.10 Liquid Media Examination Schedule

In automated systems, tubes are read continuously and flagged when positive
- Perform acid-fast bacteria (AFB) smear with Ziehl-Neelsen (ZN) or Kinyoun
- Smear result determines next steps

Visually check negative tubes for growth

Typical appearance of mycobacterial growth in instrument-negative broth culture. The organisms tend to form colony-like clumps (arrows) at the bottom of the tube, along the surface of the fluorescent indicator.


Notes:
Automated liquid culture systems are monitored for growth continuously and flagged when positive.

Liquid culture tubes which have been flagged as positive should be stained with Ziehl-Neelsen or Kinyoun stain.

All MGIT and VersaTREK negative tubes, should be examined visually for evidence of growth prior to being discarded at the end of their incubation time.
5.11 AFB Smear of Growth in Liquid Media

Notes:

As mentioned previously, all positive liquid culture tubes should be stained as soon as possible using ZN or Kinyoun.

A drop of culture is placed on a glass slide, dried in the biosafety cabinet, heat fixed, and stained according to protocol.

The slide result will dictate the remaining process.

This is shown in more detail in the next slide.
5.12 Algorithm for Growth in Liquid Media

Notes:

This slide outlines the algorithm to be followed for a positive liquid culture.

If no organisms are seen on the smear, refer to the package insert for the system you are using.

If AFB are seen, the recommended course is to subculture for purity and proceed with identification.

If non-AFB organisms are seen, reprocessing is recommended.

It's when both AFB and non-AFB are seen that the path is less clear.
5.13 Algorithm for Growth in Liquid Media (2)

Notes:

If both AFB and non-AFB are observed in the smear, there are a few paths which can be followed.

The tube can be re-processed for decontamination and culture set up again, the growth can be sub-cultured to selective media for isolation of the mycobacteria, or a molecular-based identification method can be used.

It is important to note however, contaminated tubes may not be used for growth-based drug susceptibility testing.
6. Reporting

Notes:

In the next section, we'll talk about reporting of mycobacterial cultures.
6.2 Reporting Positive Cultures

Notes:

It is important to the clinician to be provided with an interim report as soon as the culture is positive and acid fast bacilli are observed. This preliminary information should be reported with an indication that more information will follow.

The preliminary report should be updated when an identification is available.

At a minimum, the report should indicate if a MTBC or a NTM has been identified.
6.3 Reporting Negative Result

No clear guidance regarding when to issue a no growth (negative) report

- Final report issued at 6-8 weeks (e.g., No growth of mycobacteria) with typically no interim report of no growth to date
  - Automated systems incubate liquid media for 6 weeks
  - Historically, solid media has been incubated for 8 weeks

Notes:

There is currently no clear guidance on when to issue a negative report.

Typically, a final negative culture will be reported at six to eight weeks. As previously stated, recent studies have shown little value in extending the hold time of solid media beyond six weeks. Labs are encouraged to review their own data and to make this determination.
6.4 Should an Interim Negative Report be Issued?

Notes:

The question remains, "Is there value in issuing an interim negative report?"

There have been published studies which have shown that respiratory specimens which are negative at four weeks, have a 95 to 100 percent chance of being negative at six weeks.

Four weeks may be a good time to report, "No growth to date. Final report to follow."

This information can be valuable to the clinician, may help in patient management, and may reduce extra specimens, and may reduce the number of phone calls to the laboratory.
6.5 Time to Detection

Notes:

This slide is demonstrating data from the Tyrrell, et al. article in the *Journal of Clinical Microbiology* in October of 2012.

This study illustrated that when using MGIT, 99.2 percent of MTBC were recovered in 28 days, and 100 percent were recovered in 35 days.

Labs are encouraged to use their own data to determine positivity at five to six weeks and adjust their reports accordingly.
Notes:

In the next section, we'll talk about culture contamination.
7.2 Contamination

Most specimens for AFB testing come from non-sterile sites, and despite the decontamination process, some contamination of culture media is to be expected.

Notes:

Most specimens from mycobacteriological testing come from non-sterile sites, and despite the decontamination process, some contamination of culture media is to be expected.
7.3 Contamination – Solid Media

Notes:

The most common contaminant is mold. Any culture tubes with any evidence of mold should be discarded.

Some species of bacteria can cause solid media to liquefy or become discolored. Tubes showing discoloration or liquefaction should also be discarded.

If there is evidence of some contamination but the media is not discolored or liquefied, the culture can be retained.

A smear should be prepared from the surface of the contaminated media. If AFB are present, it is recommended to either re-streak for isolation or re-decontaminate and re-inoculate the culture.

Many labs choose to discard the solid contaminated tubes and proceed with the remaining liquid culture.
7.4 Contamination of Liquid Media

- More susceptible to contamination than solid media
- Needs to be supplemented with mixture of specific antibiotics to reduce contaminants
- Contamination should be suspected if homogeneous turbidity is seen

Notes:

Liquid media is more susceptible than solid media to contamination.

This is why the commercial automated liquid culture systems require the addition of antimicrobials.

If homogeneous turbidity is observed in a liquid culture tube or bottle, contamination should be suspected.
7.5 Contamination – NTMs

Notes:

Since NTM can be common in the environment, but also can be opportunistic pathogens in humans, it can be difficult to determine the significance of isolation of an NTM.

Communication between the lab and the clinician is important to discuss the need for a full identification of an isolated NTM.
7.6 Monitoring Contamination

**Notes:**

Monitoring contamination rates is a quality indicator that should be tracked by all labs.

Labs should monitor overall specimen contamination as well as contamination of just liquid or solid media.

Overall, specimen contamination is defined as contamination of both the solid and liquid media in one culture.
Notes:

The acceptable contamination rate is five to eight percent for liquid media, and three to five percent for solid media. Higher percentages may indicate incomplete specimen processing or the use of contaminated reagents or media. Lower percentages may indicate over processing of specimens or the use of harsh reagents in processing.

Contamination rates should be monitored monthly for high volume laboratories and bi-monthly or quarterly for labs with lower specimen volumes.
### Monitoring Contamination (3)

<table>
<thead>
<tr>
<th>Numerator/Denominator</th>
<th>Recommended Levels</th>
<th>Investigative Actions: Increases</th>
<th>Investigative Actions: Decreases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen</td>
<td>3-5%</td>
<td>May indicate insufficient processing, specimen delivery delays, contaminated reagents, media or equipment.</td>
<td>May indicate use of harsh specimen processing reagents or excessive processing procedures.</td>
</tr>
<tr>
<td>Liquid Media</td>
<td>5-8%</td>
<td>See above (&gt;8% for upper threshold), antibiotic supplement volume insufficient or expired.</td>
<td>See above.</td>
</tr>
<tr>
<td>Solid Media</td>
<td>3-5%</td>
<td>See above. LJ media old/expired, increase in <em>Pseudomonas</em> in cultures.</td>
<td>See above.</td>
</tr>
</tbody>
</table>

Notes:

This slide provides an overview of how to calculate contamination rates.

The recommended acceptable ranges and investigative actions if labs see an increase or a decrease from the acceptable ranges.

[List is not all-inclusive; **This calculation should not include specimens that have been re-decontaminated from aliquots of previously processed or stored specimens; *** Slants or plates used in the initial set-up.]
7.9 Monitoring Contamination (4)

- If contamination rate is increased, also consider these non-laboratory causes:
  - Specimens experiencing delays in transportation to laboratory
  - Specimens not refrigerated during shipment
  - Shifts in patient populations (e.g., more patients with cystic fibrosis)

- Carefully examining your laboratory's data may reveal possible causes and solutions to contamination problems

Notes:

Higher than expected contamination rates may also be from causes external to the lab, such as transport delays, lack of refrigerated transport, and changes in patient populations.

It is important to monitor contamination rates and address any observed trends.
8. False Positives

Notes:

In the next section, we'll talk about false positives in the laboratory.
8.2 Defining a False Positive

Notes:

A false positive result is a result reported as positive for a mycobacteria species when that organism is not actually present in the patient specimen.

While all laboratories are capable of producing false positive results, not all false positives are due to laboratory cross contamination.
8.3 Impact of False Positives

Notes:

A 1997 study by Burman and Reves showed 3.1 percent of all positive MTBC are actually false positives.

In this study, that translated into 67 percent of false positive patients being treated for TB disease that they didn't have, and in some cases, previously confirmed TB patients were thought to have failed treatment due to a false positive result.
8.4 Consequences of False Positives

Notes:

As illustrated in the previous slide, there are consequences to reporting a false positive result.

Consequences can include incorrect patient management, a decrease in credibility of the laboratory, hospital, or clinician, or an increase in laboratory workload and associated testing costs.
8.5 Impact of False Positives

Notes:

There are a number of factors that can lead to a false positive result.

These can include inadequate sterilization, mislabeling of specimens at collection or accessioning, the use of contaminated water, sharing of reagents, having more than one specimen open at one time, mixing specimen containers and lids, and the production of aerosols while processing specimens.
**8.6 Triggers for Investigating a Possible False Positive**

- Only one positive culture from a patient
  - Especially if patient is smear negative
- Culture result not compatible with the clinical picture
- Late-appearing cluster of cultures that have scanty growth or long time to detection
- Increase in the percentage of culture positivity
- Increased number of isolates of a species that is usually rare in the laboratory or is normally considered an environmental contaminant

**Notes:**

Just like there are a number of factors that can cause a false positive, there are also a number of factors that should prompt an investigation into whether the observed result is a false positive.

These factors include having only one positive from a given patient, the observed result doesn't match the clinical picture, positive cultures that appear late or with scant growth, and an increase in overall positivity rate or an increase in a rare species or one that is normally thought of as an environmental contaminant.

Any of these indicators should lead you to suspect a false positive and cause an investigation to happen.
8.7 The Role of Genotyping

The Role of Genotyping

- Can help confirm instances of false positive cultures
- TB genotyping identifies genetic linkages between MTBC isolates
- All culture-confirmed TB cases should receive genotyping through referral to a CDC-funded genotyping laboratory via state public health laboratories
- Genotyping can determine if two or more isolates are likely the same or different
  - If the genotypes match, isolates are likely the same, but this does not confirm cross contamination
- If suspected, the laboratory should expedite referring the isolate for genotyping with a notation that it is a potential false positive isolate

Notes:

Genotyping can help determine if a false positive result is a true positive or not.

Genotyping is a method of comparing genetic linkages between isolates.

All positive MTBC isolates should be sent for genotyping to a CDC-funded genotyping lab.

If a false positive is suspected, the submitting lab should contact the genotyping lab and expedite the submission and the testing process.
8.8 The Laboratory Must Be Vigilant

**The Laboratory Must Be Vigilant**

- Clinical laboratories have a low positivity rate for MTBC
  - Most episodes of false-positives are recognized only after a review of laboratory records, including genotyping results

- Laboratories need a review process to detect false positive cultures earlier
  - Single positive cultures should be reviewed on a case-by-case basis to identify possible false positive cultures

**Notes:**

Laboratories must be vigilant in finding false positives.

Since most false positives are recognized after a review of records, laboratories need to have a review process in place to detect false positives as early as possible.

Single positive cultures should be reviewed in real time to identify a potential false positive in these cases.
8.9 The Laboratory Must Be Vigilant (2)

- Laboratories need a mechanism to determine possible causes of false positive results (e.g., personnel logs, lot numbers used)
- Practices should be evaluated to determine where changes can to be made

Notes:

If a false positive result is identified, there must also be a process in place to review the possible causes of the false positive result happening.

Workflow should be evaluated and changes made if necessary to ensure this doesn't happen again.
8.10 Practices to Reduce Possibility of Cross Contamination

Notes:

There are a number of common practices which can be in place to reduce the likelihood of cross-contamination in the laboratory.

These practices include the use of fresh aliquots of reagents daily, rather than reusing reagents the next day, keeping specimen containers closed and clean, pouring slowly to minimize splashing, and not touching the container of reagents to the specimen tubes.
8.11 Practices to Reduce Possibility of Cross Contamination (2)

**Notes:**

Additional practices to reduce cross-contamination and the likelihood of false positives include opening specimen tubes gently, and only one at a time, leaving a physical space between tubes in a rack, changing gloves frequently.

Work surfaces including the inside of the biosafety cabinet should be disinfected following each use.

Proficiency testing specimens should not be handled with patient specimens.
8.12 Determination of a False Positive Result

Notes:

If a false positive result is suspected, it is important to communicate with the clinician and the TB control program as soon as possible to ensure appropriate patient management.
8.13 Thank You for Participating!

Notes:

This concludes the Mycobacterial Culture module presentation, which is part of the series from the Association of Public Health Laboratories, Essentials of the Mycobacteriology Laboratory, Promoting Quality Practices.

Please see the CDC and APHL websites for more information on the topics presented here.