“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

Earlier treatment
Isolation decisions
Public health response
Fewer transmissions
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: \textit{rpoB}
  • LOD: \(\approx 130\) AFB/ml
  • Less than ten minutes hands-on time, results in \(<120\) min
  • Requires GeneXpert system
  • Approximately \$50/cartridge
Lab Developed Test: Real-Time PCR
Mycobacteriology Testing at WSLH

- Patient Specimen
  - Decontamination and Concentration
    - Smear Microscopy
      - AFB Smear Positive
        - Same Day Nucleic Acid Amplification Testing (TB/MAC PCR)
    - AFB Smear Negative
      - NAA Testing for patients with TB risk factors
  - Culture
    - AFB positive growth
      - Mycobacteria ID
        - TB/MAC PCR
          - Negative
            - MALDI-TOF
              - Inconclusive results
                - DNA Sequencing
          - MTBC
    - Referred positive cultures
      - Conventional Drug Susceptibility Testing
      - MTBC Genotyping
      - MDDR (GeneXpert)
Mycobacteria AccuProbe alternative: WSLH TB PCR validated for use with AFB-positive cultures
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
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
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
IS6110-negative MTBC does exist!

Characterisation of *Mycobacterium tuberculosis* isolates lacking IS6110 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 Sooijen‖‖

Epidemiology of *Mycobacterium tuberculosis* strains in San Francisco that do not contain IS6110

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 IS6110 Analysis To Detect Vertebral Spondylodiscitis Caused by *Mycobacterium bovis*

Deborah Steensels,* Maryse Fauville-Dufaux,b Johan Boile; c Hans De Beenhouwer*
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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