One Stop Shop: New York State’s Approach to TB Diagnosis

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April 30, 2019
New York State Public Health Labs

NY: 19.5 million

NYC: 8.6 million

2/3 of TB cases
Upstate NY: Site of H37Rv Isolation
TB in New York

National rank #3 by number of cases; rank #5 by cases per 100,000 population

Historical TB Molecular Testing

- Spoligotyping: 1990’s
- Real-time PCR: 2007
- Pyrosequencing: 2009
Journey to WGS Implementation

Proof of Principle 2014

Validation

Assay Development

Implementation 2016

Pipeline Construction
Whole Genome Sequencing Overview

1. Extract TB DNA
2. Library Preparation
3. DNA sequencing
4. Bioinformatic pipeline
5. Report
WGS Performance Metrics

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<tr>
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<th>EMB</th>
<th>INH</th>
<th>PZA</th>
<th>RIF</th>
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<tr>
<td>Sensitivity</td>
<td>0.89</td>
<td>0.93</td>
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<td>Specificity</td>
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<td>Resistance-PV</td>
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<td>Susceptibility-PV</td>
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<td>0.98</td>
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<td># strains</td>
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<td>1522</td>
<td>1518</td>
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- Phenotype – Genotype comparisons
- MGIT DST as gold standard
- 99% if low-level resistance is included

WGS findings have prompted some research opportunities
- Rare rpoB mutations
- Unexplained PZA resistance
- Reducing phenotypic testing burden

See Posters
Molecular INH Resistance Detection Over Time

~6% of remaining strains harbor candidate mutations

Sensitivity (%)

pyrosequencing  WGS

2010 katG  2012 katG/inhA  2016 v1  2017 v2  current v3  the future...

v1, v2, v3 = version of analytical pipeline in use

~6% of remaining strains harbor candidate mutations
The role of WGS in a few interesting cases...
Case 1: Neonatal TB

- 3-month-old baby
- Born prematurely at 24 weeks
- Treated unsuccessfully for pneumonia
- Bronchial lavage, submitting lab:
  - AFB smear positive
  - GeneXpert TB+, RIF+
  - Culture positive
Initial Lab Testing Timeline

- Bronchial lavage
- MGIT culture received
- PCR: TB Complex +
  - rpoB Ser531Leu
  - katG Ser315Thr
- embB Met306Val
- ethA Trp268STOP
- pncA His137Gln
- No match in WGS database

Predicted Resistance: rifampin, isoniazid, ethambutol, ethionamide, pyrazinamide
Investigation of Potential Source

- No active pulmonary disease among contacts
- Mother had recollection of TB treatment
  - PPD negative, clear chest X-ray, no IGRA
- Further review of mother’s medical history
  - Fertility issues with no identified source
  - Pregnant through in vitro fertilization
  - Prior pelvic scan revealed an abscess
- Tissue sample is taken and sent to our lab
  - Smear-, PCR-, culture+
Updated Timeline

- 10/9/18: Collection
- 11/8/18: PCR Pyro
- 11/9/18: WGS result
- 11/13/18: DNA extraction
- 11/28/18: Culture+
- 12/17/18: WGS result
- 12/21/19: MGIT DST
- 12/31/18: 2nd line DST results
- 1/6/19: 2nd line DST invalid
- 2/5/19: 2nd line DST invalid

Tissue sample from mother
Case 1 Summary

WGS Impact
- Improved TAT (8 days)
- Results 30 days before MGIT DST
- Informed treatment
- Confirmed the strain relatedness

Treatment
- amikacin
- levofloxacin
- linezolid
- cycloserine
- p-aminosalicylic acid
  - Mother only
Case 2: Unexpected WGS findings

- 42 years old, female
- Positive sputum culture $\rightarrow$ RIPE (11/1)
- Complicated by stage 4 metastatic cancer
- Wadsworth rec’d isolate for routine WGS (11/17)

- contact investigations
- DOT
- follow-up testing
- treatment adjustment
Case 2: WGS Results

• Atypical genotype
• Should not be found in a patient strain
• Immediately alerted TB Control and the submitting lab
• Investigation revealed that the culture positivity was an erroneous result
Other Areas of Interest in our Lab
Linking Mutations with MIC’s

- Sensititre MycoTB plate
- requires solid media growth
- 12 antimicrobials
- good reproducibility

- May help overcome some limitations of critical concentration testing
Other sequencing applications

Whole-genome
- DNA baiting
- Whole-genome amplification

MinION
- Parallel isolate sequencing
- Rapid data generation
- Potential cost savings and TAT improvement

Targeted NGS

~75-85% or the data is generated in the first 24 hrs
### Acknowledgements

**Core TB WGS Team**
- Vincent Escuyer
- Kimberlee Musser
- Tanya Halse
- Pascal Lapierre

**Mycobacteriology Lab**
- Donna Kohlerschmidt
- Susan Wolfe
- Michelle Isabelle
- Ali Fiero

**Molecular Bacteriology Lab**
- Tammy Quinlan
- Justine Edwards
- Linda Gebhardt
- James Long

**Applied Genomics Technology Core**
- Melissa Leisner
- Nathalie Boucher
- Helen Ling
- Matt Shudt
- Erasmus Schneider
- Holly Schwab
- Mary Pysnik

**Bioinformatics Core**
- Mike Palumbo

**CLIMS**
- Colleen Walsh
- Alok Mehta

**Wadsworth Center**
- Jill Taylor
- Victoria Derbyshire
- Ron Limberger
- Carol Smith

**NYC Public Health Lab**
- Jennifer Rakeman-Cagno
- John Kornblum
- Yvette Francis-Morris
- Jamie Lemon
- Qinghuan Liu

**NYS TB Control**
- Cheryl Kearns
- Margaret Oxtoby
- Stephen Hughes
- Michelle Cummings
- Melanie Vavruick
- Melanie Dolan
- Fred Zielinski

**NYC TB Control**
- Herns Modestil
- Jeanne Sullivan Meissner
- Jillian Knorr
- Shama Ahuja
- Felicia Dworkin
- Diana Nilsen

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**Wadsworth Center, NYSDOH**
**Public Health Genomics Initiative**

**Establishment of Mycobacterium tuberculosis complex WGS Reference Centers**

**National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention**

**R03 NIH- Use of whole genome sequencing for tuberculosis diagnostics**

**R21 NIH- Nanopore TB project**