Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Case Study: Molecular Detection of MTBC and IS6110

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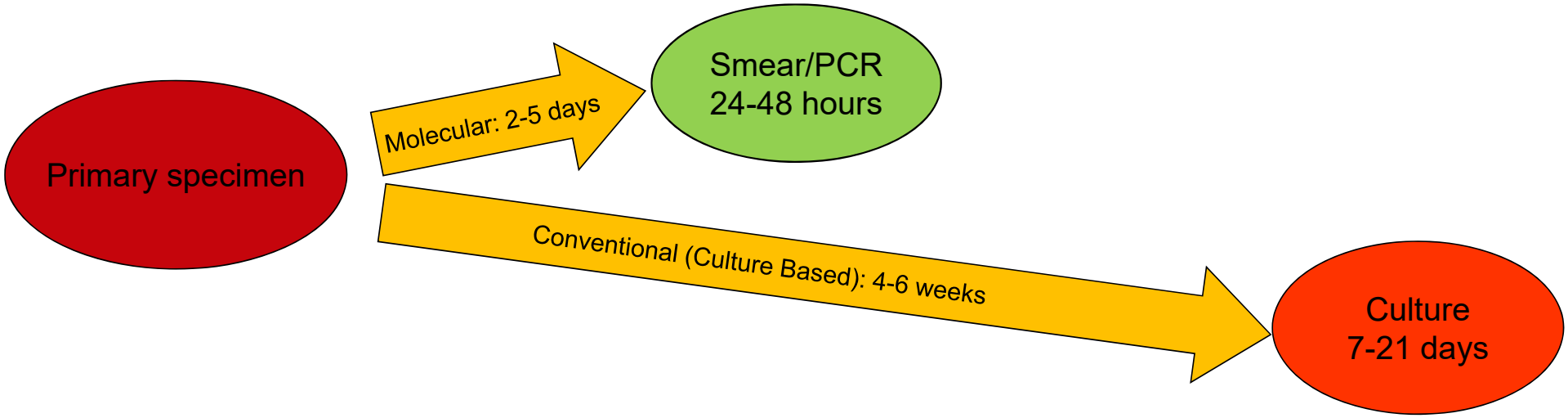
Identification of MTBC in Patient Specimens

- Identification of *Mycobacterium tuberculosis* complex is the most important finding in the mycobacteriology laboratory
- Finding of MTBC has serious clinical and public health consequences
- Almost always considered clinically significant



Molecular Detection of MTBC

- Reduction in diagnostic time from weeks to days
  - WSLH:
    - MTBC culture → average 15 days to positive
    - TB PCR → Results typically reported <24hr
  - Initiation of earlier treatment
  - Fewer transmissions

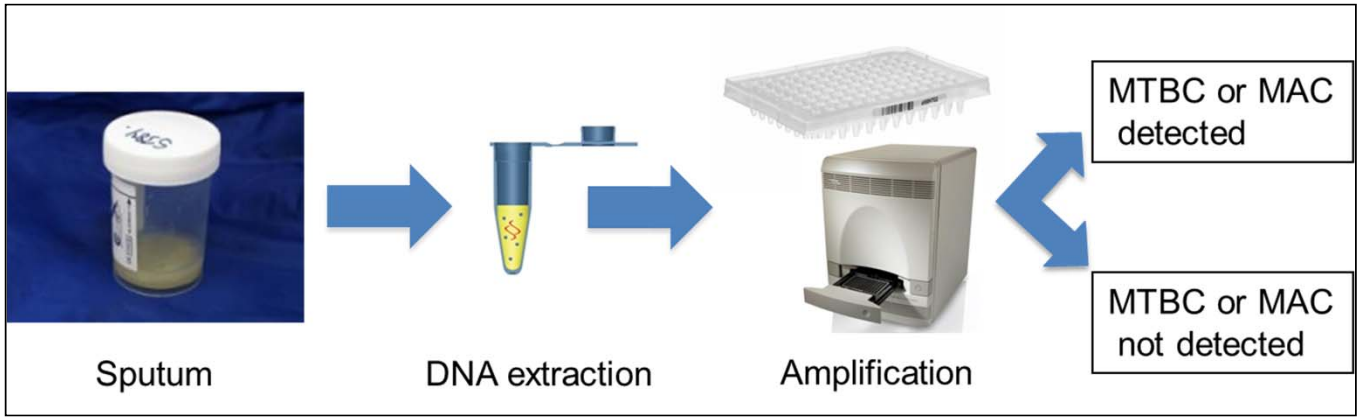
- Sensitivity much higher than smear alone
  - 5000-10000 AFB/ml vs <200 AFB/ml
  - Diagnosis in smear-negative patients
    - >95% for AFB smear-positive TB patients
    - >55% of AFB smear-negative TB patients

APHL Direct Detection of MTBC Guidance

- Laboratory should perform or have access to nucleic acid amplification testing (NAAT) to detect MTBC in **smear-positive** specimens
- Laboratory should perform or have access to NAAT to detect MTBC in high-risk individuals with **smear-negative** specimens
- Laboratory should report NAAT results within 48 hours for >75% of specimens tested
- Laboratory MTBC NAAT should contain internal controls or have other method for detecting NAA inhibitors

Direct Detection of MTBC using Nucleic Acid Amplification Testing (NAAT)

- **Not a replacement for culture**
- FDA approved: Cepheid GeneXpert MTB/RIF (sputum)
- Lab Developed Test (Realtime-PCR; individually validated)



Cepheid GeneXpert

- Cepheid GeneXpert MTB/RIF
  - Amplifies DNA from decontaminated sputum sediment
    - Target: rpoB
  - LOD: ≈130 AFB/ml
  - Less than ten minutes hands-on time, results in <120 min
  - Requires GeneXpert system
  - Approximately $50/cartridge

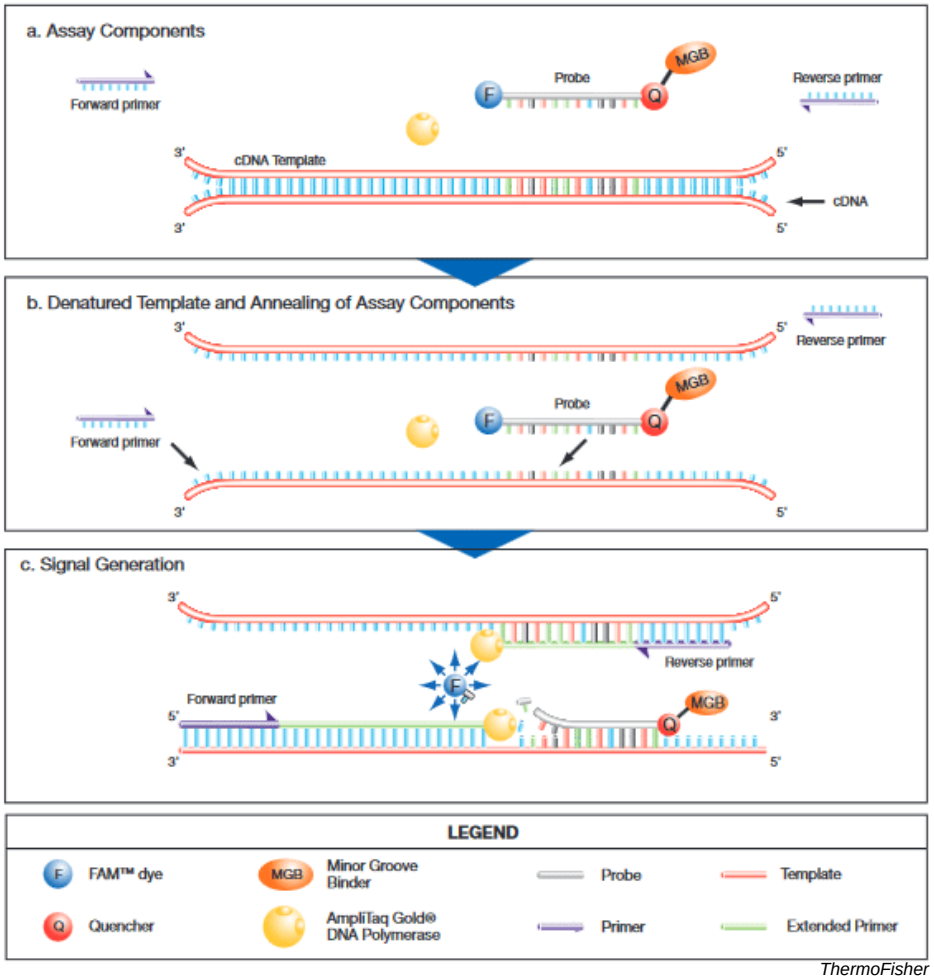
Cepheid

Hologic Amplified MTD

- Mycobacterium tuberculosis Direct (MTD) Test
  - Amplifies rRNA from decontaminated sediment
    - Target: rRNA
  - LOD: ≈50 AFB/ml
  - 3 hour hands on time
  - Requires luminometer
  - Approximately $23/sample
  - Was FDA-approved;**discontinued 7/31/2021**

Hologic

Lab Developed Test: Real-Time PCR



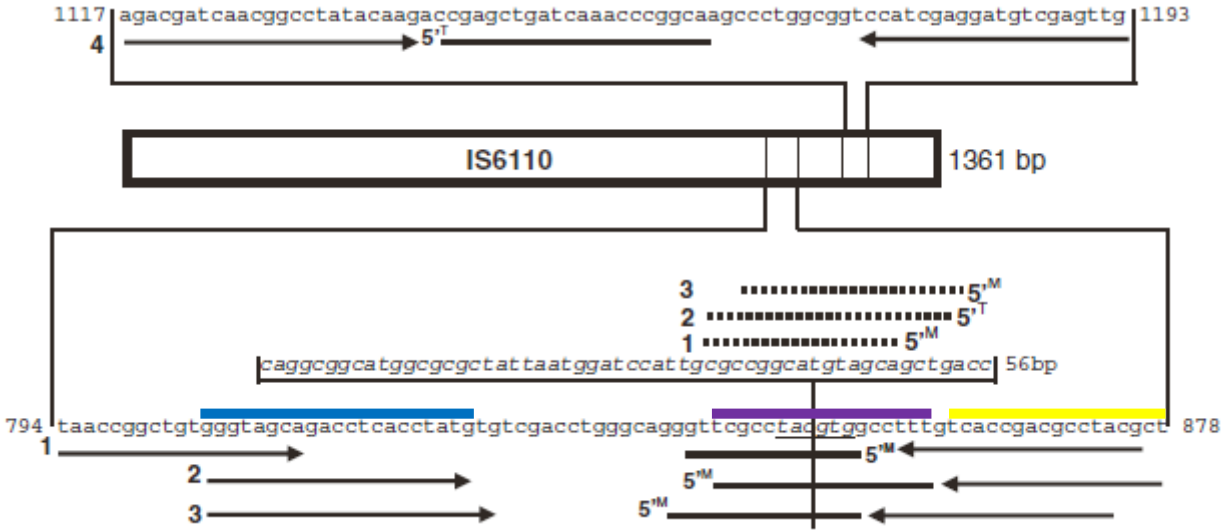
ThermoFisher

WSLH MTBC PCR Testing

- Automatically performed on all new smear-positive specimens
  - Respiratory and non-respiratory sources
  - Fee-exempt testing for smear-positive specimens and patients suspected of having active TB (approved by WI TB Program)
- Smear-negative respiratory specimens tested with submitter charge
- Sensitivity
  - >95% for AFB smear-positive, culture-confirmed TB patients
  - 55-75% of AFB smear-negative, culture-confirmed TB patients
  - LOD: <1 MTBC bacillus/reaction (≈140 AFB/ml)
- Also validated for use with AFB-positive cultures

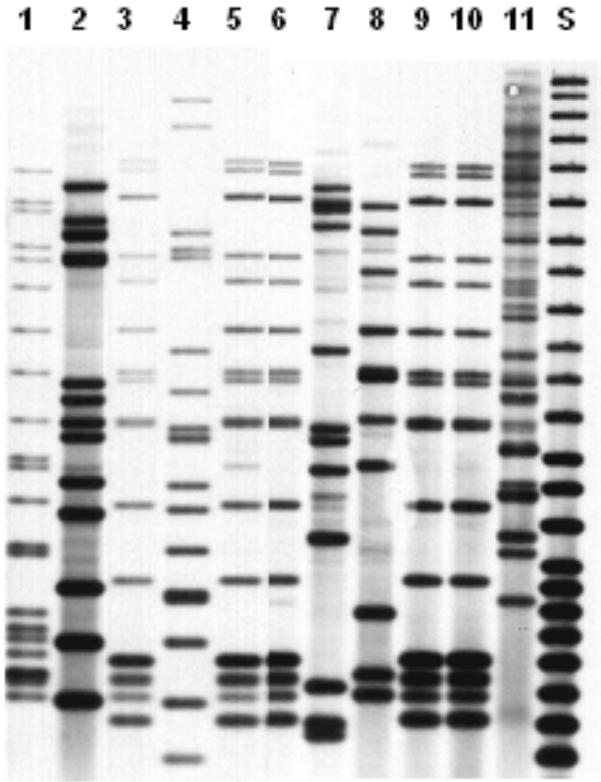
WSLH Real-Time PCR Assay

- IS6110
  - Most commonly used target in MTBC ID → sensitivity
  - 16 copies in *M. tuberculosis* H37Rv
  - 10-20 copies in other *M. tuberculosis*
  - 1 copy in *M. bovis* and *M. bovis* BCG



IS6110

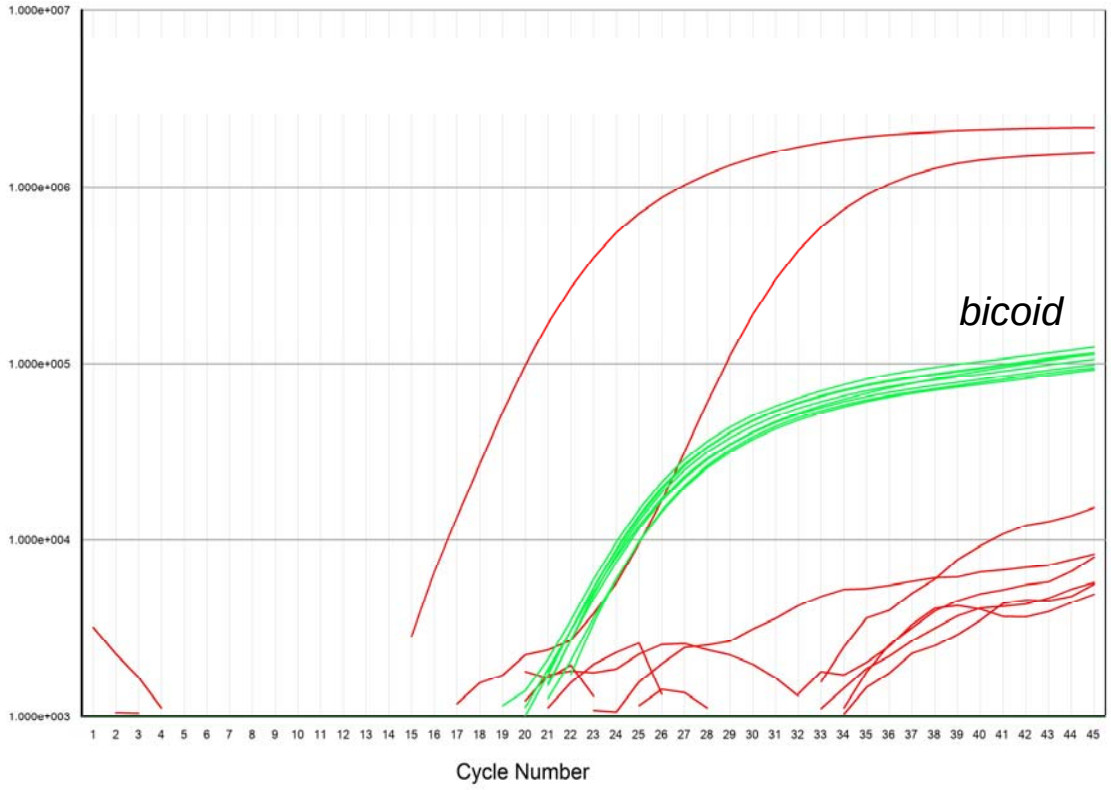
- Insertion sequence (transposon) present in MTBC, absent in NTM
  - Good target for screening AFB+ specimens
- Copy number/insertion location can be used for strain genotyping (RFLP)
- Unknown function, *may* increase virulence and antibiotic resistance



CDC DTBE: Tuberculosis Genotyping Laboratory Procedures

WSLH PCR Inhibition: Updated Internal Control

- Previously: human RNaseP PCR
- Updated: *bicoid* plasmid in PCR mastermix
  - Significantly more sensitive to inhibition
  - Reduces inter-sample variability
- Loss or reduction in *bicoid* amplification signals presence of PCR inhibitors
  - Sample is purified/ concentrated and re-analyzed



Case Study: “Undetectable” TB

- Patient History
  - 69 year-old resident of SE Asia
  - Living with family in US
  - Former smoker
- PCP visit D/T >2 months of throat pain and difficulty swallowing/speaking, sent to ER
  - Denied previous fever or chills, cough, weakness
  - Weight loss
  - Coughing up dark yellow/green mucus
  - Pneumonia and infiltrates on chest CT
  - Mass observed on bladder scan
  - Negative COVID test

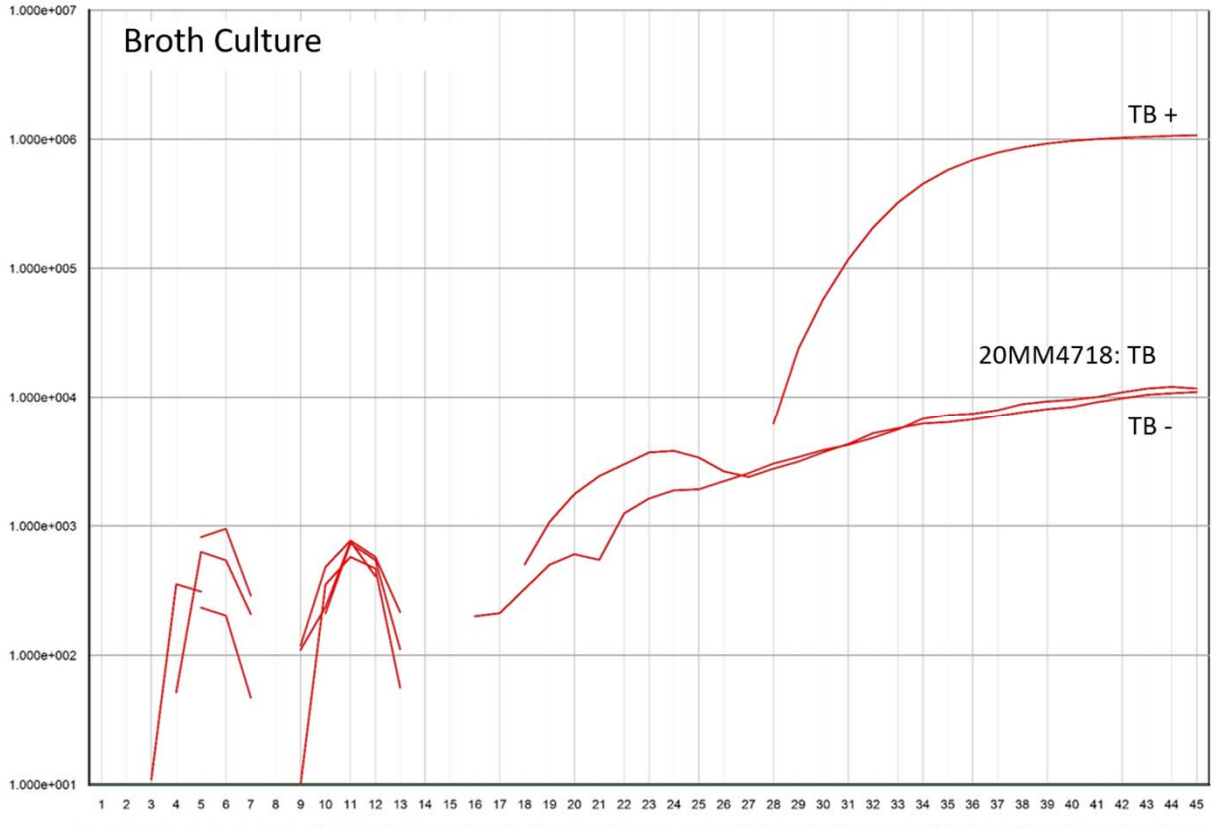
Laboratory Results

- QuantiFERON-**Negative**
- Sputum collected, **AFB smear-positive**
- Peritoneal fluid, urine also **AFB smear-positive**
- Peritoneal fluid, 2x sputum sent to WSLH for MTBC/MAC PCR
    - TB PCR negative
    - MAC PCR negative
- Specimen referred to MHD for GeneXpert MTB/Rif testing
    - MTBC positive, no rpoB mutation detected
- All cultures grew MTBC on solid media
- Isolate was pan-susceptible to 1st-line TB drugs

- Patient diagnosed with disseminated TB (and pulmonary MAC) and started on RIPE therapy with AZM

Laboratory Investigation

- TB PCR results:
  - Sputum sediment-Negative for MTBC DNA
  - Peritoneal fluid sediment-Negative for MTBC DNA
  - Broth culture- Negative for MTBC DNA
- Why was TB PCR negative?
  - PCR Inhibition?
  - GeneXpert more sensitive?
  - PCR target?



Laboratory Investigation-continued

- Consulted with NY Department of Health, Wadsworth Center
  - Use a dual-target Realtime-PCR assay for identification of MTBC
    - IS6110
    - ext-RD9 → also specific to MTBC, but only one copy
- Sent smear-positive peritoneal fluid for testing
  - Negative by IS6110 PCR
  - Positive by RD9 PCR

NEW YORK STATE DEPARTMENT OF HEALTH

WADSWORTH CENTER

FINAL LABORATORY REPORT Report Date

Clinical Mycobacteriology Laboratory
Phone: (518) 474-4158 Fax: (518) 408-2264

Testing performed at CLIA# 33D2005937

Specimen Id: IDR2000249478

Specimen Type: Other

Direct Molecular Detection - Real-time PCR

Mycobacterium tuberculosis complex DNA by real-time PCR*:	**DETECTED**	10/22/2020
Mycobacterium avium complex DNA by real-time PCR*:	**Not Detected**	10/22/2020

Molecular Identification - Real-time PCR

Mycobacterium tuberculosis complex species DNA identified*:	**Mycobacterium tuberculosis**	10/22/2020

IS6110-negative MTBC does exist!

Characterisation of *Mycobacterium tuberculosis* isolates lacking IS*6110* in Viet Nam

M. N. T. Huyen,* E. W. Tiemersma,†‡ K. Kremer,§¶ P. de Haas,¶ N. T. N. Lan,* T. N. Buu,* C. Sola,# F. G. J. Cobelens,†‡ D. van Soolingen¶,**

Epidemiology of *Mycobacterium tuberculosis* strains in San Francisco that do not contain IS*6110*

C. B. Agasino,* A. Ponce de Leon,* R. M. Jasmer,† P. M. Small*

Analysis of sequence diversity among IS6110 sequence of *Mycobacterium tuberculosis*: possible implications for PCR based detection

Sathish Sankar*, Suresh Kuppanan, Babu Balakrishnan, Balaji Nandagopal

Failure of PCR-Based IS*6110* Analysis To Detect Vertebral Spondylodiscitis Caused by *Mycobacterium bovis*

Deborah Steensels,[a] Maryse Fauville-Dufaux,[b] Johan Boie,[c] Hans De Beenhouwer[a]

IS6110-negative MTBC

- First case detected in WI since WSLH has started molecular testing
  - NY sees about 1/year (700-800 MTBC cases/year)
  - US: 0.2% of all MTBC genotyped since the mid-1990s
- **South East Asia**, particularly Vietnam: 2-4%
- **India**: 2007 study of 308 isolates → 11%
- Based on genomic analyses, thought to be a more ancient lineage of TB
- IS6110-negative strains typically susceptible to 1st-line TB drugs

Summary

- Molecular testing can drastically reduce TTD of MTBC, but it's important to understand the limitations of the test being used
  - Sensitivity and specificity depend on molecular targets
    - IS6110 → Sensitive and highly-conserved, but zero-copy strains exist
    - rpoB → Specific to MTBC, but DR mutations require careful primer design
    - rRNA → Potential cross-reactivity with NTM
  - MTBC evolves slowly, but unique combinations do exist
- If patient was resident of SE Asia, particularly Vietnam or India, and high-level MTBC suspect, consider alternative testing if IS6110-PCR is negative
- If molecular results do not agree with clinical presentation or phenotypic culture growth, investigate!

APHL Webinar

Identifying MTBC and NTM: The New Toolbox

Wednesday, November 10, 2021 | 2:00pm — 3:30pm ET

DESCRIPTION

Hologic® AccuProbe® has been used as a primary culture identification method for Mycobacterium tuberculosis complex (MTBC) and some nontuberculous mycobacteria or NTM (*M. avium, M. intracellulare, M. gordonae, and M. kansasii*) for many years. Hologic's recent announcement regarding the anticipated discontinuation of the AccuProbe line, including MTBC, has highlighted the need for many laboratories to select, evaluate, and implement a new primary identification method to replace AccuProbe®. This webinar will showcase a handful of different approaches that public health laboratories have taken or are in the midst of validating to identify MTBC and NTM. The webinar has also been extended to a 90-minute format to allow for ample question and answer time to address questions from the audience.

LEARN MORE

For more information about this webinar, please email webinars@aphl.org.

REGISTER FOR FREE

Registration via the link above is required to access the webinar. If you need assistance with registration, please email or call 240.485.3843.

Online Access

To participate in this webinar, all you need is a high-speed Internet connection to access ZOOM, the program site. To get started, check out the Top Questions section of the FAQ page.

Continuing Education

APHL is approved as a provider of continuing education programs in the clinical laboratory sciences by the ASCLS P.A.C.E.® Program. Pending approval, this webinar will provide 1.5 contact hour for participants who successfully complete this training.

Questions?

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