

# Mycobacterial Culture

Mycobacterial Culture

# **OVERVIEW AND PURPOSE**

# Mycobacterial Culture

---

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

# 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-tuberculous Mycobacteria (NTM)
  - Are 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

Mycobacterial Culture

**MEDIA**

# Culture Media

---

- Two major categories of media are routinely used
  - Solid: egg- and agar-based
  - Liquid: also often referred to as broth media
- The ideal media for isolation of mycobacteria
  - Supports rapid and robust growth of small numbers of mycobacteria
  - Permits preliminary differentiation of species on the basis of pigment production and colony morphology
  - Inhibits growth of contaminants
- No single medium meets all these requirements
  - Use of both liquid and solid media for initial culture recommended
- May be purchased commercially or prepared in-house
- Quality Control of media must be performed

# Solid Media: Egg-based

---

- Lowenstein-Jensen (LJ)
  - Selective; malachite green-Inhibits growth of contaminating bacteria and fungi
  - Supports growth of MTBC (with exception of *M. bovis*) better than NTM
  - Shelf life of 6–12 months when refrigerated
  - May have excess of water—should be removed before inoculation



# Solid Media: Agar-based

- Middlebrook 7H10 and Middlebrook 7H11
  - Media is clear, thus allows easier colony observation and quantification
  - Mycobacterial colonies can potentially be isolated if media is contaminated
  - Can be selective if antibacterial and antifungal antimicrobials are added to inhibit contaminating bacteria
  - The average time to detection of growth is earlier than with the egg-based media
  - Available as plate or slant



[www.remel.com](http://www.remel.com)

*Middlebrook plate and slant: Compared to plates, slants do not dry out or expire as quickly but growth may be more difficult to detect.*



<https://catalog.hardydiagnostics.com>



# Comparison of Solid Media

Conditions for Mycobacteria Growth and Recovery	Egg-based Media	Agar-based Media
Selective	Added malachite green	Added antibacterial and antifungal
Rate of growth	Slower	Faster and able to support INH Resistant and fastidious strains
Contamination	Usually less but if present, involves the entire surface	More frequent, but can still isolate colonies
Area of Inoculum	Relatively small	Large
Visual Examination	Difficult due to media opacity	Media is clear; allows easier colony quantification and morphology confirmation
CO <sub>2</sub>	Not required	Required
DST	Not performed on LJ in United States	Preferred due to larger surface area and faster rate of growth
Shelf Life	Long (6–12 months)	Short (1–2 months)

# Liquid Media

---

- Use is recommended standard practice for mycobacteriology laboratories
- Increased recovery of mycobacteria and decreased time to detection compared to solid media
- More easily contaminated than solid and the addition of antimicrobials is required
- Average time for growth detection of slowly growing mycobacteria is 12–16 days
- Some fastidious mycobacteria grow only in liquid media
- Shelf life is long; can be stored at room temperature
- Incubation with additional CO<sub>2</sub> is not required

Mycobacterial Culture

# **AUTOMATED SYSTEMS**

# Automated Systems

---

- Continually monitor the media for detection of mycobacteria for 6 weeks
- Most widely used FDA-cleared automated systems for rapid detection of mycobacteria using liquid media
  - Biomerieux BacT/ALERT® 3D
  - Becton Dickinson BACTEC MGIT™
  - Thermo Scientific VersaTREK™
- MGIT and VersaTrek are also FDA-cleared for susceptibility testing of MTBC

# BacT/ALERT 3D

- BacT/ALERT MP media consists of
  - Modified Middlebrook 7H9 broth
  - Reconstitution Fluid
  - Antibiotic Supplement



<http://www.biomerieux-diagnostics.com>

- Non-sterile and sterile body sites except blood
  - Blood specimens require specific bottles—BacT/Alert MB
- Detection of growth
  - Colorimetric sensor monitors changes in CO<sub>2</sub>
  - As CO<sub>2</sub> 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

# 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<sub>2</sub> by growing organisms
  - A manual fluorescent detector is also available



# VersaTREK

- Culture System consists of
  - Modified Middlebrook 7H9 broth
  - Growth supplement
  - Antibiotic supplement
  - Cellulose sponges provide unique growth matrix
- Can be used for most specimen types including urine and blood
- Detection of growth
  - Changes in headspace pressure due to O<sub>2</sub> consumption
  - Sensitive to environmental temperature variations



[www.thermoscientific.com](http://www.thermoscientific.com)

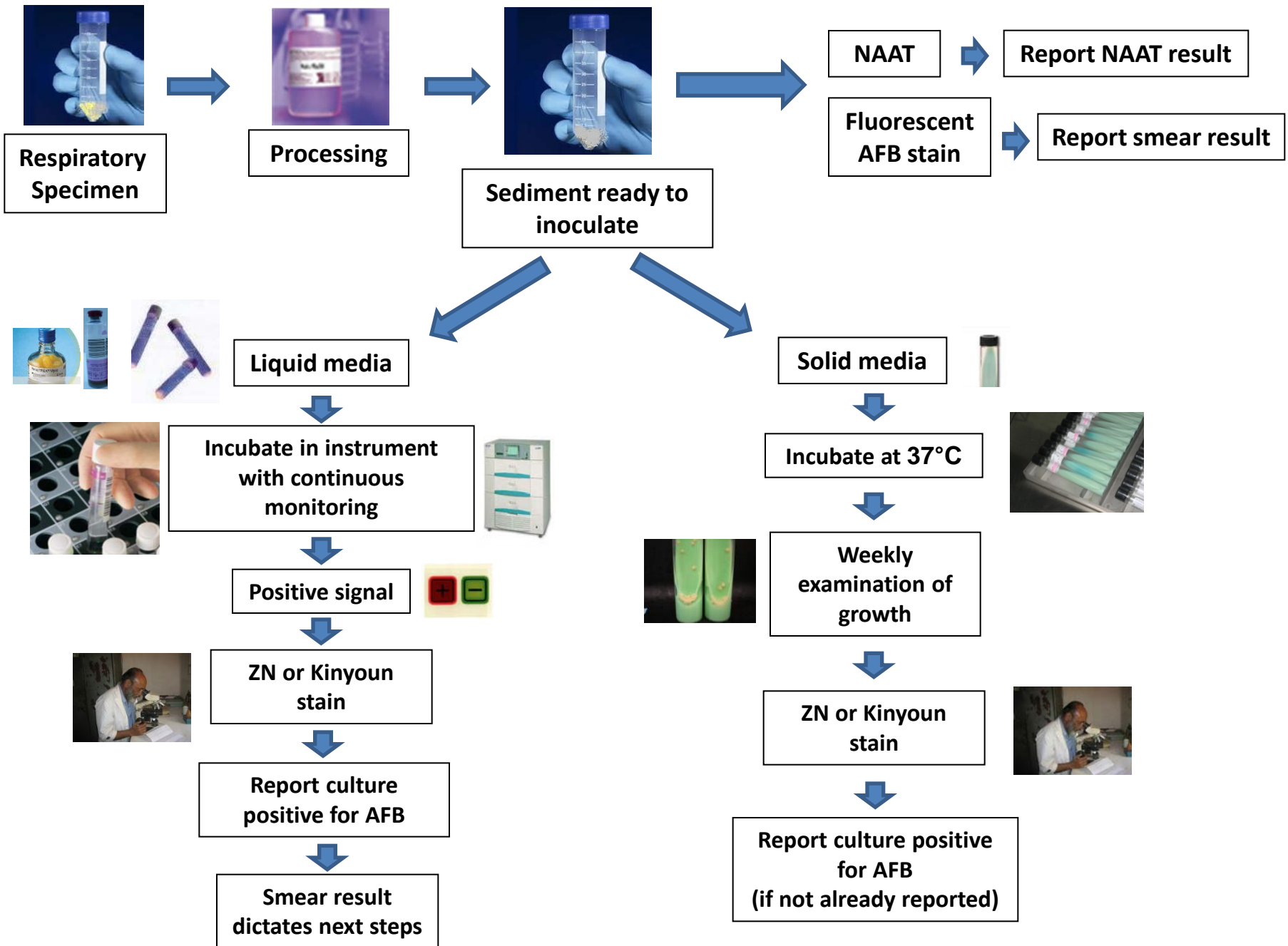
# Comparison of Automated Systems

	BacT/ALERT	MGIT	VersaTREK
Means of Inoculation	Sharps or needleless devices	Disposable pipets	Sharps or disposable pipets
Specimen types not validated	Blood-must use MB bottles	Urine, Blood	N/A
Detection	Production of CO <sub>2</sub> - color change	O <sub>2</sub> consumption-fluorescence	O <sub>2</sub> consumption - pressure change
DST (FDA Cleared)	No	Yes	Yes
Antibiotic Supplement	amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin	PANTA—polymyxin B, amphotericin B, naladixic acid, trimethoprim, and azlocillin	PVNA—polymyxin B, vancomycin, nalidixic acid, and amphotericin B



Mycobacterial Culture

# **CULTURE PROCESS**



# Inoculation

---

- Solid media
  - Tubed—approximately 2-3 drops of processed specimen
    - Allow to spread over surface
  - Plated Agar—3 drops placed separately on surface
    - Keep at room temperature to completely absorb, then seal in polyethylene bag or with shrink-wrap
- Liquid media
  - Pipette against inside of tube/bottle to reduce splash
    - BacT/Alert 3D—0.5 ml
    - MGIT—0.5 ml
    - VersaTrek—up to 1.0 ml

# Incubation

---

- Tubed solid media
  - Slanted position with screw caps loose for 5–7 days
  - After 7 days caps should be tightened and tubes may be positioned upright
- Plated solid media
  - Place in CO<sub>2</sub>-permeable plastic bags, medium-side down
  - Stack plates no more than six high
- Temperature
  - Optimal temperature for MTBC is 35–37°C
  - Some species of NTM grow best at 25–33°C
    - For skin, bone, and joint biopsies, inoculate two sets of media, one at 37°C and the other at the lower optimal temperature
  - *M. xenopi* requires 40–42°C for optimal growth

# Incubation (2)

---

- Atmosphere
  - For solid media, growth is optimized in an atmosphere containing 5–10% CO<sub>2</sub>
- Length
  - Incubation of solid media for 6–8 weeks
    - Laboratories should analyze isolation data to determine time before reporting as culture negative
  - Most commercial liquid media require a maximum of six weeks
  - Certain species of NTM, such as *M. ulcerans* (skin specimens), *M. genavense*, and *M. malmoense*, require 8–12 weeks

# Typical Growth Rates

---

- Rapid Growers will grow in 3–7 days
- MTBC is detected in an average of 14 days using MGIT versus 25 days on LJ
- Detection may be delayed by
  - Harsh specimen processing
  - Tightened caps during the first week of incubation
  - Incorrect temperature
  - Low bacterial load such as in smear-negative specimens

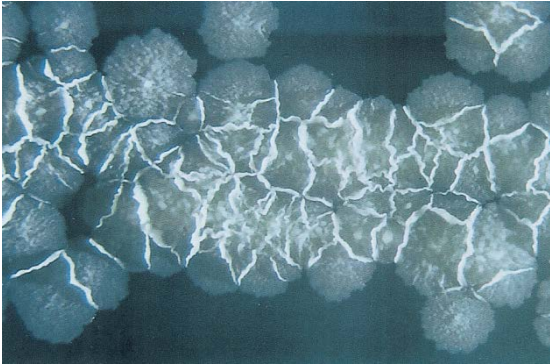
# Solid Media Examination Schedule

---

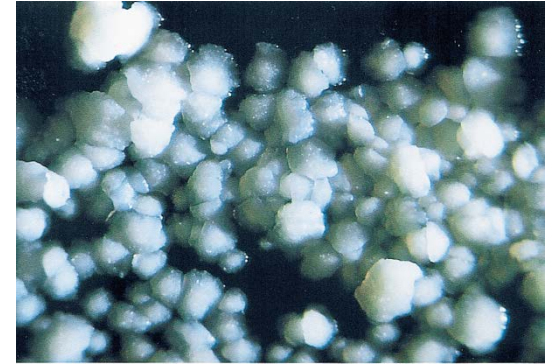
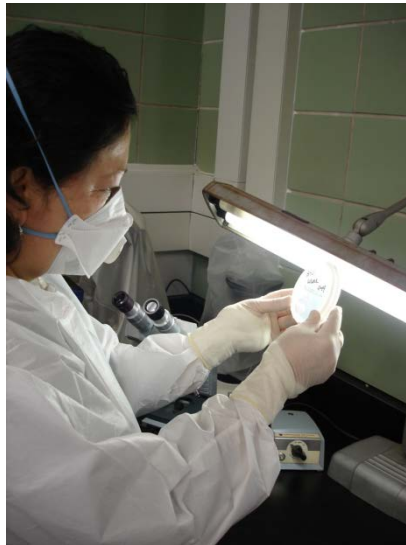
- Examine at end of week 1 to assess growth and possible contamination
- Continue to examine weekly for 6–8 weeks
- Corresponding liquid media is likely to become positive first but continue to incubate solid media until growth is observed or end of incubation time is reached
  - Growth should be identified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

# Visual Examination

- Colony Morphology on solid media
  - *M. tuberculosis* is rough and non-pigmented
  - *M. bovis* is flat, smooth and non-pigmented



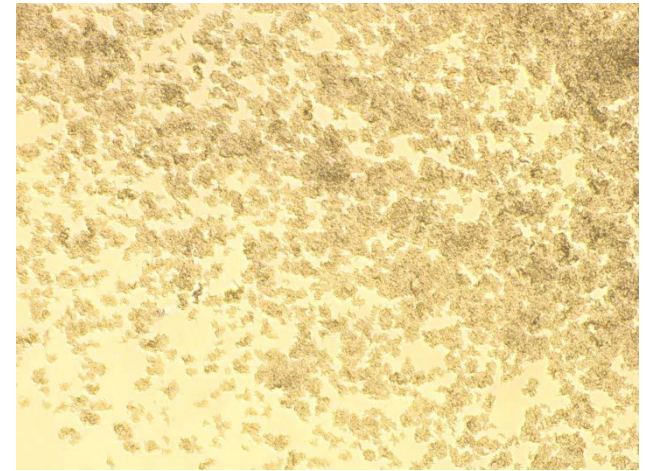
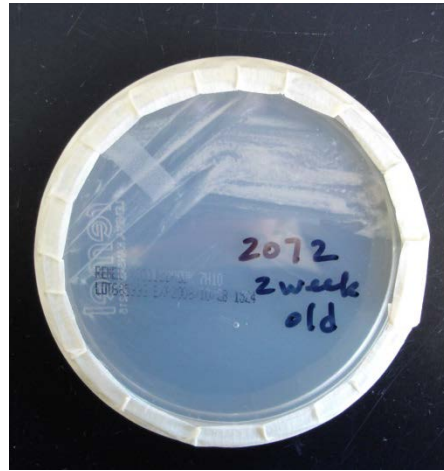
*M. tuberculosis*



*M. bovis BCG*



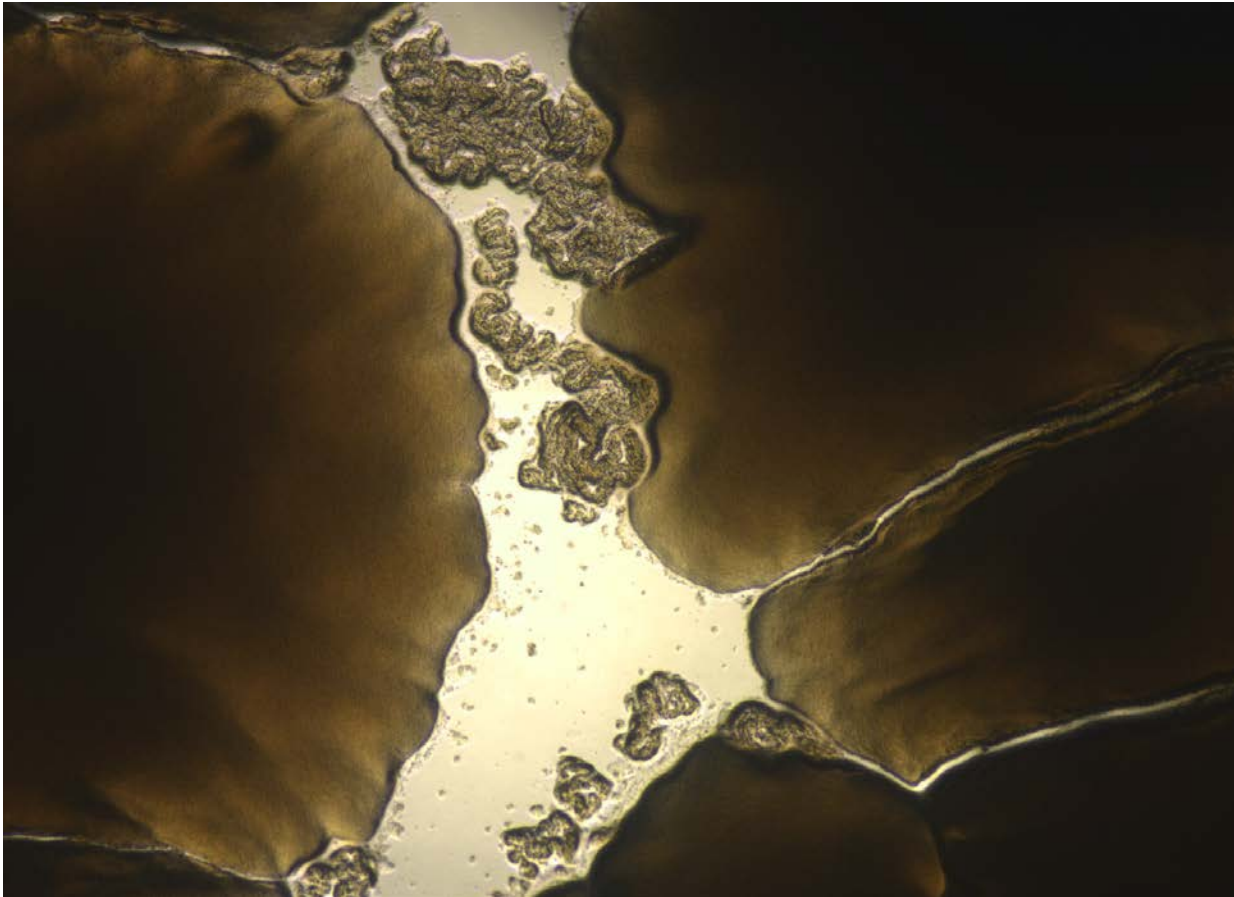
# Use of Low Power Stereoscope



Young MTBC culture; plate and colonies under stereoscope

# Mixed Culture Under Stereoscope

---



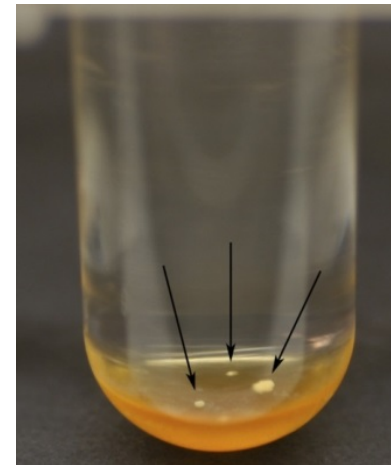
This picture shows MTBC mixed with a Gordonia species (non-mycobacteria, partially acid-fast)

# Liquid Media Examination Schedule

---

- In automated systems, tubes are read continuously and flagged when positive
  - Perform acid-fast bacteria (AFB) smear with Zeihl-Neelson (ZN) or Kinyoun
  - Smear result determines next steps
- All MGIT and VersaTREK negative tubes at end of incubation period should be visually checked for evidence of growth before being discarded

*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. (Pena, J. A., et al. (2012). "Growth detection failures by the nonradiometric Bactec MGIT 960 mycobacterial culture system." J Clin Microbiol 50(6): 2092-2095.)*

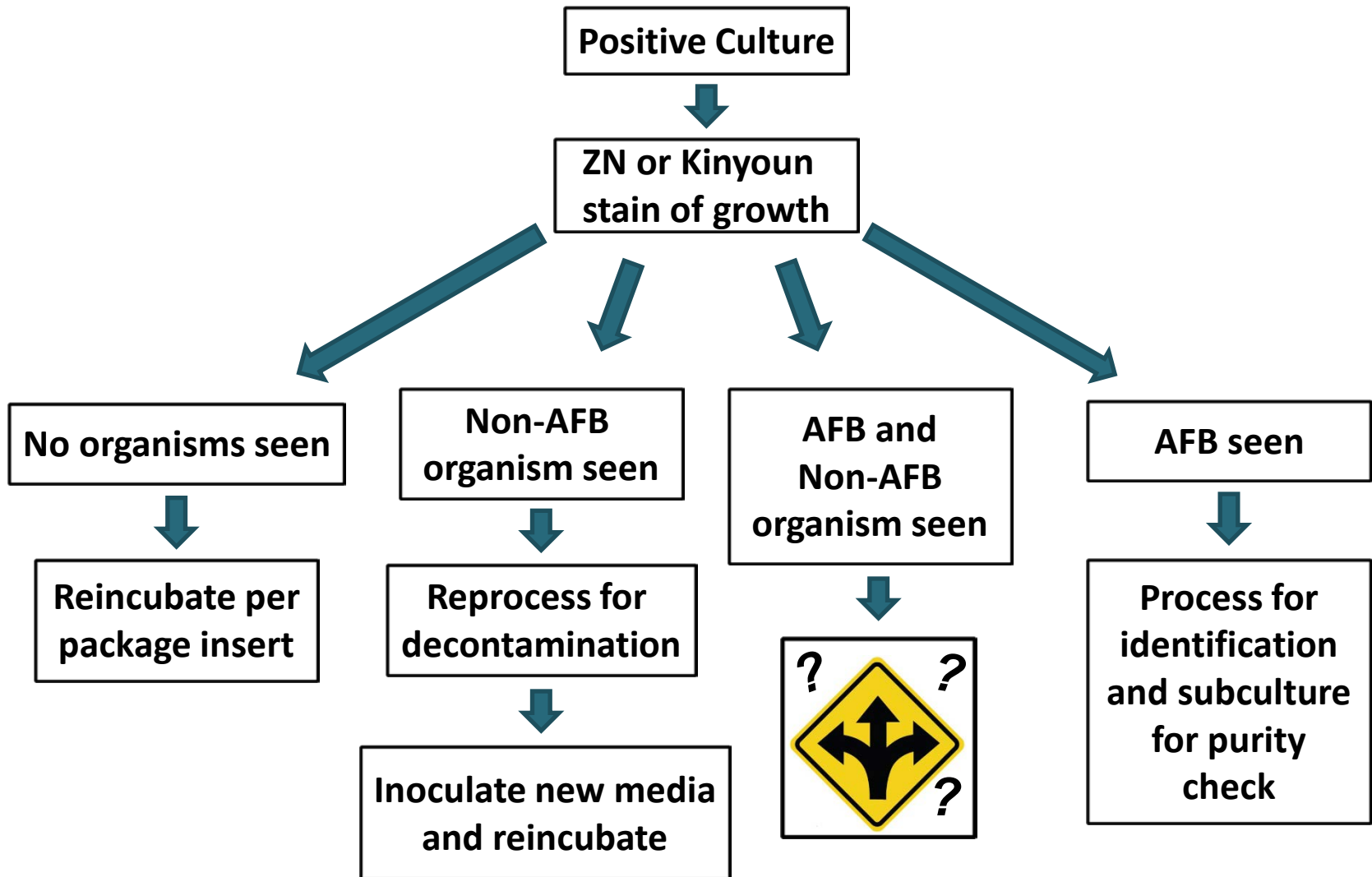


# AFB Smear of Growth in Liquid Media

---

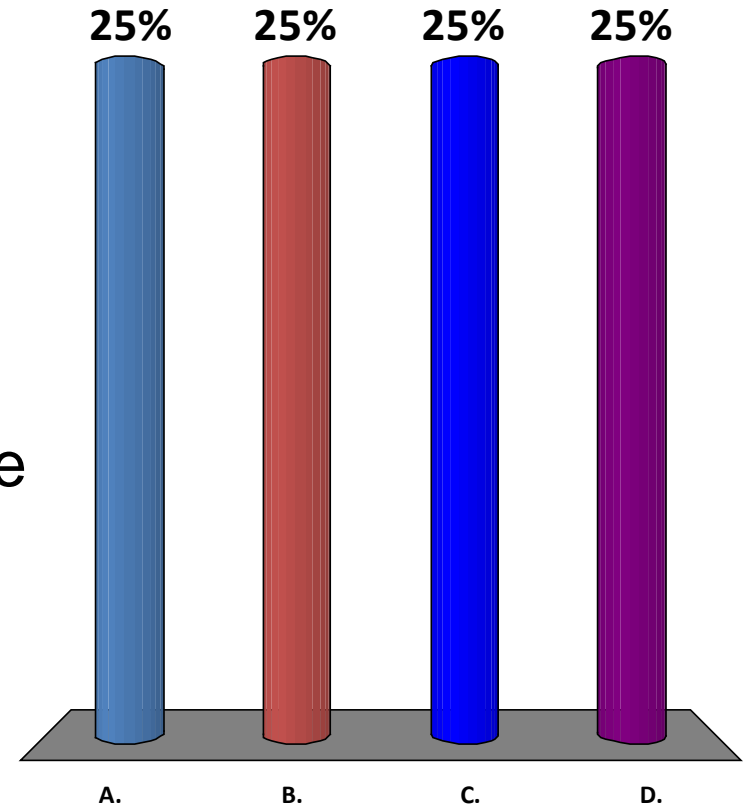
- ZN or Kinyoun staining should be performed on growth as soon as possible
- Deposit drop of culture on glass slide, let dry in biological safety cabinet, fix, and proceed with staining protocol
- After staining, culture should be handled according to the results

# Algorithm for Growth in Liquid Media



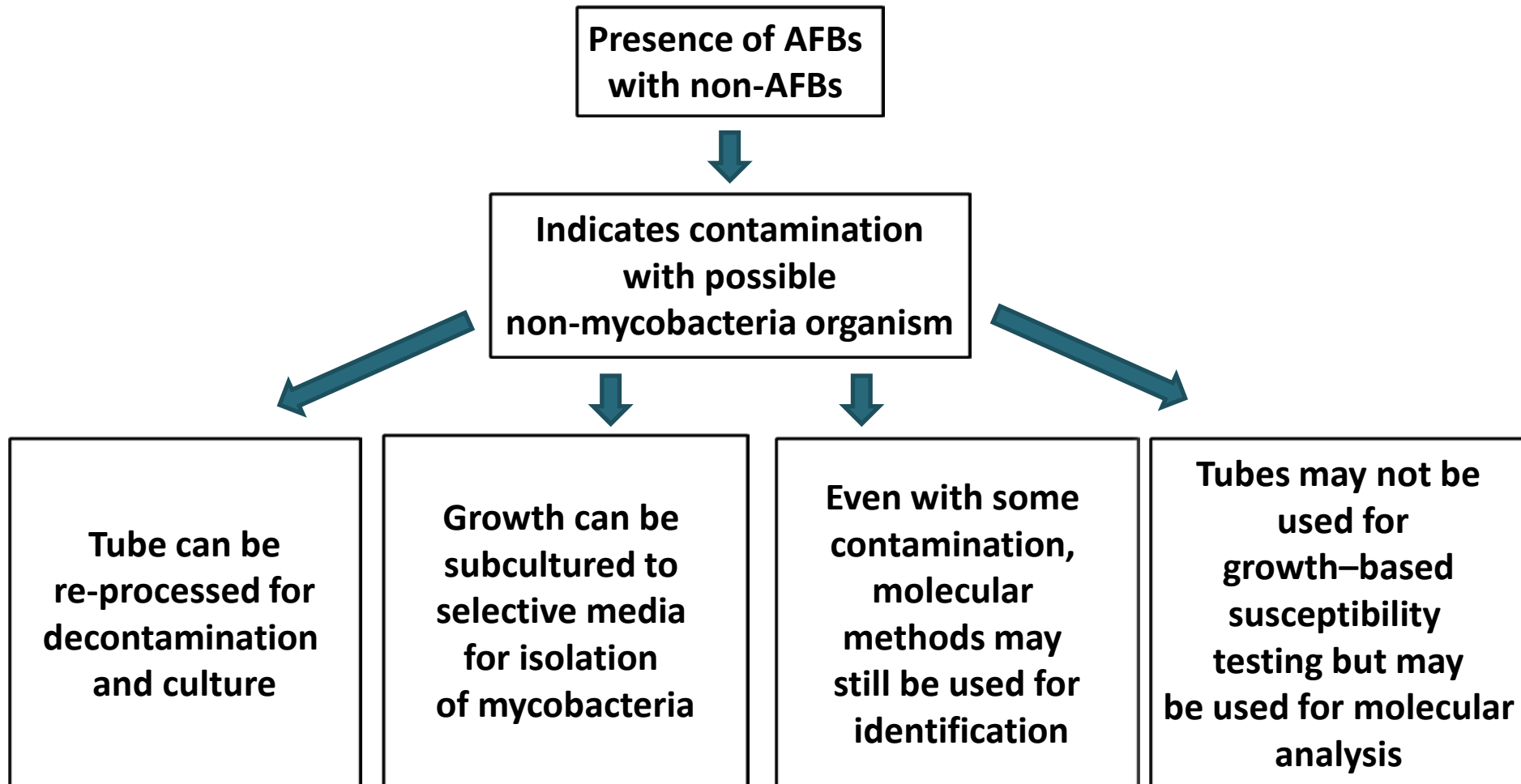
# If both AFB and Non-AFB are seen in the smear, would you...

- A. Not attempt an ID, but re-decontaminate the tube and re-inoculate a new culture
- B. Attempt an ID despite the contamination
- C. Not attempt an ID, but subculture to selective solid media
- D. Combination of the above



# Algorithm for Growth in Liquid Media (2)

---



Mycobacterial Culture

# REPORTING



# Reporting Positive Cultures

---

- Provide interim report as soon as media turns positive and AFB are observed, indicating identification to follow
- Report should be updated when identification made
- Minimally, report should indicate either MTBC or NTM

# Reporting Negative Result

---

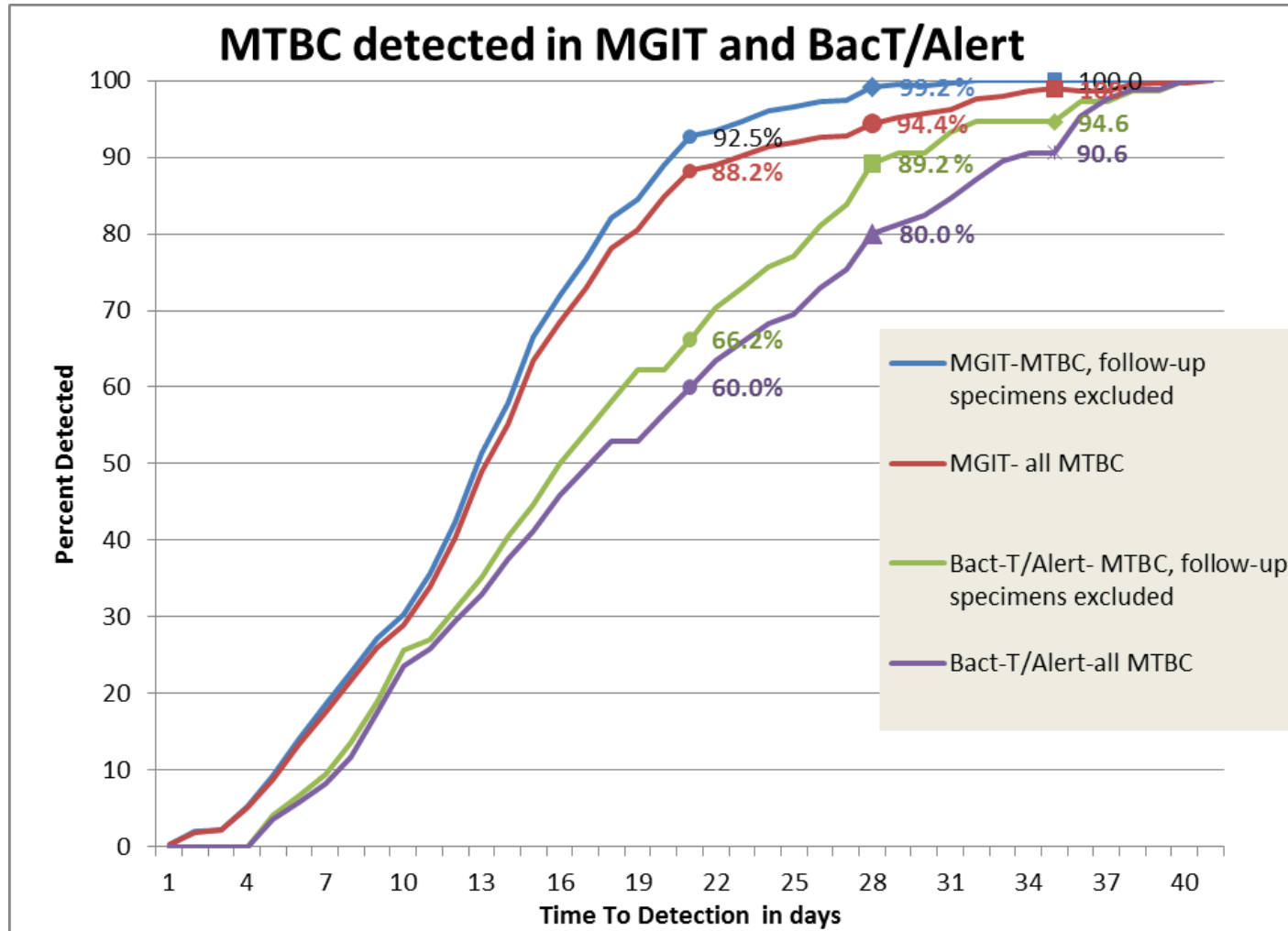
- No clear guidance regarding when to issue a no growth (negative) report
- Final Report issued at 6–8 weeks (e.g., No growth of mycobacteria) with typically no interim report of no growth to date
  - Automated systems incubate liquid media for 6 weeks
  - Historically, solid media has been incubated for 8 weeks

# Should an Interim Negative Report be Issued?

---

- Studies have shown that, using MGIT, respiratory specimens that are culture negative at 4 weeks have a 95–100% probability of a final negative result at 6 weeks
- Consider reporting interim report (e.g., No growth of mycobacteria at 4 weeks. Final report to follow)
  - May help physician with differential diagnosis and patient management decisions
  - Reduce questions or phone calls to laboratory
  - May reduce unnecessary repeat specimens

# Time to Detection



Mycobacterial Culture

# **CONTAMINATION**

# 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.

# Contamination – Solid media

---

- Most common contaminant is mold
  - Any culture showing the presence of mold contamination should be discarded
- Some bacterial species can liquefy or discolor the solid medium and those tubes should be discarded

# Contamination – Solid media (2)

---

- If partial contamination present
  - Culture can be retained if not discolored or liquified
  - Smear can be prepared from surface of the medium
    - If smear shows AFB, growth may be streaked for isolation or re-decontaminated and re-inoculated
  - Many laboratories discard contaminated solid media and continue the culture with only the corresponding liquid media



# 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

# Contamination – NTMs

---

- Many NTM are found in the environment (e.g., *M. gordonae* in tap water)
- The significance of the isolation of NTM may be difficult to assess since many species are opportunistic pathogens but may also be contaminants
- Laboratories should communicate with healthcare provider to determine need for further identification of NTM based on clinical need

# Monitoring Contamination

---

- Monitoring of contamination rates is a fundamental quality indicator
- *Specimen* contamination is defined as all media inoculated per specimen (liquid and solid) being completely compromised
- Important to monitor liquid and solid media contamination rates separately
  - Monitoring only specimen contamination rates may mask a problem with either liquid or solid media

# Monitoring Contamination (2)

---

- Acceptable rate for liquid media is 5–8% and 3–5% for solid media
  - Higher percentages may indicate incomplete processing or the use of contaminated reagents, media or equipment
  - Lower percentages may indicate the use of harsh reagents or excessive processing
- Percent contamination should be examined monthly for high volume and bi-monthly or quarterly for low volume laboratories

# Monitoring Contamination (3)

	Numerator/Denominator	Recommended levels	Investigative actions*	
			Potential causes-increases	Potential causes-decreases
<b>Specimen</b>	# cultures reported out as “contaminated” in one month/Total # of cultures reported in one month	3-5%	May indicate insufficient processing, specimen delivery delays, contaminated reagents, media or equipment	May indicate use of harsh specimen processing reagents or excessive processing procedures
<b>Liquid Media</b>	# inoculated tubes discarded due to contamination in one month/Total # of tubes inoculated in one month**	5-8%	See above (>8% for upper threshold), PANTA volume insufficient or expired	See above
<b>Solid Media</b>	# solid media (slants or plates)*** discarded due to contamination in one month/Total # solid media (slants or plates) inoculated in one month	3-5%	See above. LJ media old/expired, increase in pseudomonas in cultures.	See above

\*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.

# 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

Mycobacterial Culture

**FALSE POSITIVES**

# False Positives

---

- False positives are test results reported as positive for a *Mycobacterium* species not present in the patient specimen
  - Not all false positives are due to laboratory cross contamination
  - All laboratories are capable of producing false positive results



# False Positives (2)

---

- Burman and Reves meta-analysis showed 3.1% of positive results for MTBC from laboratories are false positives (range of 2.2–10.5%)
  - False positive results may be generated at any step between specimen collection and reporting
  - May go unrecognized in previously confirmed patients
- In the study, 67% of patients with false positive cultures were treated for TB disease
- Previously confirmed TB patients were thought to have failed treatment due to false-positive culture

# Consequences of False Positives

---

- Patients may be managed incorrectly
  - Unnecessary treatment and toxicity
  - Unnecessary isolation, hospitalization, and healthcare costs
  - Emotional repercussions to the patient
  - Unnecessary contact investigations
- Credibility of the laboratory, hospital, or clinician may be questioned
- May increase laboratory workload and testing costs

# Practices that can Lead to False Positive Cultures

---

- Inadequate sterilization of instruments such as bronchoscopes
- Mislabeling at time of collection or time of accessioning
- Use of contaminated water for specimen collection or laboratory procedures
- Shared reagents and dispensers
- Opening more than one specimen container at a time while processing
- Mix-up of testing samples or lids
- Failure to take precautions to minimize aerosol production

# Triggers for Investigating a Possible False Positive Culture

---

- Only one positive culture from a patient
- 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

# The Role of Genotyping

---

- 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
- Can help confirm instances of false positive cultures
- 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

# 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

# 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

# Practices to Reduce Possibility of Cross Contamination

---

- Use daily aliquots of processing reagents and buffers
  - Any leftover should be discarded
  - Never use common beakers or flasks when processing
- Keep the specimen tubes tightly closed and clean the outside of the tube prior to vortexing or shaking
- Pour reagents slowly against the inside of the tube to minimize splashing
- Do not touch the container of reagents to the lip of the tube at any time



# Practices to Reduce Possibility of Cross Contamination (2)

---

- Open the specimen tubes gently to avoid aerosol generation
- When adding reagents to the tubes, open one tube at a time
- Leave space between tubes in rack
- Change gloves often
- Avoid manipulation of proficiency test specimens at the same time as patient specimens
- Disinfect biological safety cabinet work surfaces routinely

# Determination of a False Positive Result

---

- Determination cannot be made by the laboratory alone
- If false positive culture is suspected, communication between the laboratory, clinician, and TB control is imperative to avoid unnecessary treatment

# References

---

- European CDC:  
[http://www.ecdc.europa.eu/en/publications/Publications/1105\\_TER\\_Basics\\_TB\\_control.pdf](http://www.ecdc.europa.eu/en/publications/Publications/1105_TER_Basics_TB_control.pdf)
- ACILT African Centre for Integrated Laboratory Training  
<http://www.cdc.gov/globalaids/resources/laboratory/Lab-Training-Center.html>
- “Monitoring the performance of mycobacteriology laboratories: a proposal for standardized indicators,” KD McCarthy et al., INT J TUBERC LUNG DIS 12(9):1015–1020.
- Manual of Clinical Microbiology, 10th Edition. Chapter 28
- [http://www.ecdc.europa.eu/en/publications/Publications/1105\\_TER\\_Basics\\_TB\\_control.pdf](http://www.ecdc.europa.eu/en/publications/Publications/1105_TER_Basics_TB_control.pdf)
- Bauer, J., et al. (1997). "False-positive results from cultures of Mycobacterium tuberculosis due to laboratory cross-contamination confirmed by restriction fragment length polymorphism." J Clin Microbiol 35(4): 988-991.
- APHL. Assessing Your Laboratory

# References

---

- Manual of Clinical Microbiology, 10th Edition. Chapter 28
- [http://www.ecdc.europa.eu/en/publications/Publications/1105\\_TER\\_Basics\\_TB\\_control.pdf](http://www.ecdc.europa.eu/en/publications/Publications/1105_TER_Basics_TB_control.pdf)
- Bauer, J., et al. (1997). "False-positive results from cultures of Mycobacterium tuberculosis due to laboratory cross-contamination confirmed by restriction fragment length polymorphism." J Clin Microbiol 35(4): 988-991.
- Review of False-Positive Cultures for Mycobacterium tuberculosis and Recommendations for Avoiding Unnecessary Treatment William J. Burman and Randall R. Reves
- Estimated Costs of False Laboratory Diagnoses of Tuberculosis in Three Patients Jill M. Northrup, et. al.
- Investigation of suspected laboratory cross-contamination: interpretation of single smear-negative, positive cultures for Mycobacterium tuberculosis V. J. Cook, et. al

# **DELETED SLIDES AND SUPPLEMENTAL MATERIAL**

Mycobacterial Culture

# **MEDIA QUALITY CONTROL**

# Quality control of commercial media

---

- Obtain QC results or certificate of quality from manufacturer, keep on file
- Perform internal QC in accordance to package insert
- Refer to CLSI M22 to determine which media are exempt from end-user QC
- Visually examine each tube or plate before using
  - Check for contamination, integrity

(CLSI M48A Jan 2012)

# Quality Control of media which has been prepared in-house

---

- Inoculate 1-3% of media with suspension of 3 organisms
- Establish an acceptable range of results
- Visually examine each tube or plate before using
  - Check for contamination, integrity



# QC strains

---

- Labs must maintain a collection of well-characterized strains from ATCC and other PT programs
- These should be maintained on LJ or 7H9 at 37 deg or room temperature
  - Sub-culture monthly
  - LJ at 4 deg can be held for 1 year
    - Not recommended for resistant organisms
- Long term maintenance in skim milk or broth at -20 or -70 deg

(Manual of Clinical Microbiology 10<sup>th</sup> edition)

# Quality Assurance

---

- Quality Assurance is vital to monitoring a lab's effectiveness
- QA plan should include personnel competency, procedure manuals, proficiency testing, QC of media, tests and reagents
- QC strain may show acceptable results but the specimen still may demonstrate unacceptable results
  - Labs should have a plan to follow up on atypical results

(Manual of Clinical Microbiology 10<sup>th</sup> edition)

# What should be included in the collection of well-characterized strains?

---

- *Mycobacterium tuberculosis complex*
  - ATCC 27294
- *Mycobacterium tuberculosis H37Ra*
  - ATCC 25177
- *Mycobacterium avium complex*
  - ATCC 25291
- *Mycobacterium kansasii*
  - ATCC 12478
- *Mycobacterium fortuitum*
  - ATCC 6841

# QC of other systems and processes

---

- Check the smear positivity rate, compare positives to other specimens of the same patient
  - Watch for increased positivity that could indicate contamination in water or reagents
- Monitor contamination rates (3-5%) is acceptable
- Including a positive control in each batch of specimens being processed is **DISCOURAGED** because of increased risk of contamination
- Document the order in which specimens are processed and media inoculated,
  - follow each batch of specimens processed with a negative control
  - negative control should remain negative throughout the process

(Kent and Kubica)

# What should be QC'd?

---

- New stain reagents should be checked against known positive and negative AFB smears
- Culture media should be checked for sterility and sensitivity
- **Digestion-Decontamination reagents**
  - culture to LJ and BAP to check TB growth vs contamination
- Water should be cultured periodically to look for contamination

(Kent and Kubica)

## Quality Control by monitoring specimen processing and handling, and isolation of Mycobacteria

---

- Good record keeping of data and periodic review of data is essential
  - Record of technicians
    - Data should be similar between technicians; changes can indicate procedural drift or shift
  - Batch sizes, record of which specimens were processed in each batch
  - Temperatures
    - Should remain constant between 35-37 degrees
  - Negative Control should remain negative throughout the whole procedure

(CLSI M48A Jan 2012)

## Quality Control by monitoring specimen processing and handling, and isolation of Mycobacteria

---

- **Good record keeping of data and periodic review of data is essential**
  - Smear and culture positivity rates
    - 20% AFB smears should be rechecked by a 2<sup>nd</sup> technician
    - ~90% of smear positive specimens should be culture positive
    - ~50% of **total** positive cultures will have been smear negative **specimens**
    - Ratio of MTBC:NTM should remain constant; if 1 increases or decreases unexpectedly it could be an indication that something is off
  - Contamination rates
  - Time to detection of MTBC and NTM

# QC General Guidelines

---

- Labs must have a written protocol
- Record all lot numbers, expiration dates, QC results
- Establish acceptable ranges/results for all media and reagents

(CLSI M48A Jan 2012)



# Contamination – Liquid media

---

- Presence of AFBs with non-AFBs indicates contamination with possible non-mycobacteria.
  - tube can be re-processed for decontamination and culture.
  - if molecular methods are used for identification, some contamination may not preclude continuing with identification of the mycobacteria present. Tubes may not, however, be used for growth–based DST.

# Contamination – Liquid media

---

- No AFBs and only non-AFBs in the deposit indicates growth of contaminants
  - some laboratories may choose to re-decontaminate and re-process these, as there may be some AFB present that is occluded by the non-AFB and are not visible on ZN
  - some labs discard the tube and continue culture with solid media only
  - reprocessing is strongly recommended if the original specimen source cannot be easily recollected; e.g. tissue specimen.

# Contamination – Liquid media

---

- Presence of only AFBs, with no non-AFBs, indicates pure growth of mycobacteria
  - medium should be processed for identification and DST
  - inoculation of a non-selective agar plate, such as blood agar, can be used for purity check

# Contamination – Liquid media

---

- No organisms seen at all
  - Consider continuing the smear examination very carefully to look for very low numbers of AFB
  - If no organisms are seen at all, consider continuing re-incubation of the liquid media tube.

# Contamination – Liquid media

---

- ZN or Kinyoun staining should be performed on instrument-positive broths
  - deposit drop of medium on glass slide, let dry in BSC, fix, proceed with staining protocol.
- After staining, culture should be handled according to the results.