Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

“TB Not Detected”

Case Study: Molecular Detection of MTBC and Use of the IS6110 Target

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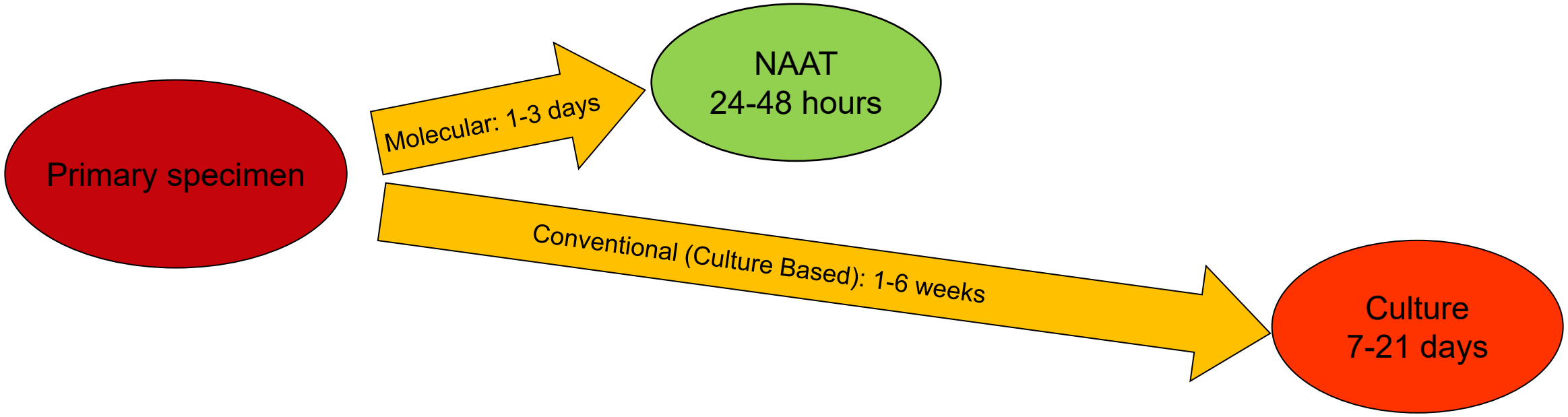
Outline

- Molecular detection of MTBC in primary specimens
  - Testing options
- WI State Laboratory of Hygiene NAAT
  - MTBC real-time PCR
- Case Study: “No MTBC DNA Detected”
  - Laboratory results
  - Investigation
  - Conclusions

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 clinically significant

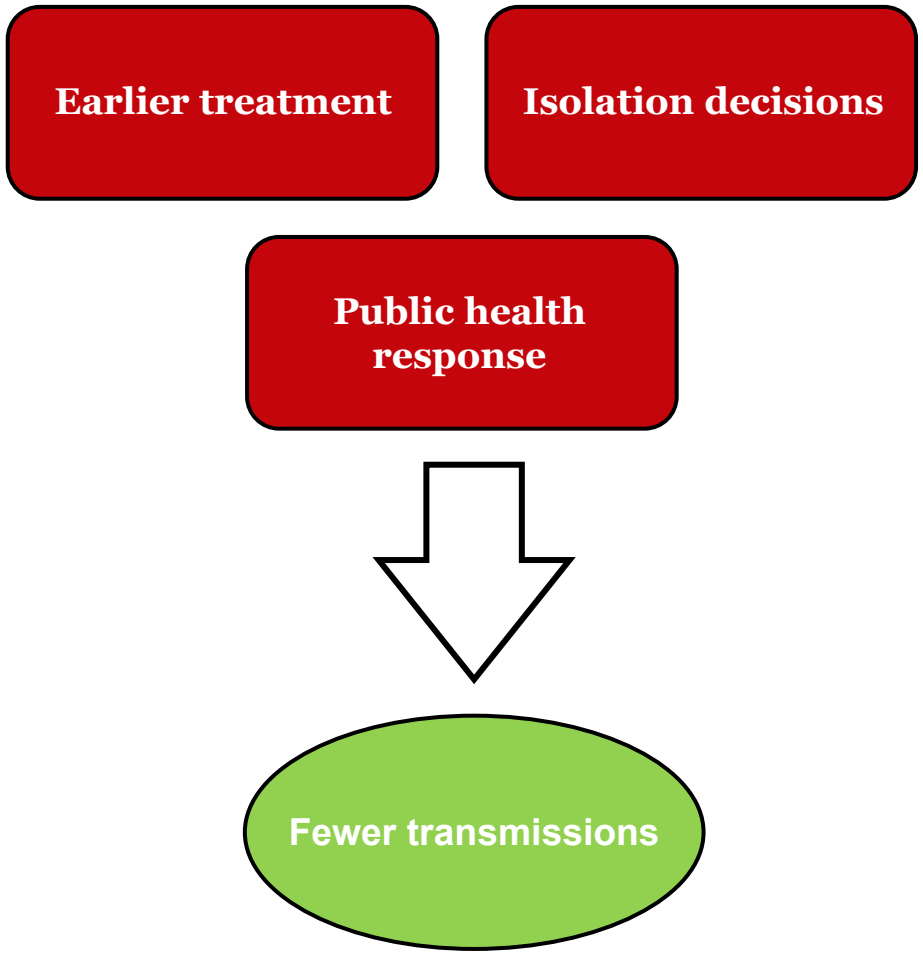


APHL Laboratory Assessment Tool: Direct Detection of MTBC

- 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

Molecular Detection of MTBC

- Reduction in diagnostic time from weeks to days
  - WSLH primary specimen:
    - AFB smear → Results in <24hr
    - MTBC culture → Average of 11 days to positive
    - MTBC PCR → Results typically reported <24hr
- Sensitivity much higher than smear alone
  - 5000-10000 AFB/ml vs <200 AFB/ml
    - >95% for AFB smear-positive TB patients
  - Diagnosis in smear-negative patients
- Specific to MTBC

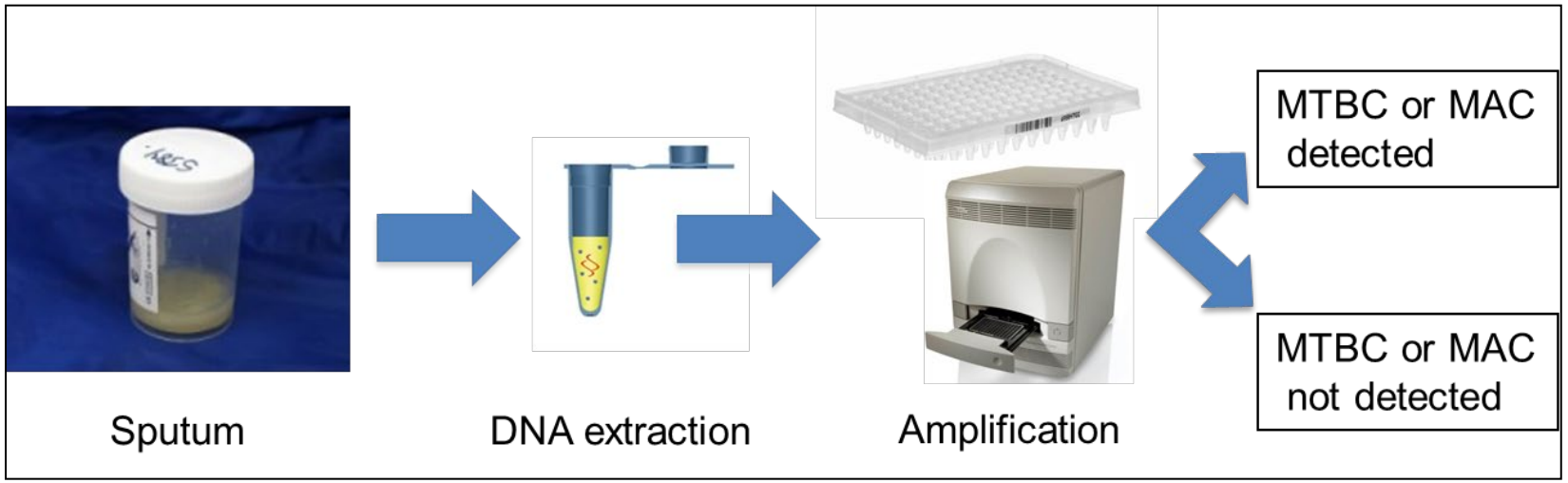


Direct Detection of MTBC using Nucleic Acid Amplification Testing (NAAT)

Not a replacement for culture

FDA approved: Cepheid GeneXpert MTB/RIF (sputum)

Laboratory Developed Test (Real-time PCR, DNA sequencing; individually validated specimen types)

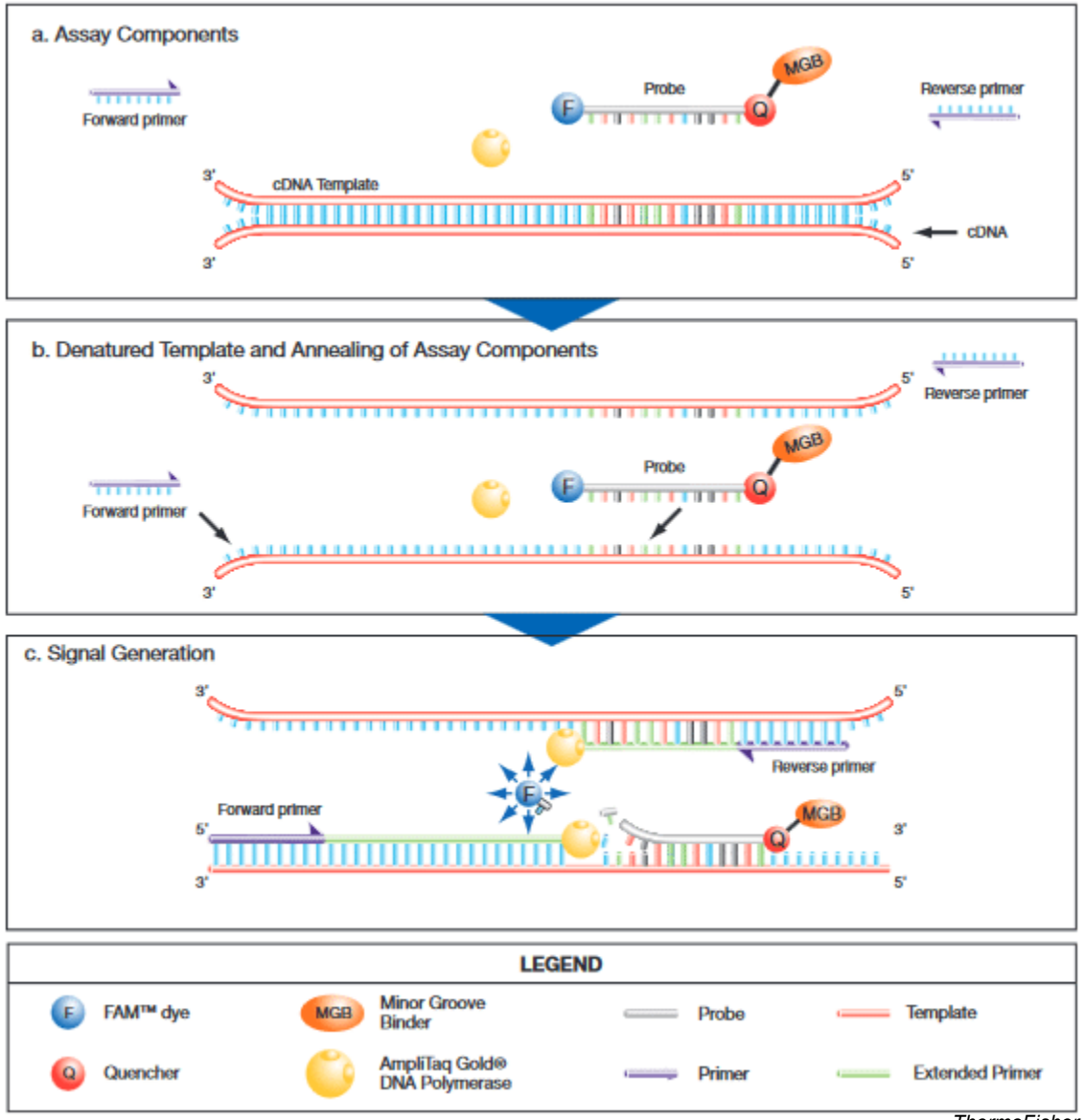


Cepheid GeneXpert

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

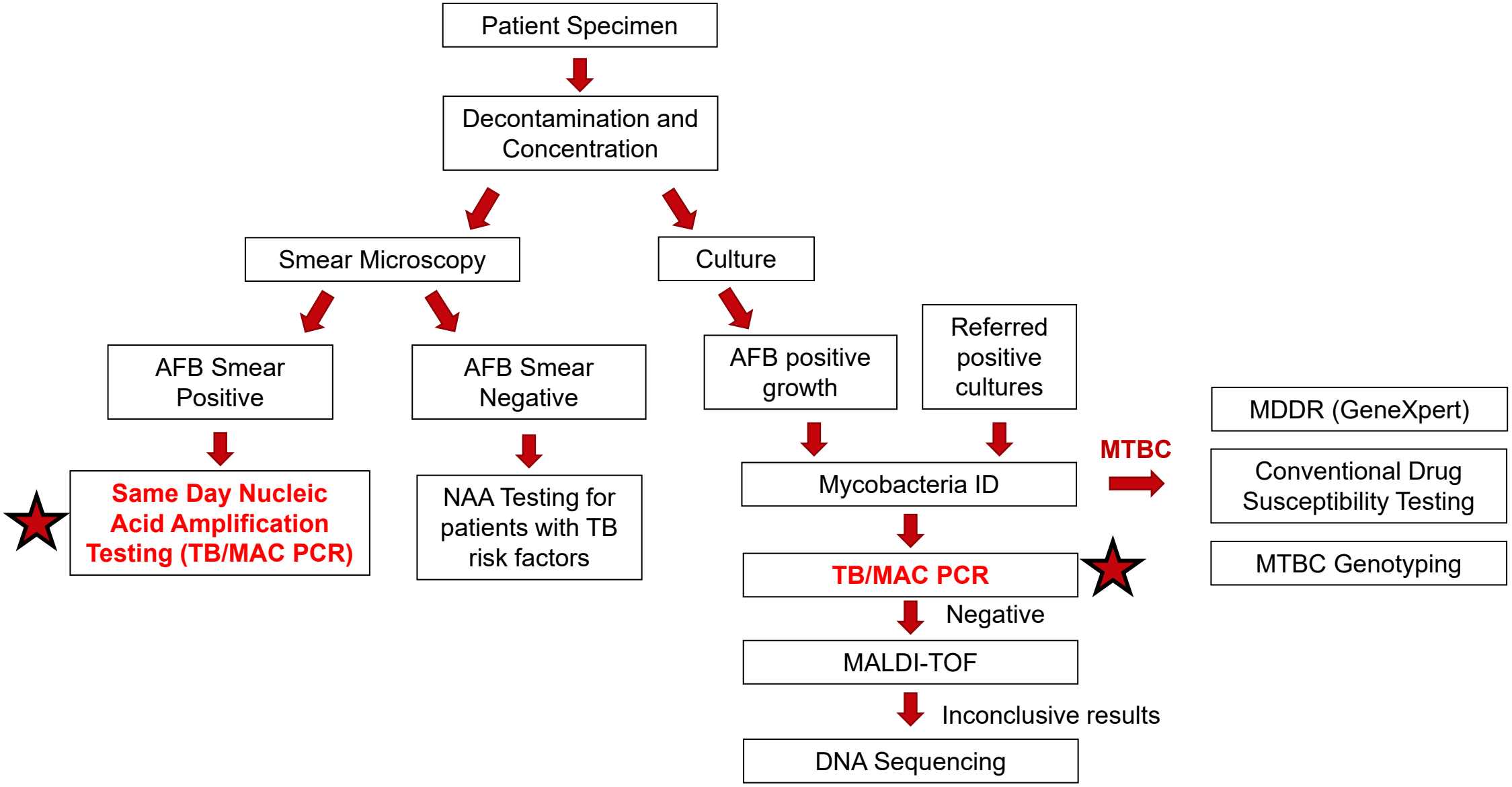
Cepheid

Lab Developed Test: Real-Time PCR



ThermoFisher

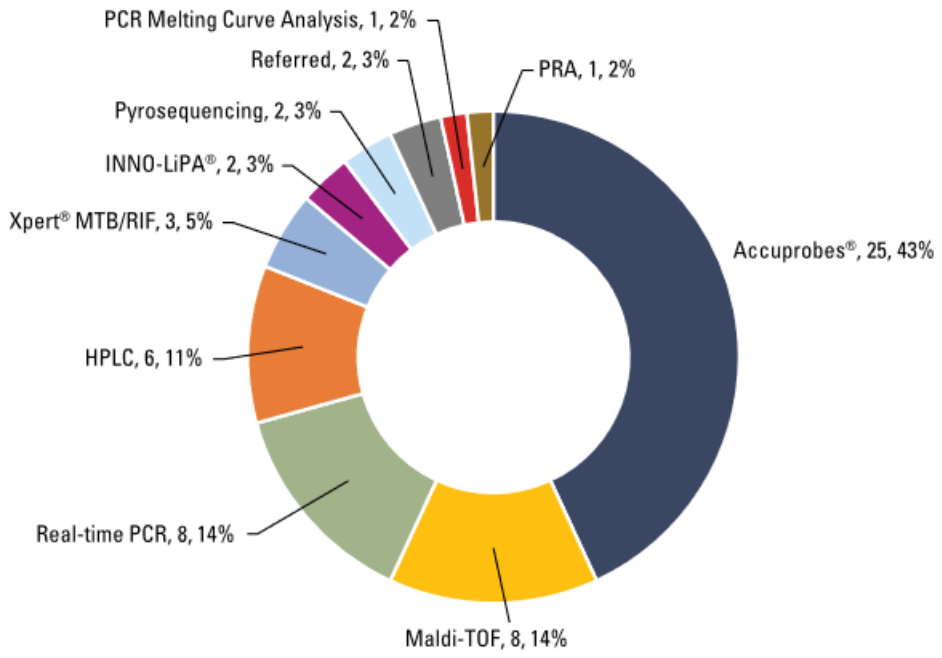
Mycobacteriology Testing at WSLH



Mycobacteria AccuProbe alternative: WSLH TB PCR validated for use with AFB-positive cultures

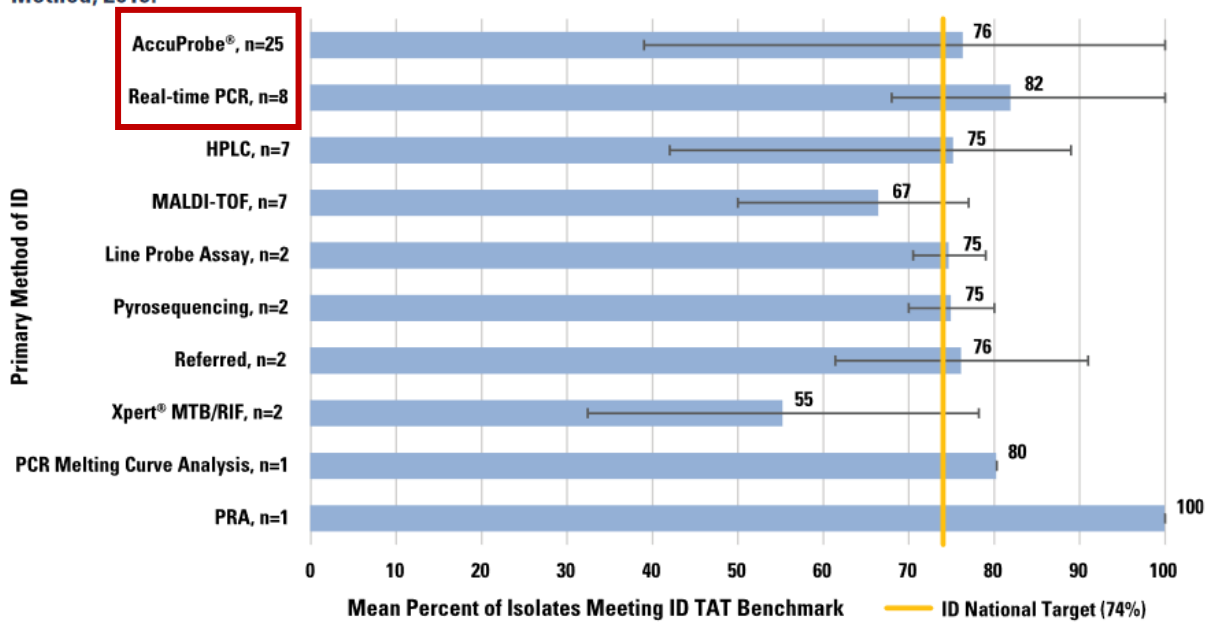
TUBERCULOSIS LABORATORY AGGREGATE REPORT
SIXTH EDITION

Figure 10. Primary ID Methods, 2020 (n=58).



Note—Data label indicates method/approach, number of laboratories, percentage

Figure 6. Mean Percent of MTBC Identified* from Culture within 21 Days of Specimen Receipt, by Primary ID Method, 2019.



n=Number of laboratories using each method
*One laboratory did not ID MTBC in 2019 and was excluded from the analysis.

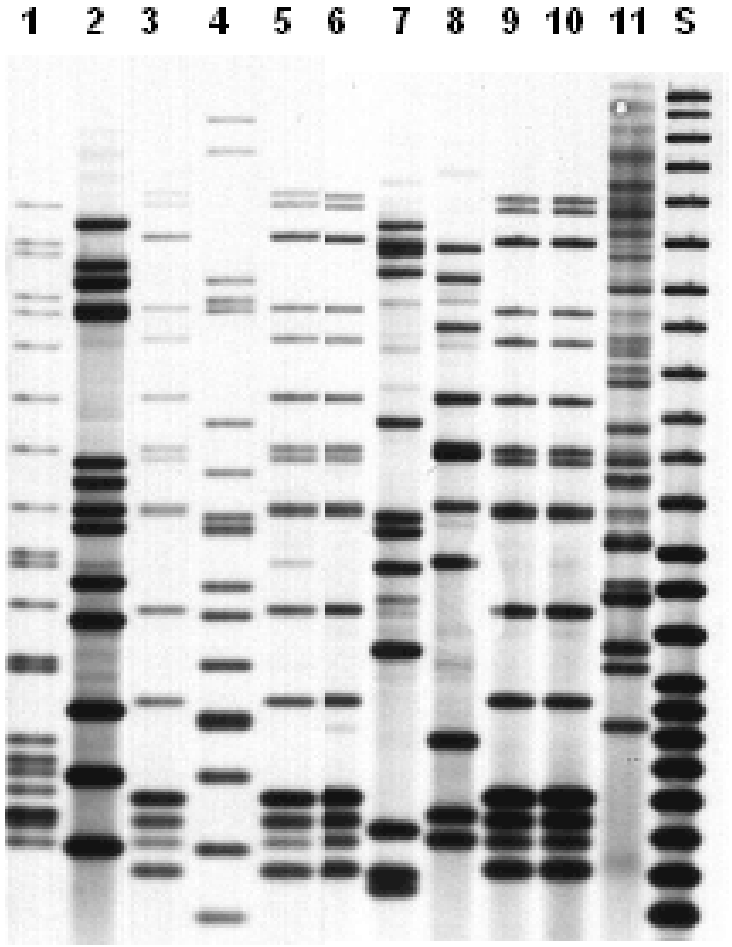
https://www.cdc.gov/tb

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
- QIAGEN spin-column extraction for removal of PCR inhibitors
- Sensitivity
  - >95% for AFB smear-positive, culture-confirmed TB patients
  - >55% of AFB smear-negative, culture-confirmed TB patients
  - LOD: <1 MTBC bacillus/reaction (≈140 AFB/ml)

WSLH Real-Time MTBC PCR: IS6110

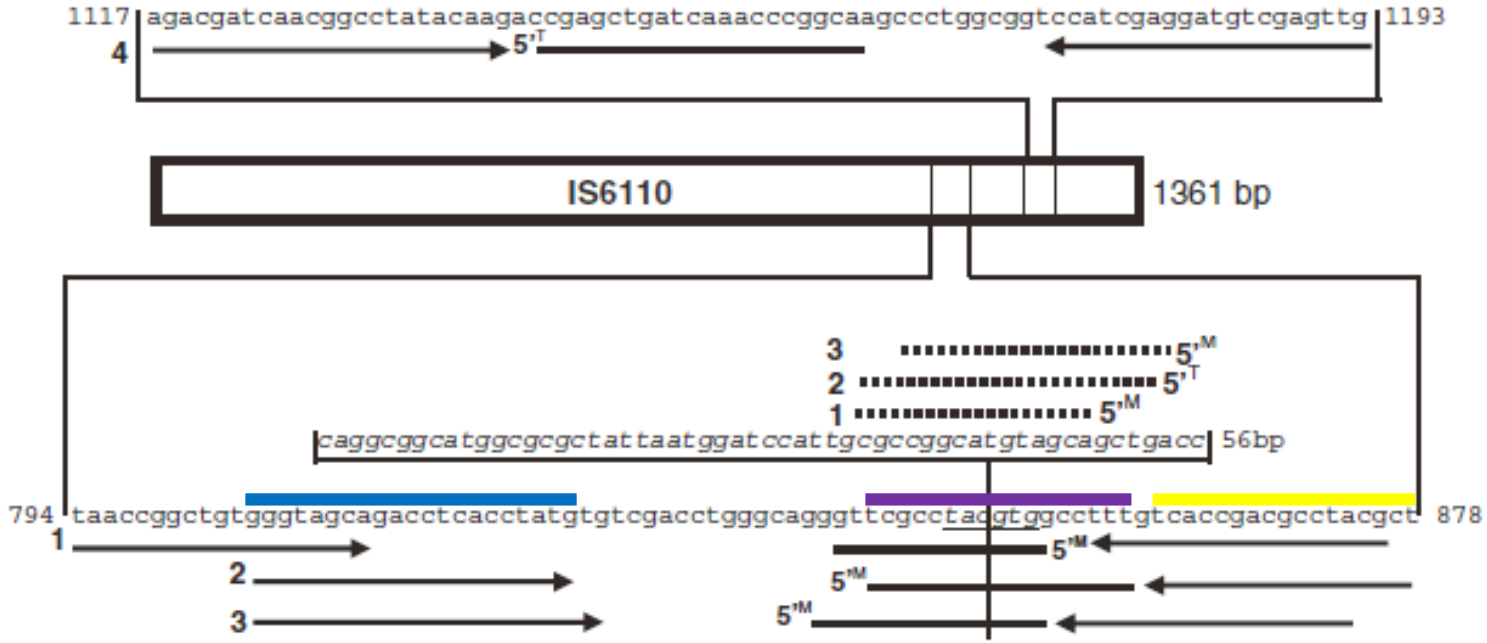
- Insertion sequence (transposon) present in MTBC, absent in NTM
  - Good target for screening AFB+ specimens
- Copy number/insertion location was used for strain genotyping (RFLP)
- Unknown function, *may* increase virulence and antibiotic resistance



CDC DTBE: Tuberculosis Genotyping Laboratory Procedures

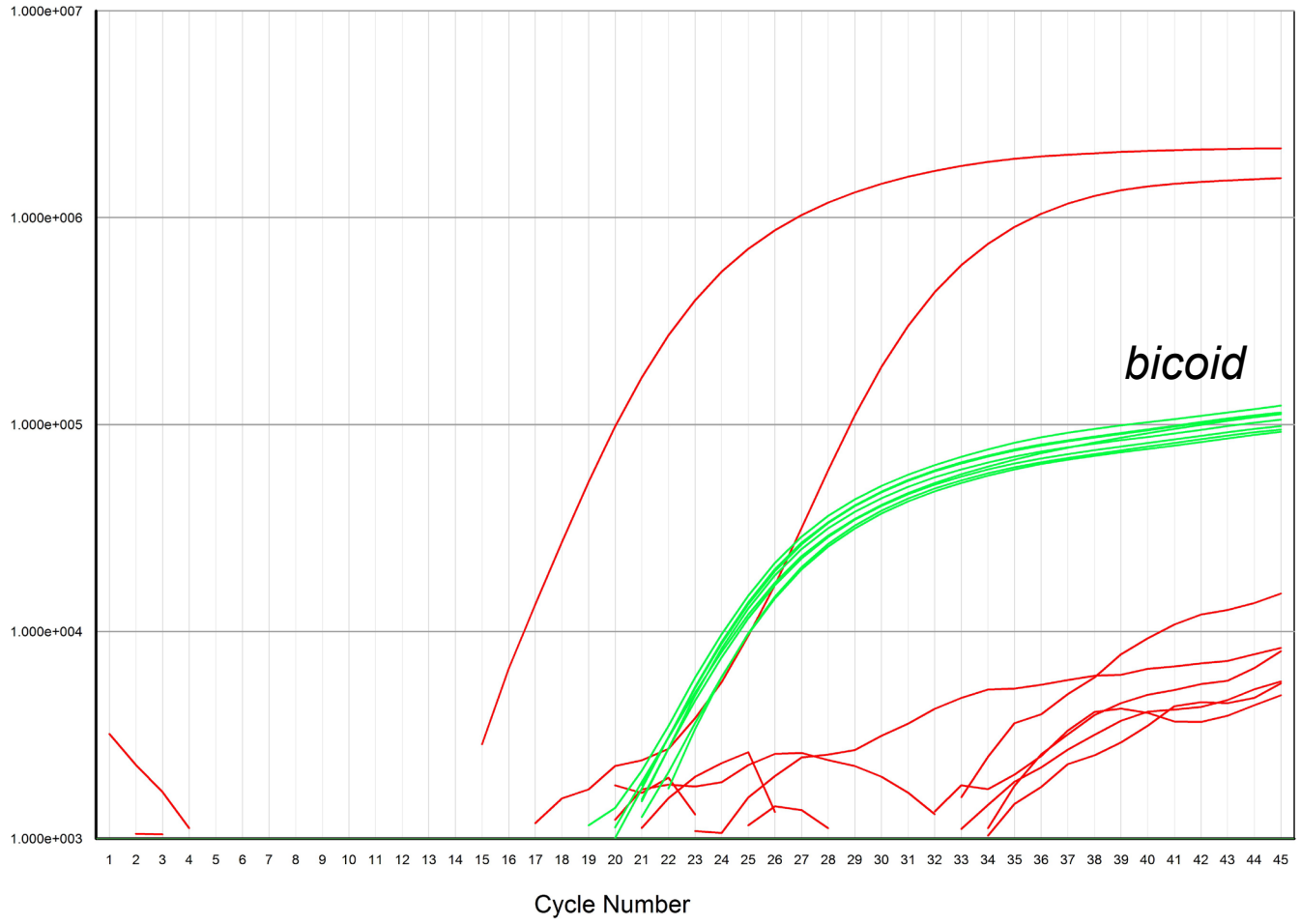
WSLH Real-Time MTBC PCR: IS6110

- Commonly used target in MTBC ID → specificity and sensitivity
  - 16 copies in *M. tuberculosis* H37Rv
  - 10-20 copies in most other *M. tuberculosis* complex members
  - 1 copy in *M. bovis* and *M. bovis*-BCG



WSLH PCR Inhibition: Internal Control

- *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
  - Specimen is purified and re-analyzed



Case Study: “Undetectable” TB

- Patient History
  - 69 year-old resident of SE Asia
  - Living with family in US
  - 1ppd smoker for >20 years
- 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: 3x negative
  - MAC PCR: negative

M tuberculosis PCR (Final result)

ID: 20MM004581	Type/Src: Sputum	
	Result	**Units**
Mycobacterium tuberculosis PCR	No Mycobacterium tuberculosis complex DNA detected.	
AFB Smear	AFB smear positive by submitting laboratory.	

Comments:
Inhibitory substances that prevent PCR amplification were tested for and not detected.

Consider testing a second specimen (not to exceed a total of two) to confirm the PCR result. A patient can be presumed to have an infection with non-tuberculous mycobacteria, pending culture results, if a second specimen is smear positive and PCR negative.

This PCR assay detects the presence of M. tuberculosis complex DNA in clinical specimens. It detects a target within an insertion sequence (IS6110), which is specific for M. tuberculosis complex.

This assay was developed and its performance characteristics determined by the Wisconsin State Laboratory of Hygiene. It has not been cleared by the U.S. Food and Drug Administration. A negative result does not rule out infection with Mycobacterium tuberculosis complex.

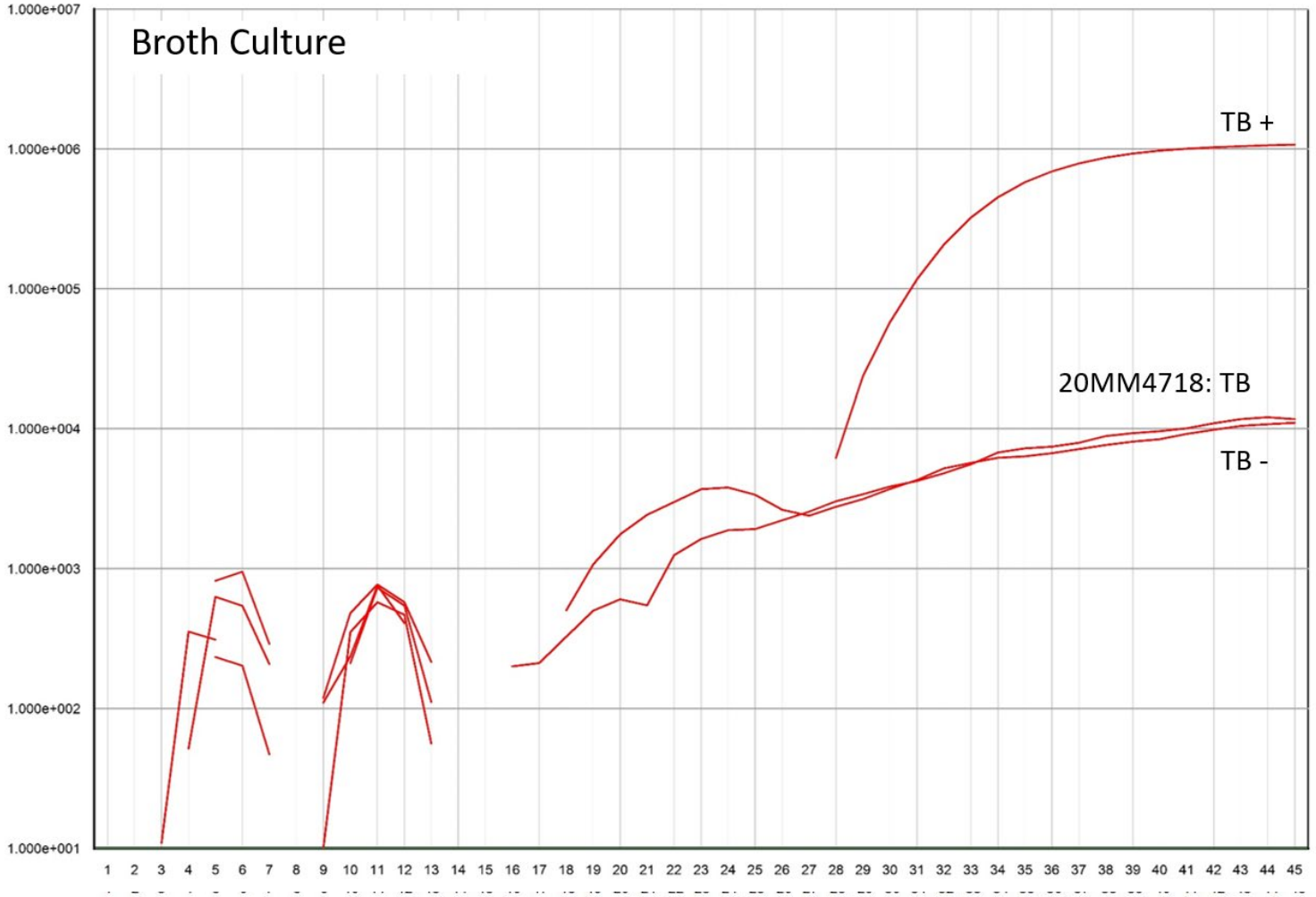
Laboratory Results

- Specimen referred to City of Milwaukee HD for GeneXpert MTB/RIF
  - MTBC positive, no rpoB mutation detected
- All cultures grew MTBC on solid media
  - ID by MALDI
- 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
  - Peritoneal fluid sediment: Negative for MTBC
  - Broth culture: Negative for MTBC
  - Solid media growth: Negative for MTBC

- 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 Real-time 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

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 IS6110 molecular testing (2011)
  - NY sees about 1/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
    - RD9 → Specific to TB, but single copy so may lose some sensitivity
    - *rpoB* → Sequence specific to TB, but mutations may require careful primer design
  - MTBC evolves slowly, but unique genetic combinations are possible
- 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 and adapt!

Questions?

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