Line It Up: Another Identification Option – the Line Probe Assay

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Minnesota Facts

- Northern most point in the lower 48 states – “Northwest Angle”
- Birthplace of the Mississippi River
Minnesota Facts

- **Population**
  - 5.5 Million
  - 54% in Minneapolis/St. Paul – “Metro Area”

- **LPH Departments**
  - 91 Total

- **Public Health Lab**
  - 1 Central PHL
  - 2 Local PHLs

- **Hospitals**
  - 142 Acute/Chronic Care
  - 78 Critical Access
MDH Public Health Laboratory

- Building is 11 years old
- Located in Downtown St. Paul
- Shared with Dept. of Agriculture
MDH Public Health Laboratory

PHL staff by program

36 Newborn Screening staff
7 Director's Office staff
19 Emergency Preparedness and Response staff
36 Environmental Laboratory staff
50+ Infectious Disease Laboratory staff

Does not include contractors.
MDH-PHL TB Laboratory Scientists

Trudy  Kerry  Scott
Tuberculosis Morbidity and Mortality, Minnesota, 2011-2015

<table>
<thead>
<tr>
<th>Year</th>
<th>No. Cases (Rate)*</th>
<th>No. Deaths** (%)</th>
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<tbody>
<tr>
<td>2011</td>
<td>137 (2.6)</td>
<td>9 (7%)</td>
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<td>2012</td>
<td>162 (3.0)</td>
<td>4 (2%)</td>
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<td>2013</td>
<td>151 (2.8)</td>
<td>2 (1%)</td>
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<td>2014</td>
<td>147 (2.7)</td>
<td>3 (2%)</td>
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<tr>
<td>2015</td>
<td>150 (2.7)</td>
<td>2 (1%)</td>
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* Cases per 100,000 population. Rates calculated using state population estimates from the U.S. Census Bureau.

**Represents only deaths due to TB disease or TB drug-induced toxicity
Tuberculosis Incidence Rates per 100,000 Population, United States and Minnesota, 2000-2016

Healthy People 2010 and 2020 objective
Tuberculosis Cases by Mycobacterial Culture Result, Minnesota, 2011-2015

- Positive for *M. tbc*: 75%
- Negative: 21%
- Not done: 4%

N = 747
MDH TB Lab Stats

- Specimens processed in 2016 = 1661
- Isolates received for ID = 523
- 1\textsuperscript{st} line susceptibilities performed = 83
- NAAT = 89
INNO-LiPA Mycobacteria v2 – Line Probe Assay

- Implemented Fall 2013
- Identification assay
- 18 clinically relevant *Mycobacterium* sp.
- Differentiates
  - *M. intracellulare/M. avium* complex
  - *M. chelonae/M. abscessus*
INNO-LiPA Mycobacteria v2 –
Line Probe Assay

- PCR from solid media or MGIT tubes
- Hybridization of amplification product onto membrane strip
- Colorization for final reaction
INNO-LiPA Mycobacteria v2 –
Line Probe Assay

Specimen Prep

- 0.75 ml MGIT broth
- 5 colonies from solid media
- 0.75 ml from blood culture bottle
- 100°C heat block for 30 minutes
- Specimen sub-culture to 7H11
INNO-LiPA Mycobacteria v2 – Line Probe Assay

**Extraction**

- Roche MagNA Pure Compact
- Can run 7 patients + control at one time
- Takes 25 minutes
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Amplification Step
- Primers amplify the 16S – 23S ribosomal RNA spacer region of Mycobacterium
- Conventional PCR
- Thermocycler 2.5 hours
- “clean”
INNO-LiPA Mycobacteria v2 – Line Probe Assay

E-gel Step (optional)

- Not routinely used anymore, except for trouble-shooting
- 15 minutes
- “dirty”
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Hybridization Step

- Reverse hybridization
- Colorization

[Diagram illustrating the hybridization step with labels for Chromogen (NBT/BCIP), Purple precipitate, Alkaline Phosphatase, Streptavidin, Biotin, Amplified target, DNA-probe, Nitrocellulose strip]
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Hybridization Step

- Prep all reagents
- PCR product + denaturation solution
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Hybridization Step

- Auto-Lipa 48
- Set program
- Several washes
- 2.0 hours
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Analysis Step
- Remove tray
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Interpretation Step

- Read bands
**INNO-LiPA Mycobacteria v2 – Line Probe Assay**

**Interpretation Step**
- Read bands
- *M. gordonae*

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<tr>
<th>Line</th>
<th>Probe Names</th>
<th>M. tuberculosis sp.</th>
<th>M. kansasii</th>
<th>M. kansasii KZN</th>
<th>M. xenopi</th>
<th>M. gordonae</th>
<th>M. genavense</th>
<th>M. simiae</th>
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<th>M. scrofulaceae</th>
<th>M. tuberculosi</th>
<th>M. chelonae</th>
<th>M. abscessus</th>
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**MDH**
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Interpretation Step
- Read bands
- *Mycobacterium* sp.
- Go to 16S sequencing
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Preliminary Reporting

- Report ID
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Preliminary Reporting

- Review solid media cultures (7H11)
- 2-3 weeks after LPA
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Final Reporting

- New patient – report out identification if it correlates with growth on plate
- Within 2 months – “morphology consistent with *Mycobacterium intracellulare* see accession #....”
- Greater than 2 months or a different source, do full ID
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Weekly workflow

- Extractions on Tuesday/Thursday
- Line probe run on Wed/Friday
  - PCR – 2.5 hours
  - Hybridization/washes – 2.0 hours
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Conclusion - Challenges

- Dirty/clean
  - Open system – after amplification step, personnel are “dirty”

- Cost
  - Approx. $40/strip
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Conclusion – Advantages

- Easy to perform
- Detection of 18 clinically relevant *Mycobacterium* sp.
- Can use MGIT liquid culture and solid media
- Quick visual interpretation
- Mostly automated using the Auto-LiPA 48
- Mixed populations easily identified
INNO-LiPA Mycobacteria v2 – Line Probe Assay

Poster for More Info

- Poster #9
- Scott Fisher
Thank you!

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