Molecular Diagnostics for Tuberculosis: What are NAATs and How Do You Use Them?

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Outline

• What are NAATs?
• What is the added value of NAATs?
• Specific NAATs that are commercially available for detecting tuberculosis (TB)
• Limitations of NAATs
• Performance of NAATs in patients who are AFB smear positive and smear negative
• Use of NAATs in California
• Case examples
• Introduction to molecular tests for DST

The Treatment of TB is Rigorous

• The clinical goal is to treat the disease if present, as early as possible, to avoid death and disability, but not expose patients to the risk of serious side effects of treatment if TB is not present

• The public health goal is to find and treat cases as early as possible in order to interrupt transmission to others
### Diagnosing TB before NAATs

- 30 years ago, only 2 tests available
  - **AFB smear**
    - False Negatives: Missed 40% of culture (+) active TB
    - False Positives: NTMs
  - Culture on solid media
    - Average time to positive growth, 3-6 weeks
    - Still needed to do biochemical tests to determine if TB or NTM, another several weeks

Clearly needed more sensitive, specific and faster tests

### What is a NAAT?

- **NAAT: Nucleic Acid Amplification Test**
- Can be done on the direct clinical specimen; sputum, CSF, lymph node aspirates, etc.
- More sensitive and more specific than the AFB smear
- Results available within **hours to a few days**
- CDC updated guidelines for NAATs in MMWR, Jan. 16, 2009:
  - “CDC recommends that NAA testing be performed on a least one respiratory specimen from each patient with signs and symptoms of pulmonary TB…” (if result will impact clinical or public health actions)
- Why aren’t we using NAATs more frequently?

### What is the added value of a NAAT?

- **For AFB smear (-) patients**:
  - Ability to confirm rapidly the presence of M. tb in 50-80% of AFB smear-negative, culture-positive specimens
  - If both the AFB smear and the NAAT are negative, the likelihood of TB becomes very low
- **For AFB smear (+) patients**:
  - Greater positive predictive value (>95%) with AFB smear-positive specimens in settings in which NTM (non tuberculous mycobacteria) are common
  - If NAAT negative, in many instances can obviate the need for contact investigation
Available NAATs

• Amplified Mycobacterium tuberculosis Direct Test (MTD, Gen-Probe)
• Xpert MTB/RIF (often called GeneXpert)
• Pyrosequencing (PSQ)
• “Home brews”

The Gen-Probe MTD Test

• The first FDA approved NAAT for M.tb
• Approved in 1995 for AFB smear positive patients
• Enhanced MTD (E-MTD) test approved in 1999 for AFB smear negative specimens
  • TMA (Transcription-mediated amplification)
  • Targets rRNA of M.tb
• Sensitivity of E-MTD in culture (+) specimens
  o Smear negative = 70%
  o Smear positive = 97%
• Specificity = 98%
• Somewhat labor intensive for the microbiologist
• Often not available in hospital labs

Enhanced Amplified Mycobacterium Tuberculosis
Direct Test (“E-MTD”)
(Gen-Probe, San Diego, CA)
**Xpert MTB/RIF**

- FDA approved in 2013 for detection of M.tb in clinical specimens
  - Can be used for both AFB smear positive and smear negative specimens
  - Using culture as the gold standard, recent meta-analysis showed pooled sensitivity estimates of 98% for smear (+), and 68% for smear (-) specimens
  - Specificity estimates were 99%
  - Because most studies included in the meta-analysis were in high TB burden countries, sensitivity may be lower in US
  - (Also tests for Rifampin resistance)


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**Xpert MTB/RIF (2)**

- Test performance in a low TB incidence setting: Montreal, Canada*
- 502 consecutive patients in a university hospital affiliated TB clinic
  - Most with abnormal CXRs, 8% symptomatic
  - 25 had culture (+) TB
  - Overall sensitivity of Xpert was 46%; 86% if smear (+), 28% if smear (-)
  - Specificity was 100%
  - Those patients with culture (+)/Xpert (-) took twice as long for cultures to grow and had minimal disease


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**Xpert MTB/RIF (3)**

- Automated cartridge-based test
- Uses PCR technology in a self-contained capsule, so contamination is eliminated
- Results available in under 2 hours
- Platform is widely available in many hospitals
Pyrosequencing (PSQ)

- Has been available to us in California since 2012
- Although usually used for detecting mutations associated with drug resistance, is also a NAAT
- Can be used on clinical specimens if at least AFB smear positive 1+
- Result in 1 day (although usually batched twice a week at the CA MDL lab, Grace Lin will run it for you any workday if urgent)

Real-Time PCR

- 2 components
  - PCR
    - A system to monitor PCR product
      - Fluorophore-labeled probes
      - An optical device to detect fluorescence
  - No post-PCR manipulations
    - Fast
      - when PCR is done, results are ready for interpretation
    - No amplicon contaminations
Limitations of NAATs

- Inhibitors
  - Direct clinical specimens can contain inhibitors that prevent amplification
  - Results in false negative results
  - Xpert MTB/RIF includes a sample processing control (SPC) to monitor for presence of inhibitors
  - MTD procedure manual includes instructions on how to test for inhibitors
  - If an AFB smear (+) specimen is NAAT negative, make sure the lab checked for inhibitors, and repeat the NAAT on another specimen
- Also, none of these tests will tell you if organisms are alive or dead
  - Should NOT be used to follow patients on treatment
  - Often NAAT will stay positive long after cultures are negative

Effects of NAAT Use on Clinical Care in California

- Since 2010, information about NAAT usage has been collected on the RVCT
- We looked at the utilization of NAAT from 2010-2013
- Overall, 39% of subsequently culture positive patients had a NAAT reported before culture results were reported
- Did use of a NAAT affect clinical care?

<table>
<thead>
<tr>
<th>Group</th>
<th>NAAT Median time* (N)</th>
<th>No NAAT Median time* (N)</th>
<th>P-value for Wilcoxon rank sums test</th>
</tr>
</thead>
<tbody>
<tr>
<td>All *</td>
<td>3.0 (1960)</td>
<td>14.0 (3101)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smear-positive*</td>
<td>2.0 (1728)</td>
<td>4.0 (1580)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smear-negative*</td>
<td>10.0 (232)</td>
<td>26.0 (1521)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Time (days) between date of earliest specimen collection and treatment initiation
How rapidly were NAATs reported?

- How rapidly were tests reported?
- Reported times vs. real world

How rapidly were NAATs reported? (2)

Among 1878 cases:
- Had NAAT
- Started treatment after specimen collection
- Started treatment before culture reported

Median TAT = 3 days
Mean = 3.77 days
IQR = 2-4 days

*NAAT TAT: working days between NAAT specimen collection and NAAT report date.

Impact of NAATs on Utilization of AIIRs and Clinical Care

- CDC sponsored retrospective multisite study* found using NAATs was associated with significant reductions in:
  - Use of AIIRs
  - Use of diagnostic procedures such as bronchoscopy and CTs
  - Initiation of CIs for smear positive, culture negative suspects
  - Time to diagnosis of TB in smear positive cases
  - Duration of presumptive TB treatment for patients who did not have TB

TB or Not TB? Case #1

48 year old man with COPD
- 1 month cough, weight loss
- Jail inmate
- Rx for TB 15 years ago

Smear positive, is this TB?
- 150 jail contacts, now what?

Laboratory Diagnosis: NAAT

- This CXR could be old TB, current reactivation, or a new process
- If NAAT positive, the diagnosis is confirmed
  - (Previous treatment 15 years ago will not give a positive result now)
Laboratory Diagnosis: NAAT (2)

- This CXR could be old TB, current reactivation, or a new process
- If NAAT positive, the diagnosis is confirmed
- A **NAAT test**, if negative, would make it much more likely that this is an **NTM**, quite common in COPD

Laboratory Diagnosis: NAAT (3)

- This CXR could be old TB, current reactivation, or a new process
- If NAAT positive, the diagnosis is confirmed
- A **NAAT test**, if negative, would make it much more likely that this is an **NTM**, quite common in COPD
- Could this be a false negative NAAT?
  - Check for inhibitors
  - Repeat the NAAT on another specimen

TB or Not TB? Case #1

48-year-old, male with COPD
- 1 month cough, weight loss
- Jail inmate
- Rx TB 15 years ago

What if he was smear negative instead?
Laboratory Diagnosis:
NAAT in Smear Negative Patient

• About 70% of smear negative specimens that subsequently grow M.tuberculosis will be NAAT positive by the E-MTD test or Xpert MTB/RIF.
• If you have a high index of suspicion for TB, a NAAT test should be ordered.

Case #2

• 56 year old white woman from a small town in northern California.
• Never traveled out of US, no known TB exposure, no case of TB in her county for past 5 years.
• 40 pack year smoker, cough for several months with recent weight loss.
• Abnormal CXR.
### Case #2 (2)
- Sputa were ordered and were AFB (+) X 3
- Health Department consulted and NAAT suggested
- NAAT negative!

**Is this a false negative NAAT or does this woman without any risk factors have TB?**

**How to answer this question?**

### Case #2 (3)
- Lab was requested to check for inhibitors, none found
- NAAT was repeated on another smear (+) specimen, also negative
- Out of caution, treatment with RIPE begun, but county held off on contact evaluation beyond the immediate family (one person)
- Culture grew in 8 days, identified as *M. kansasii*, an NTM
- No further public health action needed

### Case #3
- 44 year old man originally from Mexico applying for work as maintenance worker at a hospital
- TST (+) at 28 mm
- 20 pack year history of smoking
- C/o some cough for several weeks, occasionally productive, but no fever, sweats of weight loss
- Abnormal CXR
Case #3 (2)

- Sputum X 3 ordered for AFB; all 3 negative
- Scheduled for bronchoscopy because of concern for malignancy
- Xpert MTB/RIF ordered; Positive!
- Bronchoscopy canceled, RIPE begun

- CXR 2 months later:
**Case #3 (3)**

- Marked improvement in CXR
- Culture positive after 21 days, M.tb
- Patient spared invasive procedure, fewer persons potentially exposed to TB

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**Summary**

- NAAT technology for diagnosing TB is underutilized
- NAATs are more sensitive than AFB smears for detecting TB
- Time for treatment initiation is shorter when NAATs are used
- More frequent use of NAATs for TB diagnosis may affect both clinical care and public health action
- Recent FDA approval of Xpert MTB/RIF will likely increase NAAT use

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**The Treatment of TB is Rigorous**

**And MDR TB Treatment is Even More so**

- The clinical goal is to treat the disease if present, as early as possible, to avoid death and disability, but not expose patients to the risk of serious side effects of treatment if TB is not present
- The public health goal is to find and treat cases as early as possible in order to interrupt transmission to others
Drug Susceptibility Testing (DST)

- Phenotypic DSTs:
  - Gold standard is agar proportion method on solid media: very slow and seldom used now
  - MGIT liquid system is now commonly used. Takes about 1 week after a positive culture for results

- Molecular DSTs:
  - Pyrosequencing
  - Line probe assays (Hain test)
  - Xpert MTB/RIF
  - MDDR Service

Pyrosequencing (PSQ)

- Available at the California state lab since 2012.
- Performed on cultures or clinical specimens if at least 1+ AFB positive
- Result in 1 day (although usually batched twice a week at the CA MDL lab, Grace Lin will run it for you any workday if urgent)
- Tests for INH (inhA, katG, ahpC) and Rifampin (rpoB) resistance mutations, as well as mutations found in resistance to quinolones (gyrA) and injectable drugs (rrs)

PSQ (2)

- Sensitivity: Will pick up about 90% of INH resistant isolates and over 95% of Rifampin resistant isolates
- Specificity is over 95% for both INH and Rif resistance
Molecular Diagnostics for TB: January 21, 2015
What are NAATS and How Do You Use Them?

**Hain Test**
- Commercially available and used in much of the world
- Not FDA approved in the US
- Line Probe Assay, detects INH and Rifampin resistance. Second generation test also looks for resistance to quinolones and injectable drugs

**Xpert MTB/RIF**
- Xpert MTB/RIF does not just detect M.tb DNA; it also tests for rifampin resistance using a molecular beacon technology
- In Chang’s meta-analysis, using cultures and phenotypic drug susceptibility tests as the gold standard, the Xpert assay had a pooled sensitivity of 95% and specificity of 98% for detecting rifampin resistance

Molecular Diagnostics for TB: What are NAATS and How Do You Use Them?

MDDR Service from CDC
- Launched in 2010 by CDC
- **Molecular Detection of Drug Resistance**
- Intended primarily for patients with MDR TB who will be treated with second line drugs
- Uses DNA sequencing to find mutations associated with
  - INH and Rifampin,
  - Ethambutol and PZA,
  - Fluoroquinolones (Levo/Moxi)
  - Aminoglycosides (Kanamycin/Amikacin)
  - Capreomycin
- Excellent turn around time of < 48 hours
- Drawback: Done on positive cultures, not clinical specimens

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REFERENCES

Molecular Diagnostics for TB: What Are NAATs and How Do You Use Them?
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