TB SEQUENCING IN MASSACHUSETTS

Laboratory Aspects of TB

July 13, 2022

Tracy Stiles
Microbiology Division Director, Massachusetts State Public Health Laboratory
TB testing in Massachusetts

• Only full-service TB lab in Massachusetts
• 17,000 specimens annually
• 10,000 patients
• 150-250 new cases of TB annually
• 1 MDR in 2021
• 2022: 1 MDR and 1 pre-XDR
Sequencing Timeline

2016

PulseNet: Listeria mainly, STEC and Sal

2018

Legionella Unknown Respiratory Pathogens (URP)

PulseNet announcement — live sequencing in 2019
Began to explore CRO to support Epi investigations

2020

Live with PulseNet in January 2019
Real time sequencing and uploading of all PulseNet pathogens
- CLIA validated BRR and Rabies training
WNV Sequencing

2022

COVID

TB Sequencing Development

MDRO
Sequencing of all ARLN and HAI
CLIA validated wet lab
Began to explore TB
## Sequencing Volumes to date

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>PulseNet</td>
<td>447</td>
<td>951</td>
<td>1017</td>
<td>1462</td>
<td>951</td>
<td>1149</td>
<td>433</td>
<td>6410</td>
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<td>GenomeTrakr</td>
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<td>0</td>
<td>2</td>
<td>34</td>
<td>231</td>
<td>118</td>
<td>397</td>
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<tr>
<td>AR (CRO)</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>54</td>
<td>206</td>
<td>248</td>
<td>99</td>
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<td>HAI (GAS/MRSA)</td>
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<td>9</td>
<td>17</td>
<td>25</td>
<td>30</td>
<td>81</td>
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<tr>
<td>TB</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>37</td>
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<td>SARS-CoV-2</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>674</td>
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<td>10583</td>
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<td>Monkeypox</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Rabies</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24</td>
<td>31</td>
<td>0</td>
<td>55</td>
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<tr>
<td>Other*</td>
<td>29</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>13</td>
<td>289</td>
<td>4</td>
<td>355</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>447</strong></td>
<td><strong>963</strong></td>
<td><strong>1027</strong></td>
<td><strong>1527</strong></td>
<td><strong>1906</strong></td>
<td><strong>9202</strong></td>
<td><strong>3114</strong></td>
<td><strong>18186</strong></td>
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</table>

* Legionella, Burkholderia cepacia complex, Achromobacter, Yersinia, Neisseria meningitidis, Neisseria gonorrhoea*
Wet Lab Procedure

*Validation Pending...

**BSL-3/TB Lab**

**Heat Inactivation**
- Solid: 1000uL LC-MS grade water
  - Half-loop (10uL loop) growth
  - Swirl, Vortex, Spin
  - 100°C +/- 5°C for 30 minutes

**Extraction**
- Liquid: 1.2mL from the bottom
  - Spin >13000rpm for 3 minutes
  - Remove sup, resuspend pellet
  - 1000uL LC-MS grade water
  - Vortex, Spin
  - 100°C +/- 5°C for 30 minutes

**Library Prep**
- Nextera XT (4-5 hours)
  - tagmentation,
  - amplification,
  - normalization,
  - pooling

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**BSL-2/Sequencing Lab**

**Heat Inactivation**

**Extraction**

**Library Prep**
## Experimenting with Extractions

A ("small" loop, X), B ("medium" loop, 2X), C ("large" loop, 3X)

<table>
<thead>
<tr>
<th>Extraction Procedure</th>
<th>Concentration</th>
<th>Nanodrop Reading</th>
<th>Qubit Reading</th>
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<tr>
<td><strong>Target Ranges</strong></td>
<td></td>
<td>1.75-2.05</td>
<td>&gt;1ng/uL</td>
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<tr>
<td><strong>DNeasy Gram positive protocol - Solid Culture</strong></td>
<td></td>
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<tr>
<td>A</td>
<td>1.7</td>
<td>3.53 ng/ul</td>
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<tr>
<td>B</td>
<td>1.76</td>
<td>25.5 ng/ul</td>
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<tr>
<td>C</td>
<td>1.69</td>
<td>44.6 ng/ul</td>
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<tr>
<td><strong>FastPrep24/InstaGene Protocol - Solid Culture</strong></td>
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<tr>
<td>A</td>
<td>1.19</td>
<td>2.64 ng/ul</td>
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<td><strong>B</strong></td>
<td>1.87</td>
<td>16.8 ng/ul</td>
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<tr>
<td>C</td>
<td>1.74</td>
<td>31.4 ng/ul</td>
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<td><strong>FastPrep24/InstaGene Protocol - 7H9 Broth</strong></td>
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<tr>
<td>A</td>
<td>1.86</td>
<td>4.03 ng/ul</td>
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<tr>
<td><strong>B</strong></td>
<td>1.76</td>
<td>7.90 ng/ul</td>
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<tr>
<td>C</td>
<td>2.21</td>
<td>19.8 ng/ul</td>
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</tbody>
</table>
Sequencing Capacity

4 Illumina MiSeq
1 Illumina NextSeq 550
1 Illumina iSeq
1 Clear Labs instrument
1 ONP MinION

Illumina MiSeq
500 cycle v2 kits
Tb genome 4.4 Mbp
16 per cartridge
1-2 cartridges/week when live

Goal: Mixed runs on the NextSeq
APHL AIMS Platform Pilot of Wadsworth TB Pipeline

• Project in work 2019-2022
• WC shared pipeline
• 3 pilot state labs now have access - FL, MA, NC
• Available soon at Datapult
Piloting the AIMS (NY) Pipeline

- 615 Sequences have been analyzed
  - 574 raw data files from MI
  - 41 sequenced in MA and analyzed in parallel
- MI: NextSeq/MA: MiSeq
  - Early issues with fastq files generated from the NextSeq since resolved
Piloting the AIMS (NY) Pipeline: lineage determination

Count of Samples by Lineage

- Lineage 1 (Indo-Oceanic)
  - Count: 5
- Lineage 2 (Beijing)
  - Count: 11
- Lineage 3 (Central-Asian)
  - Count: 1
- Lineage 4 (Euro-American)
  - Count: 24

Lineages: Lineage 1 (Indo-Oceanic), Lineage 2 (Beijing), Lineage 3 (Central-Asian), Lineage 4 (Euro-American)
Analysis Comparison-Lineage

Count of Samples by Lineage

<table>
<thead>
<tr>
<th>Lineage</th>
<th>MA</th>
<th>MI</th>
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<tbody>
<tr>
<td>Lineage 1 (Indo-Oceanic)</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Lineage 2 (Beijing)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Lineage 3 (Central-Asian)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lineage 4 (Euro-American)</td>
<td>9</td>
<td>9</td>
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</tbody>
</table>

Lineage:
- Lineage 1 (Indo-Oceanic)
- Lineage 2 (Beijing)
- Lineage 3 (Central-Asian)
- Lineage 4 (Euro-American)
Genotypic vs Phenotypic Susceptibility

- 8/41 contained molecular resistance genes
- 4 failed coverage at a target site
  - unable to determine resistance
- 1 discordant result

<table>
<thead>
<tr>
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<th>WGS Resistance (MA)</th>
<th>Conventional DST</th>
<th>WGS Resistance (MA)</th>
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<tbody>
<tr>
<td>1</td>
<td>Isoniazid=RESISTANT, Ethionamide=RESISTANT</td>
<td>Isoniazid,</td>
<td>Isoniazid,</td>
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<tr>
<td></td>
<td></td>
<td>ethionamide R</td>
<td>ethionamide R</td>
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<tr>
<td>2</td>
<td>Isoniazid=RESISTANT, Streptomycin=Not Determined, Kanamyc</td>
<td>Isoniazid R</td>
<td>Isoniazid R</td>
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<tr>
<td></td>
<td>cin/Amikacin=Not Determined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Streptomycin=RESISTANT</td>
<td>Strep R</td>
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</tr>
<tr>
<td>4</td>
<td>Streptomycin=Not Determined</td>
<td>pan S</td>
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<tr>
<td>5</td>
<td>Isoniazid=RESISTANT, Ethionamide=RESISTANT</td>
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<td>ethionamide R</td>
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<td>8</td>
<td>Streptomycin=Not Determined</td>
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## Next Steps

<table>
<thead>
<tr>
<th>Wet Lab</th>
<th>Analysis</th>
<th>QC</th>
<th>Communication</th>
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</thead>
<tbody>
<tr>
<td>Optimize and validate extraction and library prep from primary clinical specimens</td>
<td>Pipeline is command line heavy; make user friendly; parse out the summary section</td>
<td>Finalize QC metrics and run acceptance criteria</td>
<td>What goes into the LIMS</td>
</tr>
<tr>
<td>V2 chemistry vs v3 chemistry</td>
<td>CLIA Validation lineage and MDDR</td>
<td>Compare against theiaprok and reference free pipeline</td>
<td>Do we report to providers or epi? And How?</td>
</tr>
<tr>
<td>CLIA Validation</td>
<td>How do we integrate real time surveillance?</td>
<td>Version control of pipelines</td>
<td>Concurrent testing for genotypic and phenotypic AST; messaging to providers and TB Control</td>
</tr>
</tbody>
</table>

**Next Steps**
Acknowledgements

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- Jasmine Guillet
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Wadsworth Center
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https://blog.mass.gov/publichealth

www.mass.gov/dph
Questions?