TB Genotyping: Laboratory Aspects

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Benefits of TB Genotyping

When combined with epidemiologic data:

• Confirm epidemiologic links

• Outbreaks detected earlier; controlled more rapidly

• Detect unsuspected transmission

• Transmission between patients living in different jurisdictions are detected more readily

• Detect or confirm false positive cultures

• Able to monitor progress toward the elimination of TB transmission
CDC Tuberculosis Genotyping Program

- Two genotyping laboratories, Michigan and California, have been contracted by the CDC to provide genotyping services to TB control programs in the United States.

- Each participating TB program may submit 1 culture positive isolate per patient within their jurisdiction.

- Isolates suspected to be the result of a false-positive culture may also be submitted.
Michigan TB Genotyping Laboratory

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Isolate Submission

• All isolates must be clearly labeled with an identifier corresponding with the TB Genotyping Isolate Submission Form

• CDC provides a standard Excel submission form for the following information:
  - state ID
  - name of lab
  - date shipped
  - identifier

• Hardcopy sent with isolate shipment; electronic copy is sent via e-mail
Isolate Submission (cont.)

- **Accepted:**
  - 1 ml samples of broth culture in a 2 ml screw cap vial with o-rings
  - L-J or other solid medium slants
    **Cultures must be sealed with parafilm / tape and surrounded by absorbent material**

- **Not accepted:**
  - clinical samples or sediments from processed clinical samples
  - cultures not yet identified as *M. tuberculosis* complex
  - Obviously contaminated cultures; suspected mixed cultures
Receiving

• Inner shipping containers are cautiously opened in a BSL-3 laboratory

• Inspected for leaks

• Each isolate is wiped-down and disinfected prior to assigning accession numbers (e.g. 08RF4023)
Handling of Isolates

- Submitted isolates are aliquoted for
  - extraction for PCR
  - subculture in 7H9 broth
    potential RFLP requests
  long term freezer (-70ºC) storage
Genotyping Methods

**Primary Genotyping:** PCR based; all isolates

- Spoligotyping (spacer oligonucleotide typing)
- MIRU (mycobacteria interspersed repetitive units)

**Secondary Genotyping:** select isolates

- RFLP (restriction fragment length polymorphism)
Discrimination of Genotyping Methods

- Single method
  RFLP > MIRU > Spoligotype

- Spoligotype and MIRU combined are comparable to RFLP

- All three methods = best discrimination
Turn Around Time

• Spoligo & MIRU results are to be reported within 10 working days, from receipt, for > 90% of submitted isolates

• RFLP (as needed) is to be reported within 30 working days from time of request
DNA Extraction

• Several methods utilized for extracting DNA
  - Qiagen M48 BioRobot (MagAttract Kits)
  - Fast Prep
  - CTAB (higher quality DNA for RFLP)
Qiagen M48 BioRobot

- Fully enclosed robot uses magnetic beads to extract DNA out of solution and minimizes human contact with the sample.
- Decreases risk of contamination of DNA product
- Increase throughput
- Allows for multi-tasking
Spoligotyping

- Hybridization assay
- Variability in direct repeat region
- Direct repeats separated by unique “spacer” sequences
- 43 spacer sequences

Example 1

Spoligotype pattern
Bio-Plex

- Bio-Plex (BioRad)
- 43 different regions
- 96 well plate
- Decreases subjectivity
- Standardized results
How It Works

- Oligonucleotide capture probe covalently attached to bead
- Reporter fluorochrome bound to hybridized PCR product

- red - identification
- green - quantification
Spoligotype Pattern

Example 1

Original banding pattern
1111111111111111110011111111110001111111

Binary code

14 + 1 grouping
Octal designation
7 7 7 7 7 7 4 7 7 7 6 0 7 7 1
MIRU-VNTR

- Mycobacterial interspersed repetitive units
- Variable number tandem repeats

- Number of repeats are determined by the size of the amplicon
MIRU

• Each MIRU loci amplified individually; pooled post PCR

• Dye-labeled primers

• 24 samples
MIRU Instrumentation

• CEQ 8000 (Beckman Coulter)
  Capillary Electrophoretic Genetic Analysis System

• Fragment Analysis
MIRU Output

- **MIRU 26**
  - 165 bp
  - 2 copies

- **MIRU 40**
  - 220 bp
  - 2 copies

- **MIRU 20**
  - 365 bp
  - 2 copies
MIRU Results

- 12 digit pattern

<table>
<thead>
<tr>
<th>MIRU</th>
<th>02</th>
<th>04</th>
<th>10</th>
<th>16</th>
<th>20</th>
<th>23</th>
<th>24</th>
<th>26</th>
<th>27</th>
<th>31</th>
<th>39</th>
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<tbody>
<tr>
<td>Size (bp)</td>
<td>283</td>
<td>332</td>
<td>201</td>
<td>168</td>
<td>365</td>
<td>287</td>
<td>414</td>
<td>303</td>
<td>226</td>
<td>264</td>
<td>226</td>
<td>220</td>
</tr>
<tr>
<td>No. of copies</td>
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<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

MIRU pattern: 232234253322

- Designations a,b,c…for greater than 9 repeats
- Occasionally the repeat number is 0
Genotyping Results

- Access database (CDC)

- MI676763777777600232234253322
  
<table>
<thead>
<tr>
<th>ID</th>
<th>Spoligo</th>
<th>MIRU</th>
</tr>
</thead>
</table>

- Matching genotype patterns form PCR cluster (e.g. MI_012)

- PCR type (e.g. PCR0003014)
Reporting Results

• Genotype results reported to TB control programs (not submitting laboratories) via e-mail
  – Submitter no. (submitting lab)
  – Accession no. (genotype lab)
  – Genotype pattern
  – Cluster designation (if applicable)
  – Report date

• TB control program will determine if secondary typing by IS6110-RFLP is required
Requesting RFLP

PCR clustered isolates, as needed

YES
  Indirect epi links (e.g. same zip code)

NO
  Strong epi links (e.g. household members)
  Absolutely no suspected epi links
Requesting RFLP (cont.)

- CDC provides standard Excel submission form for the following information:
  - state id
  - submitter no.
  - genotype accession no.
  - assigned PCR cluster
  - date requested
  - additional comments

- Electronic form to be sent via e-mail
Restriction Fragment Length Polymorphism

TB DNA → Restriction Digest → Agarose Gel → Transfer to Membrane

IS6110 Probe → Expose to film
IS6110 RFLP
Film

• Image scanned for computer analysis

• Compare new isolate results with previously analyzed isolates
IS6110 RFLP Results

• IS6110 fingerprints will only be compared to those of other isolates within the same PCR cluster

• RFLP results include
  – IS6110 pattern (e.g. 08RF2025-11)
  – Number of bands
  – IS6110 cluster, if clustered (e.g. MI_025A)
  – IS6110 Comments
  – Date reported
Upcoming Changes

• MIRU 2 (24 loci)

• Genotyping Information Management System (TB GIMS)
Acknowledgements

CDC Mycobacteriology Laboratory
Lauren Cowan, Ph.D.

Michigan Genotyping Laboratory
Jim Rudrik, Ph.D.; Dale Berry B.S., Laura Guild B.S., Rebecca Kramer B.S.

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Frances Downes, Dr.PH.; Laura Mosher, M.S.
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