

Rapid detection of *Mycobacterium tuberculosis* complex from positive MGIT broth cultures: an alternative workflow

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Disclosures

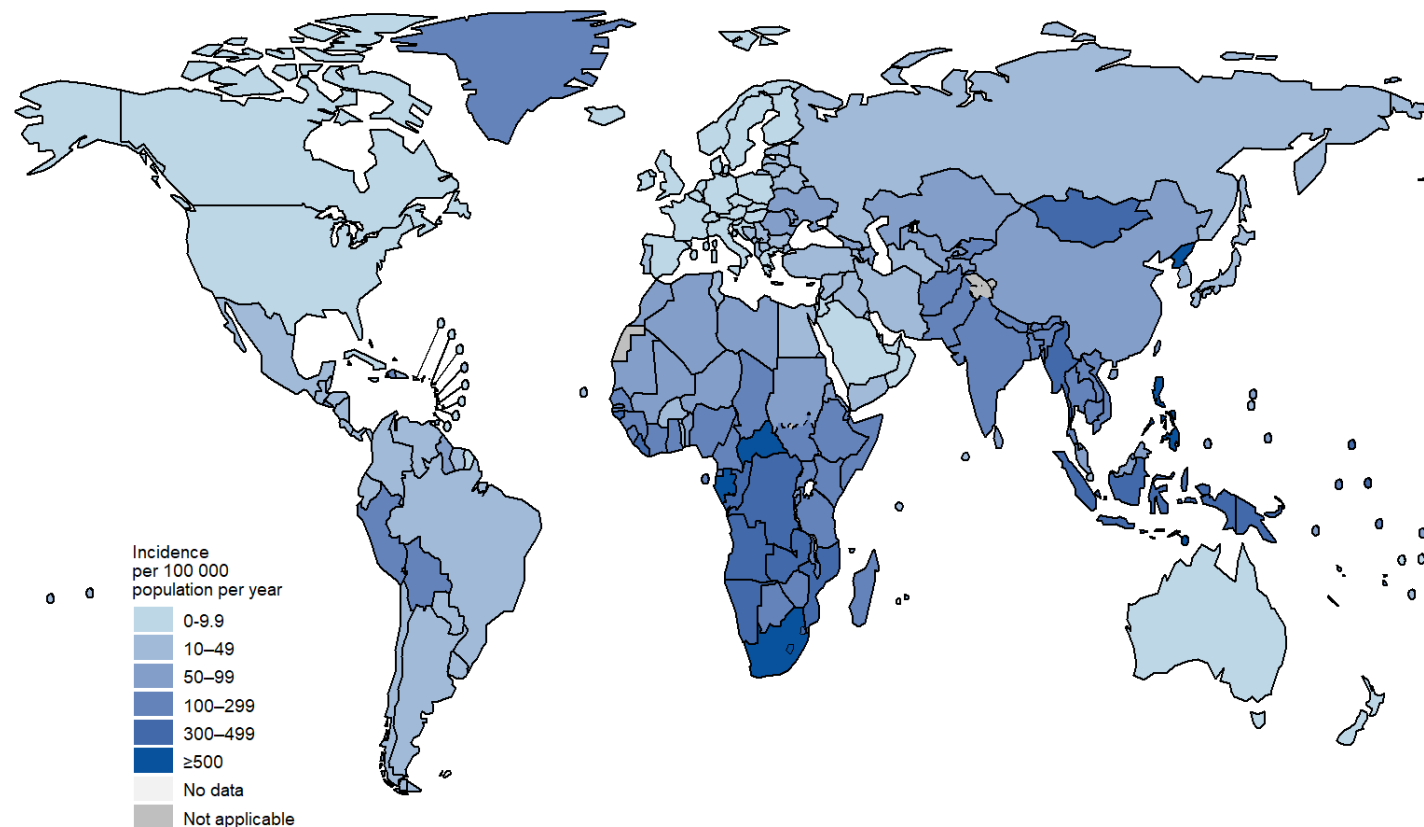
- None

Objectives

- Understand the isolation and identification workflow in the clinical Mycobacteriology/AFB laboratory
- Understand the use of the Cepheid Xpert TB/RIF PCR in the clinical AFB laboratory
- Understand the use of Xpert TB/RIF PCR for detection of *Mycobacterium tuberculosis* from positive mycobacterial growth indicator tubes as an alternative workflow

Global incidence of tuberculosis (WHO 2021)

An estimated 2 billion latently infected with MTBC (23% of world's population)
1.6 million attributable deaths



Mycobacterium tuberculosis

- Extremely low ID₅₀ of <10 bacilli
- Direct detection from clinical specimens is critical for patient care and public health efforts
- Multidrug therapy
 - » First line: Rifampin, Isoniazid, Pyrazinamide, and Ethambutol
- Increase in Multidrug resistance
 - » Rifampin and Isoniazid
 - » Second line: Fluroquinolone, Bedaquiline, Linezolid

MTB Testing Recommendations

- Robust screening guidelines
- Increased TB diagnosis and treatment initiation
 - » AFB smear microscopy for patients suspected of pulmonary TB disease
 - » Liquid and solid culture for identification and antimicrobial susceptibility testing
 - » Rapid nucleic acid amplification tests on initial respiratory specimens
- Isolation of patients suspected to have TB with smear-positive results pending culture or treatment response
- WHO-recommended rapid diagnostic tests (mWRDs) may be used to improve the accuracy of symptom screening in populations at high risk of TB.
 - » Cost-effective in areas of high TB incidence
 - » Improved accuracy and effectiveness in people with HIV

WHO 2021

Role of the Clinical Microbiology Lab

Use of state-of-the art methods

- fluorescence microscopy
- liquid and solid culture media
- rapid detection methods

Reporting of AFB smear results within 24 hours*

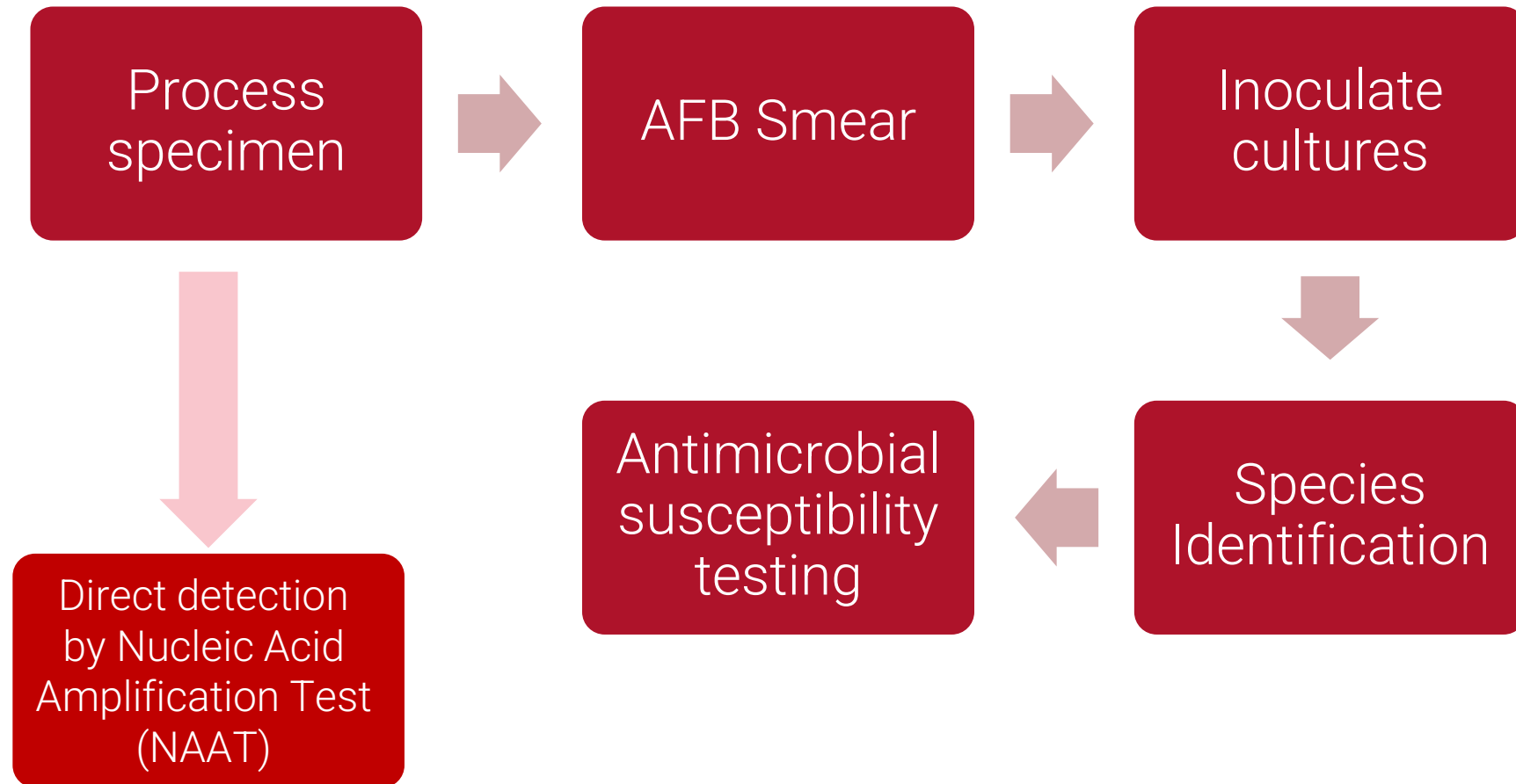
Reporting of *M. tuberculosis* complex within 21 days*

Reporting of conventional susceptibility testing within 28 days*

Verbal reporting of test results to ordering provider within 24 hours of availability

*CAP recommendations for Turn-around-time and reporting

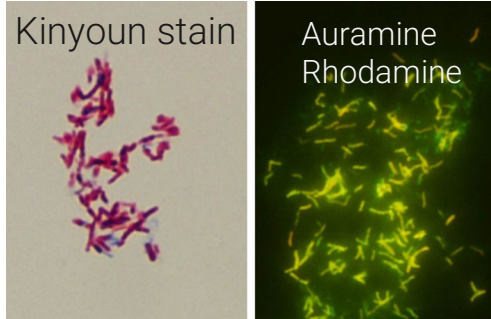
Mycobacterium laboratory workflow



Process specimen

Mycobacterium Laboratory Workflow

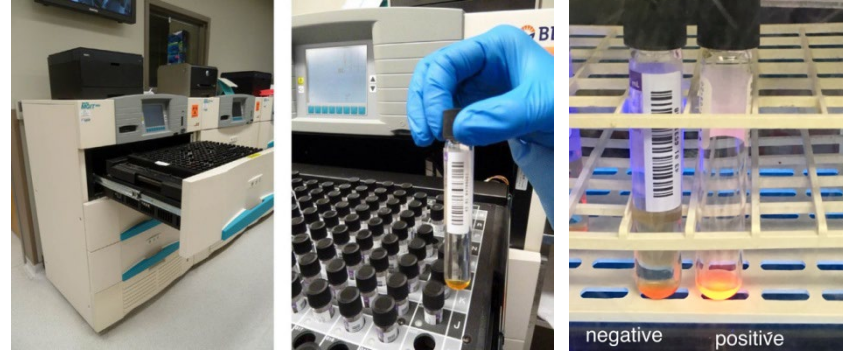
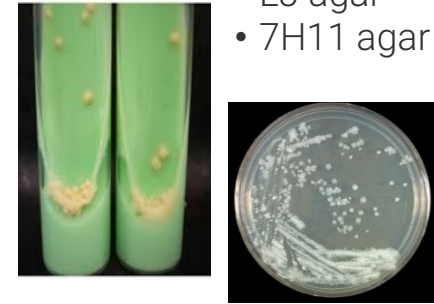
AFB Smear



Automated Monitoring Culture using BACTEC 960 system
Mycobacterium Growth Indicator Tube (MGIT)

Solid Culture
(4-6 weeks)

- LJ agar
- 7H11 agar



Direct Detection

Xpert MTB/RIF PCR



Identification

MTB-Accuprobe

Susceptibility

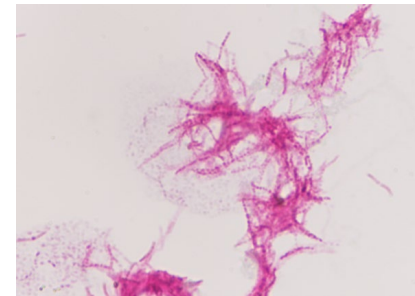
MALDI-TOF or
Sequencing

MTB
Negative

Liquid Culture
(2-5 days)



Positive



AFB smear

Hologic TB AccuProbe

- Rapid DNA probe test that utilizes nucleic acid hybridization for identification from culture
 - » *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, and *M. canetti*
 - » Does not differentiate between members of MTB complex (MTBC)
- Allows identification of MTBC isolated from solid media and broth culture
 - » Approximately 4 hours

*APHL Molecular Detection and Identification of Mycobacteria; Caulfield, A. J., & Wengenack, N. L. (2016). Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. *Journal of clinical tuberculosis and other mycobacterial diseases*, 4, 33–43.

Gene Xpert MTB/RIF

- Can detect as little as 10 bacilli per sample
- Sample to Result < 120 minutes
- CDC recommends NAAT on at least 1 respiratory specimen from each patient
- Detects gene mutations associated with rifampin (RIF) resistance (*rpoB*)
- Globally, approximately 82% of RIF resistant TB is multidrug resistant (MDR)
 - MDR-TB = resistance to at least RIF and isoniazid (INH)
- When testing a single sputum (Cochrane review 2013)
 - Detects 98% (95% CI; 97-99%) smear positive cases
 - Detects 80% (95% CI; 87 – 88%) smear negative cases



<https://www.youtube.com/watch?v=mIsBLmjus6Q&t=138s>

Gene Xpert MTB/RIF

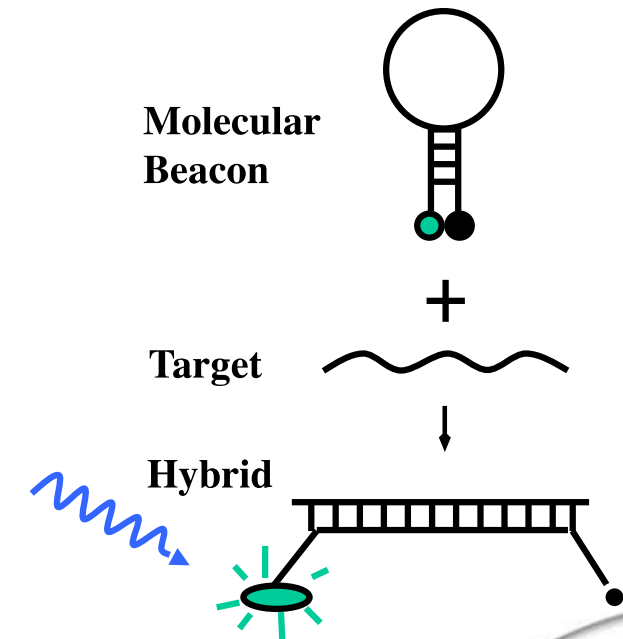
rpoB GENE 81 bp RIF RESISTANCE DETERMINING REGION



5-Probes bind to wild type (do not bind to mutant sequence)

1-Probe for SPC (*Bacillus globigii*)

6-fluorescent dyes detected simultaneously



Gene Xpert MTB/RIF

- Lower total healthcare costs per patient (\$2673) compared to microscopy and culture alone (\$2728)
 - » Implementation of the MTB/RIF increased lab costs by >60% per patient
 - » Total healthcare costs decreased through reduction of unnecessary treatment and prolonged isolation in hospitals
- Quality-adjusted life-year (QALY) study
 - » Molecular testing resulted in less empiric treatment and shorter hospitalizations
 - » Reduction in time of diagnosis for TB cases from 16.3 days to 2.7 days
 - » Incremental 6.32 QALYs gained per 1000 patients

COVID-19 Pandemic Challenges

- Manufacturing capacity strained worldwide
- Shortages in culture-based and direct detection laboratory supplies
- Hologic AccuProbe MTB discontinuation notice in Q3 2020
- No other FDA-approved assays available
- Focus on ruling out MTB



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MYCOBACTERIOLOGY AND
AEROBIC ACTINOMYCETES



Clinical Validation of the Xpert MTB/RIF Test for Identification of the *Mycobacterium tuberculosis* Complex in Acid-Fast Bacillus Smear-Positive MGIT Broth Cultures

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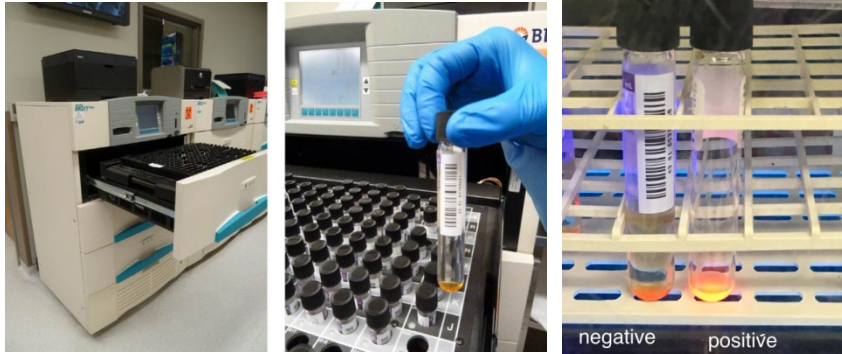
Study Summary

- 347 MGIT cultures tested on the Xpert MTB/RIF, compared with MALDI
 - » 177 tested with standard 3:1 reagent:sample ratio, 170 tested with 2:1 reagent:sample ratio
 - » Previously tested clinical isolates (n=100) and spiked MGIT (112) at various dilutions
- 100% categorical agreement for MTBC detection and rifampin resistance prediction
- No cross-reactivity detected with NTM (n=125)
- No false-positive with non-mycobacterial bacteria or NTM
- Mixed cultures detected MTBC
- Evaluated AFB smear-positive MGIT cultures only ($>10^4$ CFU/mL)
 - » Smear-negative cultures not tested

[J Clin Microbiol.](#) 2022 Feb; 60(2): e02164-21

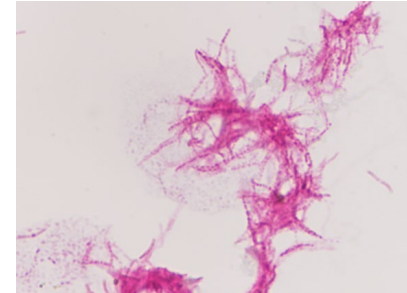
Proposed Mycobacterium Laboratory Workflow

Automated Monitoring Culture using BACTEC 960 system
Mycobacterium Growth Indicator Tube (MGIT)

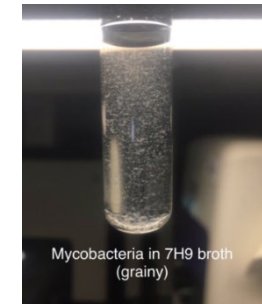


Positive

AFB smear



Liquid Culture
(2-5 days)



Identification

Xpert MTB/RIF PCR



Identification

MALDI-TOF

MTBC ID

Susceptibility

No ID

Sequencing

MTBC ID

Susceptibility

Comparison of MTBC detection methods

Test platform	Probe	MTB/RIF	MALDI	Sequencing
Sample type	Positive culture	Direct specimen, positive culture (validated)	positive culture	Positive culture
Assay Cost	\$\$	\$\$\$	\$	\$\$\$
Testing time	2-4 hr	2 minutes hand-on Time to result < 2hr	20 min	~24 hr
Labor	Labor intensive, not automated, testing in dedicated areas	Simple, sample-to-result closed system, Limited biosafety concerns	Moderate hands-on time for extraction, limited biosafety concerns	Moderate hands-on time for extraction, extensive analysis time
Rifampin detection	N/A	Predicts Rif resistance	N/A	Sequencing of <i>rpoB</i> gene (not available in most labs)

MALDI-TOF

- From positive culture (M7H9 or solid media)
- Rapid identification
- Cheaper supply costs
- Difficult extraction protocol
- Database limitations
- Initial cost investment high

ID of MTBC

No AST results

Need to correlate results with smear and culture

Xpert MTB/RIF

- Direct identification from respiratory specimens, CSF, pleural fluid, tissue
- Provides information on possible RIF resistance
- Automated closed system
- Rapid, limited hands-on time
- More expensive supply costs



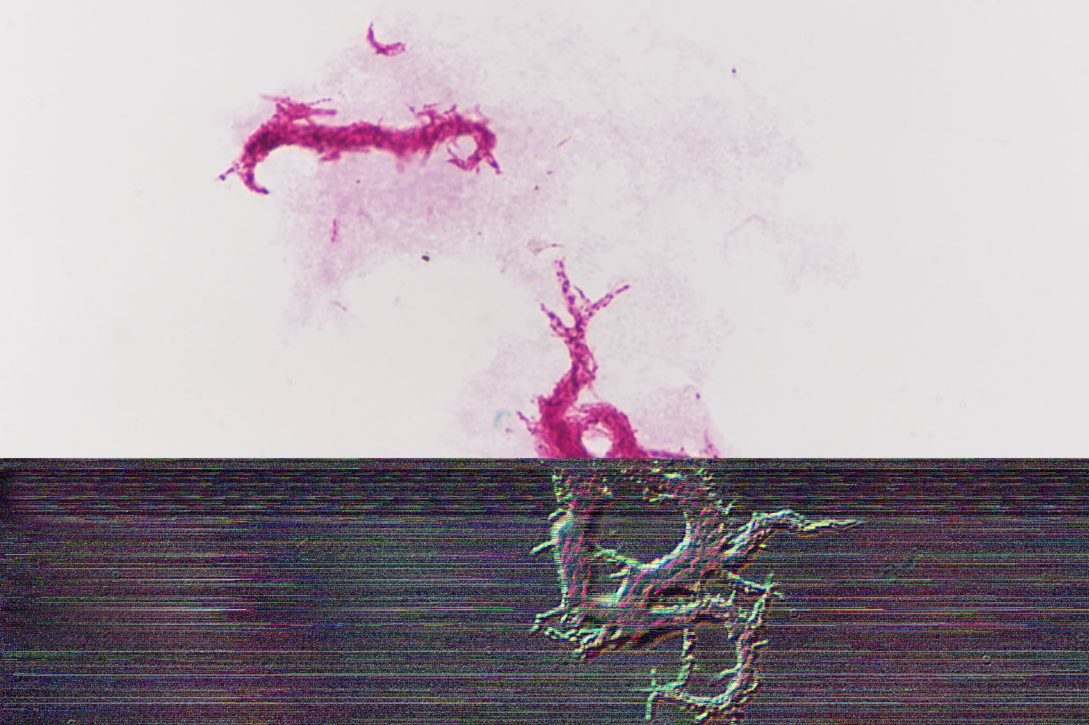
Rationale for validating GeneXpert on AFB positive MGIT

- Rapid detection
- Rifampin resistance prediction
- Easy implementation in laboratory workflow

But....

- Not cost-effective for laboratories with large testing volumes
- Multiple samples received

Cording in *Mycobacterium tuberculosis*



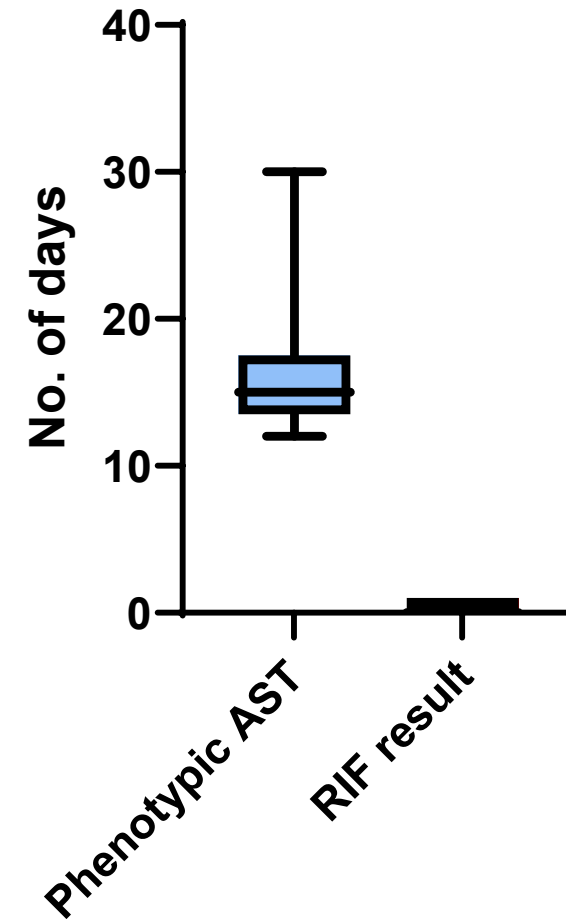
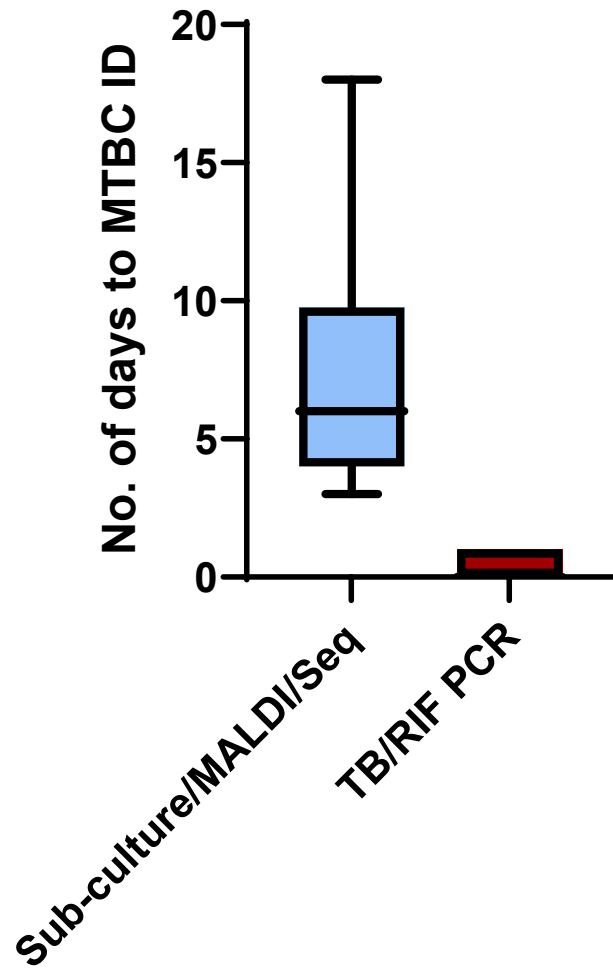
Validation method

- 45 specimens
 - » 40 prospectively-collected, 5 spiked specimens
- Processed by standard laboratory work-up
- Positive MGIT 960 broth cultures tested by Kinyoun AFB staining
- 0.5 mL of MGIT cultures exhibiting cording were tested on Xpert MTB/RIF
- Positive MGIT 960 cultures also sub-cultured in M7H9 media for downstream laboratory applications (MALDI-TOF, AST)
- Smear morphology, time to positive MTBC result, and susceptibility were noted

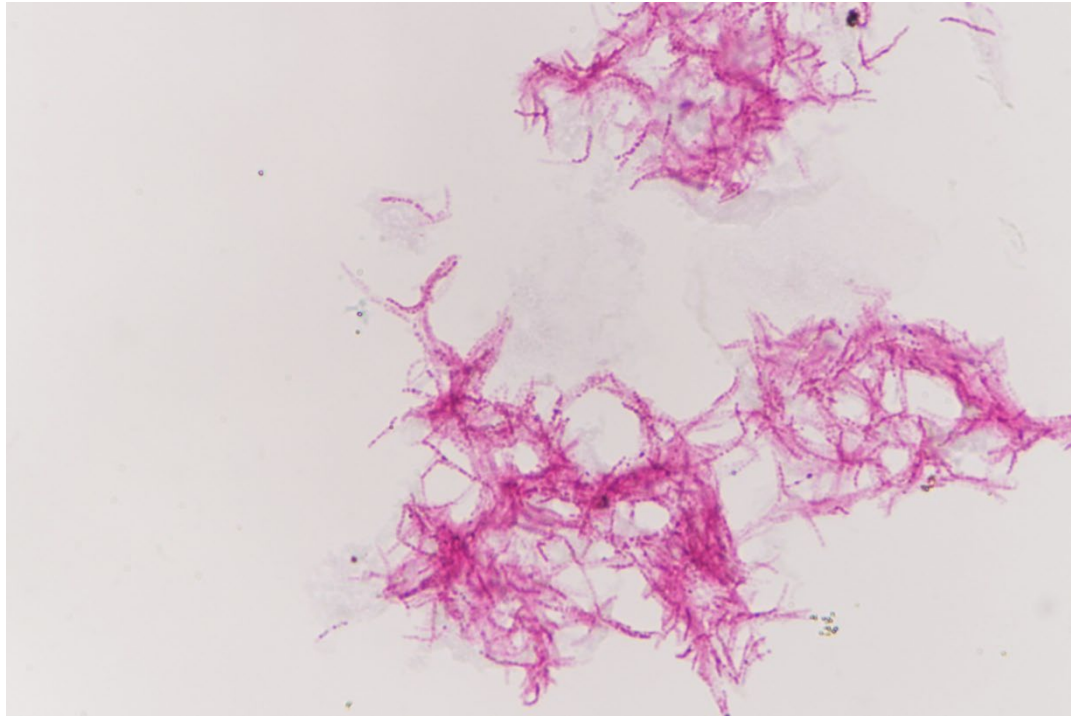
Results

- Overall, 28/30 specimens were positive for MTBC alone
- 2 specimens were dual positive for MTBC and MAI
- 10 specimens were negative for MTBC and positive for non-tuberculous Mycobacteria (*M. kansasii* (2), *M. abscessus* (7) and *M. avium intracellulare* (MAI) complex (1))
- 100% categorical agreement MTBC identification (30/30) to MALDI-TOF or 16S rRNA sequencing-based identification from 7H9 broth
- 100% categorical agreement for prediction of rifampin susceptibility
- No false-positive results or cross-reactivity were noted

Time to Result: Identification and RIF prediction



Cording phenomenon in Non-tuberculous *Mycobacterium* (NTM)

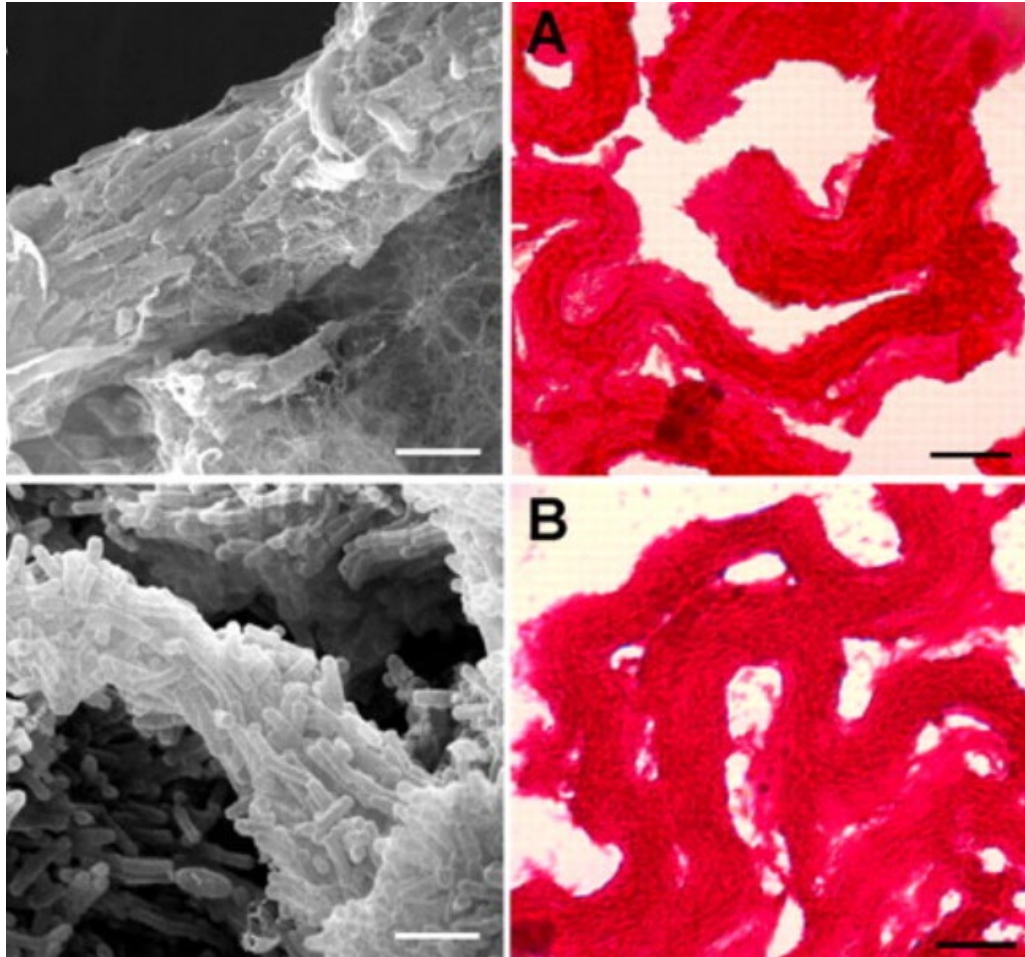


M. kansasii “cross-barred cording”



M. tuberculosis “serpentine cording”

Cording phenomenon in NTM



- Slow growing Mycobacteria
 - » *M. marinum*
 - » *M. kansasii*
- Rapidly growing Mycobacteria
 - » *M. abscessus* (rough colonies)
- Other non-pathogenenic NTM

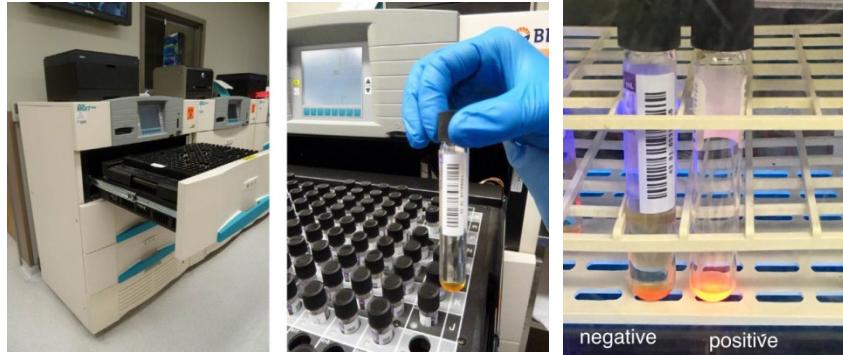
[J Clin Microbiol.](#) 2011 Jun; 49(6): 2293–2295

Time to positive MGIT culture

- The median time for MGIT positivity for MTBC was 7 days compared to *M. abscessus* (rapid grower) which was 4 days
- The median time for MGIT positivity for MTBC was 7 days compared to *M. avium* (slow grower) which was 12 days

New Mycobacterium Laboratory Workflow

Automated Monitoring Culture using BACTEC 960 system
Mycobacterium Growth Indicator Tube (MGIT)



Positive



AFB smear positive with cording
Time to positivity ≥ 7 days

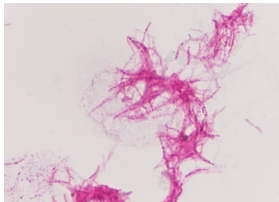
First positive specimen

Identification



Xpert MTB/RIF PCR

AFB smear



Liquid Culture
(2-5) days



MALDI-TOF
Sequencing
(1-2 days)

MTB ID

Susceptibility

MTB detected
RIF resistance
predicted
Reported
same day

Advantages

- Rapid, accurate alternative
- Same day result with positive MGIT broth
- Provides RIF resistance prediction result
- Potential improvement for patient management

Limitations

- Time to positivity may vary depending on organism burden
- Subjectivity with recording smear results
- Duplicate results with direct specimen testing
- Need for culture for phenotypic susceptibility testing
- Increase cost to the lab (\$50 compared to MALDI-TOF)

Conclusions

- Creative solutions needed with supply challenges during COVID-19 pandemic
- Need for alternative, rapid-diagnostic methods to identify or rule-out MTBC from positive culture
 - » More rapid patient management
 - » Public health measures
- Rapid NAAT assays like Xpert MTB/RIF can be used as an alternative
- Applying MTBC phenotypic characteristics such as cording and time to positivity (≥ 7 days) may streamline the workflow
- Laboratories should consider testing volumes and costs

Acknowledgments

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- APHL ID CON

Thank you!





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