TB or not TB, that is the question.

Jennifer L. Lemer, MLS(ASCP)\textsuperscript{CM}

Microbiologist II
Objectives:

<table>
<thead>
<tr>
<th>Review</th>
<th>North Dakota tuberculosis workload and national data summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explain</td>
<td>Sputum sample collection and the importance of a high-quality sample.</td>
</tr>
<tr>
<td>Discuss</td>
<td>Current testing algorithm used to identify <em>Mycobacterium</em> samples at the NDDoH Microbiology Lab.</td>
</tr>
<tr>
<td>Understand</td>
<td>Methodology of MALDI-TOF testing and how it relates to <em>Mycobacterium</em> identification.</td>
</tr>
</tbody>
</table>
Background of TB

- TB has affected humans for millennia
- Historically known by a variety of names, including:
  - Consumption
  - Wasting disease
  - White plague
- TB was a death sentence for many
Tuberculosis
A serious re-emerging infectious disease
Latent TB – Treatment needed for latent TB infection to prevent TB disease

- Have no symptoms
- Don’t feel sick
- Can’t spread TB bacteria to others
- Usually have a positive TB skin test reaction or positive TB blood test
- May develop TB disease if they do not receive treatment for latent TB infection
Active TB – Treatment needed to treat TB disease

Symptoms may include

- a bad cough that lasts 3 weeks or longer
- pain in the chest
- coughing up blood or sputum
- weakness or fatigue
- weight loss
- no appetite
- chills
- fever
- sweating at night
## Turnaround Time Indicators, 2015

<table>
<thead>
<tr>
<th>Benchmark</th>
<th>Specimen receipt within 1 day of collection</th>
<th>AFB smear result within 1 day of receipt</th>
<th>ID of MTBC within 21 days of receipt</th>
<th>DST within 17 days of ID of MTBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Target (%) of specimens that should meet benchmark</td>
<td>67%</td>
<td>92%</td>
<td>74%</td>
<td>69%</td>
</tr>
<tr>
<td>No. laboratories meeting or exceeding National Target*</td>
<td>12</td>
<td>31</td>
<td>25</td>
<td>20†</td>
</tr>
<tr>
<td>National average (%) of specimens meeting benchmark</td>
<td>45%</td>
<td>90%</td>
<td>69%</td>
<td>61%</td>
</tr>
<tr>
<td>No. laboratories meeting or exceeding national average*</td>
<td>31</td>
<td>33</td>
<td>29</td>
<td>26†</td>
</tr>
</tbody>
</table>

*Number of laboratories reporting = 57. 110 PHL referring isolates to the DST Reference Center for first-line DST are excluded from analysis of the DST TAT for 2015.
North Dakota averages 2500 samples each year for TB testing, which is also roughly the National Testing Average.
In 2015, North Dakota had the lowest ratio of positive samples per number of patients tested.
2493 clinical samples received at the Department of Health for TB testing
- 1260 total patients

9 positive patients for TB
- 6 of which were ND residents

82 patients tested on GeneXpert for TB presence and Rifampin resistance
- 4 positive TB samples, none with Rifampin resistance

2843 specimens processed for IGRA testing (primarily QuantiFERON)
- 166 positive samples
Sputum Specimen Collection

• Quality specimens are vital for the laboratory diagnosis of TB and sputum is the most frequent specimen collected for TB testing.

• Sputum specimens are preferably collected under the direction of a trained health care professional. Specimens should be collected in containers that are sterile, clear, plastic, and leak-proof, for example a 50-ml screw-cap centrifuge tube.

• Ideally, 5-10 ml in volume, although smaller quantities are acceptable if the quality is satisfactory.
Sputum Quality

Thick, Mucopurulent

Hemoptysis (Bloody Sputum)

Watery (acceptable if induced)

Salivary
Containers used for the collection of AFB samples
Possible Rejection Criteria

- Labeling of specimen does not match identifiers on requisition form
- Insufficient volume
- Dried swabs – swabs in general are not optimal
- Provide limited material
- Broken specimen containers or leaking specimens
- Excessive delay between specimen collection and receipt in the laboratory
- Tissue or abscess material in formalin
TB Testing for Initial Diagnosis

AFB Smear

When tuberculosis faces an even stronger opponent - labor&moresuccidia AG - labor&more

<table>
<thead>
<tr>
<th>No. of AFB seen</th>
<th>CDC guideline</th>
<th>Study definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>1–2 per whole smear</td>
<td>Doubtful positive</td>
<td>Scanty</td>
</tr>
<tr>
<td>1–9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per 100 fields</td>
<td>1+</td>
<td>Positive</td>
</tr>
<tr>
<td>Per 10 fields</td>
<td>2+</td>
<td>Positive</td>
</tr>
<tr>
<td>Per single field</td>
<td>3+</td>
<td>Positive</td>
</tr>
<tr>
<td>&gt;9 per single field</td>
<td>4+</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**NOTE.** CDC, Centers for Disease Control and Prevention.

* From Kent and Kubica [15].
MTB/RIF Screen on Cepheid GeneXpert
Application of AFB Sputum Smear Microscopy to Xpert Results

Positive Xpert result:
- *M. tb* complex detected
- TB likely
- Stop Xpert testing and continue A.I.I.

Two negative Xpert results
- *M. tb* complex not detected

Review AFB smear results

- AFB positive on 2 tests:
  - TB not likely; NTM likely*
- AFB negative on 2 tests:
  - TB not ruled out; continue diagnostic evaluation
- AFB discordant 1 positive and 1 negative result:
  - Infectious TB not likely; NTM possible*

Follow up final AFB culture results

A sample is positive for AFB: Now what?

Ziehl-Neelsen Stain
Gen-Probe AccuProbe tests

- Based on hybridisation of nucleic acids
- 4 steps
  - Sample preparation
  - Hybridisation
  - Selection of the hybrid
  - Detection of the hybrid

Identification of NTM

- Gen-Probe Accuprobe
  - M. avium complex, M. gordonae and M. kansasii
  - Quick identification

- Biochemicals
  - May take 3 days to 3 weeks to complete test

* Piecing a puzzle together or finding the best possible match
Laboratory Workflow for MALDI-TOF MS ID of *M. tuberculosis* complex after growth in culture

10ul loop-ful of organism → Beads+500ul 70% Ethanol → Bead Beat 2 minutes

BSL3 Activities

BSL2 Activities

Centrifuge 5 min → Decant supernatant → Speed Vac 10 min → 70% Formic Acid & Acetonitrile

Spot 1ul sample + 2ul of Matrix

MALDI-TOF

start to finish takes ~2 hrs for 24 samples
MALDI-TOF MS

Principle of MALDI-TOF

Time of Flight

Molecular masses

Matrix assisted laser desorption ionization — ppt video online SlidePlayer

Implementation of MALDI-TOF Tecnoology in Portugal Quilaban
Use of MALDI-TOF mass spectrometry for identification of bacteria ... ScienceDirect.com
MALDI-TOF results report

Identification results

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Organism (best match)</th>
<th>Score Value</th>
<th>Organism (second-best match)</th>
<th>Score Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV (+++)(A)</td>
<td>Mycobacterium spp-1 (mycobacterium)</td>
<td>Mycobacterium brumii</td>
<td>2.45</td>
<td>Mycobacterium tuberculi</td>
<td>2.28</td>
</tr>
<tr>
<td>DS (+++)(A)</td>
<td>BTS (213b)</td>
<td>Escherichia coli</td>
<td>2.45</td>
<td>Escherichia coli</td>
<td>2.36</td>
</tr>
</tbody>
</table>

Adapted thresholds for increased sensitivity

<table>
<thead>
<tr>
<th>Rank (Quality)</th>
<th>Matched Pattern</th>
<th>Score Value</th>
<th>NCBI Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+++)</td>
<td>Mycobacterium duvalii CCUG 46278 CCUG b</td>
<td>2.26</td>
<td>127729235</td>
</tr>
<tr>
<td>2 (+++)</td>
<td>Mycobacterium duvalii 1144456 IUDB b</td>
<td>2.24</td>
<td>127729255</td>
</tr>
<tr>
<td>3 (+++)</td>
<td>Mycobacterium duvalii DSM 43585 DSM b</td>
<td>2.07</td>
<td>127729235</td>
</tr>
<tr>
<td>4 (+++)</td>
<td>Mycobacterium duvalii DSM 43544 DSM b</td>
<td>1.93</td>
<td>127729235</td>
</tr>
<tr>
<td>5 (+)</td>
<td>Mycobacterium duvalii DSM 44244T DSM b</td>
<td>1.77</td>
<td>127729235</td>
</tr>
<tr>
<td>6 (+)</td>
<td>Mycobacterium duvalii DSM 43544 DSM b</td>
<td>1.67</td>
<td>127729235</td>
</tr>
<tr>
<td>7 (+)</td>
<td>Mycobacterium duvalii DSM 43543 DSM b</td>
<td>1.64</td>
<td>127729235</td>
</tr>
<tr>
<td>8 (+)</td>
<td>Mycobacterium duvalii DSM 43543 DSM b</td>
<td>1.56</td>
<td>127729235</td>
</tr>
<tr>
<td>9 (+)</td>
<td>Mycobacterium chuii DSM 45694T DSM b</td>
<td>1.47</td>
<td>127729235</td>
</tr>
<tr>
<td>10 (+)</td>
<td>Mycobacterium gastri WC14.9114 NYDH b</td>
<td>1.45</td>
<td>127729235</td>
</tr>
</tbody>
</table>
## Drug Susceptibility at the CDC

### MTBC Agar Proportion Susceptibility

<table>
<thead>
<tr>
<th>Drug</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid 0.2 μg/mL</td>
<td>100 %</td>
<td>Resistant</td>
</tr>
<tr>
<td>Isoniazid 1.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Isoniazid 5.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Rifampin 1.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ethambutol 5.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Streptomycin 2.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Streptomycin 10.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Rifabutin 2.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ciprofloxacin 2.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Kanamycin 5.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ethionamide 10.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Capreomycin 10.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>PAS 2.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ofloxacin 2.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Amikacin 4.0 μg/mL</td>
<td>0 %</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

**Comments and Disclaimers**
- Susceptibility testing method: Biodisk agar proportion, 7H10 medium. Resistance is defined as >1% (growth on drug-containing medium compared to drug-free medium).

### MTBC Pyrazinamide Susceptibility

<table>
<thead>
<tr>
<th>Drug</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrazinamide 100 μg/mL</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

**Comments and Disclaimers**
- Susceptibility testing method: Mycobacterium Growth Indicator Tube (MGIT)
Genome Testing at Michigan Department of Health

G00011 in North Dakota
Results received 1/8/2019
Analysis updated with 18RF7917

Node A
12L6537
12L7456
12L9367
12L9368
12L9369
13L0496
13L1156
13L2106
14RF1653
14RF5749
14RF6681
14RF6682
16RF7689
18RF7051
TB Testing for Release from Isolation
TB Testing to Monitor the Course of Treatment
Jennifer L. Lemer, MLS(ASCP)\textsuperscript{CM}
Microbiologist II

701.328.6289 • 701.328.6280(f)

• jllemer@nd.gov • ndhealth.gov/microlab