Genotyping
Understanding and utilizing this dynamic tool in your TB program
Lisa Y Armitige, MD, PhD
November 18, 2015

Tuberculosis Intensive
November 17-20, 2015
San Antonio, TX

Lisa Armitige, MD, PhD has the following disclosures to make:

- No conflict of interests
- No relevant financial relationships with any commercial companies pertaining to this educational activity
Genotyping

Understanding and utilizing this dynamic tool in your TB program
Lisa Y Armitige, MD, PhD
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Heartland Tuberculosis
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University of Texas at Tyler

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Learning Objectives

• Describe the basis for genotyping of *M. tuberculosis*

• Describe the utility of genotyping in management of TB patients

• Describe the utility of genotyping in conducting outbreak investigation responses
What is TB genotyping?

• Genotyping or ‘fingerprinting’ utilizes the fact that there are regions of the DNA that are different in different TB isolates.

• Allows comparison of relatedness of *M. tuberculosis* isolates based on genetic make-up.

• Following differences in strains can show patterns and dynamics of TB transmission

Genotyping Methods

• **RFLP**
  – Restriction Fragment Length Polymorphism

• **Spoligotyping**
  – Spacer oligonucleotide typing

• **VNTR-MIRU or MIRU**
  – Variable-Number Tandem Repeat of Mycobacterial Interspersed Repetitive Units
Spacer Oligonucleotide Typing  
(Spoligotyping)

- Exploit DNA polymorphisms within the direct repeat (DR) locus of MTB
- Locus contains multiple, well-conserved 36 bp DRs interspersed with non-repetitive spacer sequences 34-41 bp long
- Variation in the 43 spacers reflects the polymorphisms studied
**Spoligotyping Nomenclature**

Octal code: 000 = 0, 001 = 1, 010 = 2, 011 = 3, 100 = 4, 101 = 5, 110 = 6, 111 = 7

- Gel Image
- Binary
- Triplet
- Octal

**Mycobacterial Interspersed Repetitive Units (MIRU)**

- 41 different MIRUs loci
- 12 or 24 separate loci are targeted
- Number of repeats at the 12 or 24 loci are determined using 12 or 24 different PCR assays (MIRU1 or MIRU2)
- Use high-throughput automated analysis
MIRU Nomenclature

**MIRU Example 1**

<table>
<thead>
<tr>
<th>MIRU Locus</th>
<th>02</th>
<th>04</th>
<th>10</th>
<th>16</th>
<th>20</th>
<th>23</th>
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**MIRU Example 2**

<table>
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<td>5</td>
<td>4</td>
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Restriction Fragment Length Polymorphism - **RFLP**

- Detects IS6110 patterns

- Detect strain difference in both the number of copies and the position of IS6110 in the genome

- Results look like a ‘bar code’
### RFLP “Bar Code” Patterns

<table>
<thead>
<tr>
<th>Band Size (kb)</th>
<th>3</th>
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<td></td>
</tr>
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</table>

(84) (96) (4) (118) (5) (167) (27) (28)

No. of patients
Limitations of RFLP

- Comparison of strains requires a large database and standards
- Use in areas of high genetic diversity
- Relative labor intensive

How To Read a Genotyping Report

Sample Genotype

<table>
<thead>
<tr>
<th>Patient</th>
<th>collected</th>
<th>spoligotype</th>
<th>Miru</th>
<th>Miru2</th>
</tr>
</thead>
<tbody>
<tr>
<td>John Q. Public</td>
<td>4/16/2010</td>
<td>7777677760771</td>
<td>225325033223</td>
<td>733424423336</td>
</tr>
</tbody>
</table>
Program Genotype Report (sent by DSHS)

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>DOB</th>
<th>Spoligotype</th>
<th>MIRU</th>
<th>Cluster Name</th>
</tr>
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<tbody>
<tr>
<td>Doe</td>
<td>Jane</td>
<td>8/1/1950</td>
<td>777777777760771</td>
<td>233326153321</td>
<td>TX_100</td>
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<tr>
<td>Doe</td>
<td>Sarah</td>
<td>12/12/1975</td>
<td>777777777760771</td>
<td>233326153321</td>
<td>TX_100</td>
</tr>
<tr>
<td>Smith</td>
<td>John</td>
<td>5/5/1975</td>
<td>000000000003771</td>
<td>223321153623</td>
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</table>

- Each unique combination of spoligotype and 12-locus MIRU-VNTR results has been assigned a PCRTypetype, “PCR” followed by some numbers (PCR00002)

- Each unique combination of spoligotype and 24-locus MIRU-VNTR results has been assigned a GENType, “G” followed by 5 digits (GENType00003)
PCRTyp e vs. GENTyp e

- There can be multiple GENTyp es in the same PCRTyp e……

<table>
<thead>
<tr>
<th>Line#</th>
<th>GENType</th>
<th>PCRTyp e</th>
<th>Spoligotype</th>
<th>24-locus MIRU-VNTR</th>
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1 Unique combination of spoligotype and 24-locus MIRU-VNTR

PCRTyp e vs. GENTyp e

- There can be multiple GENTyp es in the same PCRTyp e……

<table>
<thead>
<tr>
<th>Line#</th>
<th>GENType</th>
<th>PCRTyp e</th>
<th>Spoligotype</th>
<th>24-locus MIRU-VNTR</th>
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<td>G00014</td>
<td>PCR00051</td>
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<td>223125163324</td>
</tr>
</tbody>
</table>

1 Unique combination of spoligotype and 24-locus MIRU-VNTR
PCRTYPE vs. GENType

- There can be multiple GENTypes in the same PCRTYPE......

### Comparative Examples for GENType and PCRTYPE

<table>
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<tr>
<th>Line#</th>
<th>GENType</th>
<th>PCRTYPE</th>
<th>Spoligotype</th>
<th>24-locus MIRU-VNTR</th>
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<td>G00014</td>
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<td>223125163324</td>
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</tbody>
</table>

1. Unique combination of spoligotype and 24-locus MIRU-VNTR

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Goal of TB genotyping

Identify recent transmission
- Identify unsuspected relationships between patients
- Locate unusual transmission settings
- Uncover transmission between jurisdictions
- Evaluate completeness of contact investigation
- Promptly identify false-positive cultures
- Detect and investigate outbreaks sooner
Goal of TB genotyping

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Case #1

• G.S. is a 43 y/o black man with history of HIV (last CD4 count 753).
  – He presented to the Emergency Room with symptoms suggestive of a TIA
  – He undergoes a workup which reveals elevated BP and elevated cholesterol. He is started on drugs to lower his blood pressure and his cholesterol and discharged home
Case #1 (cont)

• 5 weeks after he is discharged, a sputum collected during admission grows Mtb......

Now what?!

Case #1 (cont)

• Upon questioning, you discover:
  
  – The sputum was collected on the order of a training physician....who can’t quite come up with a reason why.....

  – The patient is asymptomatic. He has had no fevers, cough or weight loss either then or now.

  – His BUN is 45 and his creatinine is 3.8.
Case #1 (cont)

Again...Now what?!

• A call to the lab reveals there were 4 sputums that grew Mtb that were processed that day. All were processed at the same time.

• While re-assessing the patient you ask your state lab for a genotype analysis of each of the patients processed on the same day as your patient. You also note:
  – His IGRA and TST are both negative
  – His CXR shows no abnormalities
  – He has no symptoms and has gained 5 lb since his admission

<table>
<thead>
<tr>
<th>Patient</th>
<th>collected</th>
<th>spoligotype</th>
<th>Miru</th>
<th>Miru2</th>
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<td>G.S.</td>
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</table>
National TB Genotyping Service

• Began in January, 2004
• CDC contracted with 2 reference laboratories initially, now only one in Michigan
• Genotype at least 1 isolate for each new culture-positive case of TB
  – Primary typing for all isolates is PCR-based methods (spoligotyping, MIRU)
  – Secondary typing of clustered isolates by IS6110 - RFLP upon program or clinician request
TB Genotyping Information Management System (TB GIMS)

- Stores and manages genotyping data on TB patients in the United States
- Allows users to submit and track *Mycobacterium tuberculosis* isolates to and from the contract genotyping labs
- Provides immediate notification of genotyping results and updates to TB labs and programs
- Links isolate data to patient-level surveillance data
- Provides reports on genotype clusters, including national genotype distribution
- Provides national, state, and county maps of genotype clusters

Isolate and Data Flow

- Other Labs
- Isolates & Data
- Genotyping Lab
- Results
- State Lab
- TB Program
- CDC
- Patient names
Centralized National TB Database

- Centralized national database allows for detection of:
  - Interstate transmission
  - Cluster growth at a regional and national level
  - Tracking
    - MDR-TB, XDR-TB
    - Outbreak Strains
    - Special populations (e.g. homeless, prison strains)

Using genotyping in practice

- Identifying outbreaks
- Tracking transmission in your community
- Identifying source cases
- Recognizing false-positives
- Laboratory contaminants
When genotypes match and epidemiologic links are identified

- Supports same chain of recent transmission
- Did one person transmit to the other?
- Is it possible that both became infected by a third person?

When genotypes match but no epidemiologic links are identified

- Incomplete contact investigation?
- Did you ask the right questions?
- Casual contact?
- RFLP needed?
- Endemic strain?
- Distant transmission?
- Cross-contamination?
Investigating on-going transmission

- Expanding contact investigations
- Initiating (expanding) outbreak investigations
  - Increase in the expected number of cases
  - Transmission continues despite adequate control efforts by the TB program
  - Contact investigation has grown to a size that requires additional outside help

Investigating on-going transmission

False-positive culture investigations

- Confirm (or refute) the suspicion that ≥1 patients in a genotyping cluster has been falsely diagnosed with TB on the basis of a false-positive culture result
- Common collection or processing points
- Identify patients in the cluster who, despite a diagnosis of TB, do not fit the typical clinical picture of the disease
  - Normal chest radiographs
  - Only one positive specimen
Investigating on-going transmission

False-positive culture investigations

- Possible sources and locations:
  - Bronchoscopes
  - Sputum collection areas
  - Laboratory processing areas

Questions?
Interferon-Gamma Release Assays (IGRAs)

Lisa Y. Armitige, MD, PhD
Medical Consultant
Heartland National TB Center

Associate Professor
Internal Medicine/Pediatrics/Adult Infectious Disease
University of Texas Health Science Center at Tyler

Overview

• Development of interferon-gamma release assays (IGRAs)

• FDA-approved IGRAs

• Current recommended use: CDC guidelines
Development of IGRAs

The Tuberculin Skin Test (TST)

• Where we started......
  100 years ago

• 0.1 ml of 5 TU PPD tuberculin injected intradermally

• Induration in millimeters read 48-72 hours after injection
TST Limitations

- Technical problems in administration and reading
- Interpretation based on pretest probability
- >1 visit needed
- False-negative responses
  - Anergy (compromised immunity)
  - TST reversion at old age
- Repeated TSTs boost the immune response
  - Need 2-step approach in serial testing
- False positives
  - Nontuberculous mycobacteria (NTM)
  - Bacille Calmette-Guerin vaccination (BCG)

TST vs In-vitro Assays

IFN-γ release assays (IGRAs)

Original QuantiFERON-TB (QFT) versus TST

<table>
<thead>
<tr>
<th>QFT</th>
<th>TST</th>
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<tbody>
<tr>
<td>1 patient visit</td>
<td>2 patient visits</td>
</tr>
<tr>
<td>Measurement of IFN-γ by machine (more objective)</td>
<td>Induration measured by human (more subjective)</td>
</tr>
<tr>
<td>Antigen: PPD</td>
<td>Antigen: PPD</td>
</tr>
</tbody>
</table>
What is PPD (Purified Protein Derivative)?

- **Old tuberculin**: a sterile solution of a concentrated filtrate of *M. tuberculosis* in culture
- **PPD**: purified protein fraction precipitated from old tuberculin
- **PPD contains many antigens**
  - Some are also found in BCG and NTM
- **IGRA that uses PPD does not address issue of false-positive results related to BCG or NTM cross-reactions**

Antigens Specific to *M. tuberculosis*

Genetic Region of Difference 1 (RD-1)

- Not found in BCG or most NTM
  - NTM exceptions: *M. kansasii, M. szulgai, M. marinum*

- Codes for 9 proteins

- Two found to produce strong immunologic responses in persons infected with *M. tuberculosis*
  - 10-kDa culture filtrate protein (CFP-10)
  - 6-kDa early-secreted target antigen (ESAT-6)

Antigens for Newer Generation IGRAs

- Negative control or nil (e.g., saline, heparin)

- Positive control or mitogen: non-specific immune response stimulator (e.g., phytohemagglutinin)

- *M. tuberculosis*-specific antigens
  - Unlike PPD used in TST, do not cross-react with BCG or NTM (some exceptions)
  - ESAT-6, CFP-10, TB 7.7 (actually simulated using overlapping peptides)
## Antigens for Gamma-Release Assays

<table>
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<th>Tuberculosis complex</th>
<th>Antigens</th>
<th>Environmental strains</th>
<th>Antigens</th>
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<td>CFP</td>
<td>ESAT</td>
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<tr>
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<td>+</td>
<td>-</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BCG substrain</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>gothenburg</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>moreau</td>
<td>-</td>
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<tr>
<td>tice</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>tokyo</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>danish</td>
<td>-</td>
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</tr>
<tr>
<td>glaxo</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>montreal</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pasteur</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- M abcessus
- M avium
- M branderi
- M celatum
- M chelonae
- M fortuitum
- M gordonii
- M intracullarare
- M kansasii
- M malmoense
- M marium
- M oenave
- M scrofulaceum
- M smegmatis
- M szulgai
- M terrae
- M xenopi

- FDA-Approved IGRAs
FDA Approved IGRAs

- **QuantiFERON®-TB Gold In-Tube (QFT-GIT)**
  - FDA approved Oct 2007

- **T-Spot®.TB (T-Spot)**
  - FDA approved July 2008

### QuantiFERON®-TB Gold In-Tube (QFT-GIT)

**Stage 1: Whole Blood Culture in special blood collection tubes**

- Collect 1mL of blood in 3 tubes
- Incubate at 37°C for 16-24 hours
- Centrifuge 5 minutes to separate plasma above gel

**Stage 2: Measure [IFN-γ] & Interpret**

- Collect 50 µL of plasma for ELISA
- Measure [IFN-γ] in 'Sandwich' ELISA
- Software calculates results and prints report

*Mtb = ESAT-6 + CFP-10 + TB 7.7
**T-Spot.TB (T-Spot)**

- Collect blood in CPT tube
- Recover, wash, & count PBMCs
- Aliquot 250,000 PBMCs to 4 wells with anti-IFN-γ
- Add saline, PHA, ESAT-6 or CFP-10 & incubate
- Wash away cells
- Develop & count spots where cells produced IFN-γ

**What Result is Considered Positive?**

- Depends on the test
- Based on calculation of IFN-γ response to TB antigens relative to IFN-γ response to nil
- Unlike TST, not risk stratified (i.e., there are not multiple cutoffs for different risk groups)
- Still somewhat complicated
  - Mitigated by software that performs calculations
### Interpretation Criteria for the QFT-GIT Test

<table>
<thead>
<tr>
<th>Nil (IU/mL)</th>
<th>TB Antigen minus Nil (IU/mL)</th>
<th>QFT-GIT (IU/mL)</th>
<th>Mitogen</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 8.0</td>
<td>≤ 0.35 or &lt; 25% of Nil value</td>
<td>Negative</td>
<td>≥ 5.0</td>
<td><em>M. tuberculosis</em> infection <strong>unlikely</strong></td>
</tr>
<tr>
<td>≤ 8.0</td>
<td>≥ 0.35 and ≥ 25% of Nil value</td>
<td>Positive</td>
<td>ANY</td>
<td><em>M. tuberculosis</em> infection <strong>likely</strong></td>
</tr>
<tr>
<td>≥ 8.0</td>
<td>ANY</td>
<td>Indeterminate</td>
<td>ANY</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>≤ 8.0</td>
<td>≤ 0.35 and or &lt; 25% of Nil value</td>
<td>Indeterminate</td>
<td>&lt; 5.0</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>Test</td>
<td>Result</td>
<td>Flag</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>--------</td>
<td>------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>PH QUANT TB</td>
<td>0.160</td>
<td></td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT NIL</td>
<td>0.164</td>
<td></td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT ESAT-6</td>
<td>0.203</td>
<td></td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT MIG</td>
<td>5.256</td>
<td></td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT ESAT-NIL</td>
<td>0.007</td>
<td></td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT CFP10-NIL</td>
<td>0.043</td>
<td></td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT MIG-NIL</td>
<td>0.096</td>
<td></td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT INTERF</td>
<td>NEGATIVE</td>
<td>L</td>
<td>NEGATIVE</td>
<td></td>
</tr>
</tbody>
</table>

**Note:**

- NO ESAT-6 OR CFP-10 RESPONSIVENESS DETECTED.
- M. TUBERCULOSIS INFECTION VERY LIKELY.
- ESAT-6 AND/OR CFP-10 RESPONSIVENESS DETECTED.
- M. TUBERCULOSIS INFECTION LIKELY.
- NOTE: QUANTIFERON TB GOLD IS AN INDIRECT TEST FOR
**QuantiFERON-TB Gold**

<table>
<thead>
<tr>
<th>Cutoff, IFN-γ (IU/ml)</th>
<th>CFP-10 Specificity (%)</th>
<th>CFP-10 Sensitivity (%)</th>
<th>ESAT-6 Specificity (%)</th>
<th>ESAT-6 Sensitivity (%)</th>
<th>CFP-10 and/or ESAT-6 Specificity (%)</th>
<th>CFP-10 and/or ESAT-6 Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>92.5</td>
<td>81.4</td>
<td>94.8</td>
<td>94.9</td>
<td>89.4</td>
<td>97.5</td>
</tr>
<tr>
<td>0.10</td>
<td>94.4</td>
<td>77.1</td>
<td>96.2</td>
<td>90.7</td>
<td>92.0</td>
<td>95.8</td>
</tr>
<tr>
<td>0.15</td>
<td>95.8</td>
<td>72.9</td>
<td>97.6</td>
<td>88.1</td>
<td>93.9</td>
<td>93.2</td>
</tr>
<tr>
<td>0.20</td>
<td>96.7</td>
<td>71.2</td>
<td>99.1</td>
<td>86.4</td>
<td>96.2</td>
<td>91.5</td>
</tr>
<tr>
<td>0.25</td>
<td>97.2</td>
<td>67.8</td>
<td>99.1</td>
<td>84.7</td>
<td>96.7</td>
<td>91.5</td>
</tr>
<tr>
<td>0.30</td>
<td>97.7</td>
<td>66.6</td>
<td>99.1</td>
<td>83.1</td>
<td>97.2</td>
<td>89.8</td>
</tr>
<tr>
<td>0.35</td>
<td>98.6</td>
<td>65.3</td>
<td>99.5</td>
<td>83.4</td>
<td>98.1</td>
<td>89.0</td>
</tr>
<tr>
<td>0.40</td>
<td>98.6</td>
<td>61.9</td>
<td>99.5</td>
<td>79.7</td>
<td>98.1</td>
<td>88.1</td>
</tr>
<tr>
<td>0.45</td>
<td>98.6</td>
<td>60.2</td>
<td>100.0</td>
<td>78.8</td>
<td>98.6</td>
<td>86.4</td>
</tr>
<tr>
<td>0.50</td>
<td>99.1</td>
<td>60.2</td>
<td>100.0</td>
<td>75.4</td>
<td>99.1</td>
<td>83.9</td>
</tr>
</tbody>
</table>

Sensitivity was determined on the basis of data from 118 patients with culture-positive tuberculosis, and specificity was determined on the basis of data from 213 low-risk subjects. The chosen cutoff (0.35) is in boldface.

---

**T-Spot.TB**

[Diagram showing T-Spot.TB results and controls]
## Interpretation Criteria for the T-Spot.TB

<table>
<thead>
<tr>
<th>Result</th>
<th>Nil*</th>
<th>TB Response#</th>
<th>Mitogen++</th>
<th>Interpretation+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≤ 10 spots</td>
<td>≥ 8 spots</td>
<td>Any</td>
<td>M. tuberculosis infection likely</td>
</tr>
<tr>
<td>Borderline</td>
<td>≤ 10 spots</td>
<td>5, 6, or 7 spots</td>
<td>Any</td>
<td>Uncertain likelihood of M. tuberculosis infection</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 10 spots</td>
<td>≤ 4 spots</td>
<td>Any</td>
<td>M Tb infection unlikely</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&gt; 10 ≤ 10</td>
<td>Any</td>
<td>Any</td>
<td>Uncertain likelihood of M. tuberculosis infection</td>
</tr>
</tbody>
</table>

---

Thank you to Marilyn Richardson.
Indeterminate and Borderline Results

- **Indeterminate**
  - Negative control result is too high
    - High background production of IFN-γ
  - Positive control result is too low
    - Immunocompromised patients may not respond to mitogen

- **Borderline (T-Spot only)**
  - Falls within borderline zone close to negative/positive cut point

CDC Guidelines
Previous U.S. Guidelines for FDA-Approved IGRAs

Guidelines for Using the QuantiFERON®-TB Test for Diagnosing Latent Mycobacterium tuberculosis Infection

Prepared by:
Gerald H. Mansukh, M.D.,
Margaret L. Vilanui, M.D.
Division of Tuberculosis Elimination
National Center for HIV, STD, and TB Prevention

2003

Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection — United States, 2010

MMWR
Morbidity and Mortality Weekly Report
www.cdc.gov/mmwr

June 25, 2010 / Vol. 59 / No. RR-6
Data Reviewed

• Over 150 published articles

• Supplemented by unpublished data presented at August 2008 consultation

• Only published articles used and cited as evidence basis in guidelines

Special Situations and Populations
Children

• Limited data, especially children < 5 y.o.
• Some studies show increased percentage of indeterminate results
• Blood drawing more difficult in very young children
• More difficult to confirm diagnosis of TB disease in children

Periodic Screening
(e.g., Healthcare Workers)

• Some studies have shown considerable variation in IFN-γ response with serial testing over time
• Uncertainty about magnitude of change in result that is likely caused by new infection versus expected test variation
• Questionable significance of conversions and reversions when initial test result is near cut point
• Frequency of false-positive conversions may be higher with IGRAs because of less stringent criteria for conversion compared to TST
Recommendations

• TST or IGRAs should be used as aids in diagnosing infection with *M. tuberculosis*
  • Both the standard qualitative test interpretation and the quantitative assay measurements should be reported

• As with the TST, IGRAs generally should not be used for testing persons who have a low risk of infection and a low risk of disease due to *M. tuberculosis*
CDC Recommendations

• Selection of the most suitable test or combination of tests for detection of *M. tuberculosis* infection should be based on the reasons and the context for testing, test availability, and cost effectiveness of testing.

• IGRAs may be used in place of (and not in addition to) TST in all situations in which CDC recommends tuberculin skin testing as an aid in diagnosing *M. tuberculosis* infection, with preferences and special considerations as follow.

• Despite the indication of a preference, use of the alternative test (IGRA or TST) is considered acceptable medical and public health practice.

CDC Recommendations

• Populations/situations in which IGRAs are preferred
  – testing persons from groups that historically have poor rates of return for TST reading
  – testing persons who have received BCG (as a vaccine or for cancer therapy)

• Populations/situations in which TST is preferred
  – testing children younger than 5 years old

• Populations/situations in which there is no preference between IGRAs and TST
  – testing recent contacts of persons with infectious tuberculosis
  – periodic screening that addresses occupational exposure to TB (e.g., surveillance programs for healthcare workers)
Additional Considerations for Serial Testing

- **IGRA advantages** include obtaining results in a single visit and no need for two-step testing (IGRAs don’t boost subsequent test results)

- **Disadvantages** include a potential greater risk of false test conversion
  - IGRA conversion is defined as a change from negative to positive without any consideration of magnitude
  - Using lenient criterion to define conversion might produce more conversions than are observed with the more stringent criteria applied to TSTs
  - Recent published studies appear to validate this concern

U.S. Healthcare Worker Study (Published After Guidelines)

- 6,530 HCWs screened with QFT-GIT at Univ. of Illinois
- 287 positive by QFT-GIT: 123 prior positive TST, 164 prior negative TST
- 135 of 164 retested within 4 weeks with QFT-GIT and TST
- Only 2 were positive by TST and 66 reverted to negative by QFT-GIT

Gandra et al. Infect Control Hosp Epidemiol 2010; 31(12)
**Potential sources of variability and their impact on results in IGRAs**

<table>
<thead>
<tr>
<th>Source of variability</th>
<th>QFT</th>
<th>T-SPOT.TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturing issues</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>Between-batch variability</td>
<td>Up</td>
<td>Up</td>
</tr>
<tr>
<td>Prenatal issues</td>
<td>No impact</td>
<td>No impact</td>
</tr>
<tr>
<td>Time of blood draw (am vs pm)</td>
<td>Up</td>
<td>Up</td>
</tr>
<tr>
<td>Site distillation</td>
<td>No impact</td>
<td>No impact</td>
</tr>
<tr>
<td>Temperature control</td>
<td>No impact</td>
<td>No impact</td>
</tr>
<tr>
<td>Blood vol (10-12 ml)</td>
<td>Down</td>
<td>NA</td>
</tr>
<tr>
<td>Heating of tuberculin to virulence</td>
<td>No impact</td>
<td>No impact</td>
</tr>
<tr>
<td>T cell and APC source</td>
<td>Up</td>
<td>No impact</td>
</tr>
<tr>
<td>Transportation temp</td>
<td>No impact</td>
<td>No impact</td>
</tr>
<tr>
<td>Delay to incubation (16-24 h)</td>
<td>No impact</td>
<td>No impact</td>
</tr>
<tr>
<td>Incubation time (36-38 h)</td>
<td>Possible effect</td>
<td>No impact</td>
</tr>
<tr>
<td>Plasma separation time (seconds to hours)</td>
<td>No impact</td>
<td>No impact</td>
</tr>
<tr>
<td>Plasma storage (4°C-8°C)</td>
<td>No effect</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Movement Toward a Change in IGRA Criteria for ‘Positive’**

**Int J Tuberc Lung Dis. 2009 January; 13(1): 84-92**

CDC Recommendations

- Routine testing with both TST and an IGRA is not recommended

- Results from both tests may be useful *when the initial test is negative* if increased sensitivity is desired (considered infected if either test is positive)
  - risk of infection, the risk of progression, and the risk of a poor outcome are increased
  - clinical suspicion of active tuberculosis and confirmation of *M. tuberculosis* infection is desired

- Results from both tests may be useful *when the initial test is positive* if increased specificity is desired (considered infected only if both tests are positive)
  - additional evidence of infection is required to encourage compliance (such as in foreign-born healthcare workers who believe their positive TST is due to BCG)
  - in healthy persons who have a low risk of both infection and progression

CDC Recommendations

- Repeating an IGRA or performing a TST may be useful *when the initial IGRA result is indeterminate, borderline, or invalid*, and a reason for testing persists
(Most important) CDC Recommendation

- Each institution and TB control program should evaluate
  - the availability,
  - overall cost effectiveness, and
  - benefits of IGRA in prioritizing IGRA use in their setting

Pearls for TST vs. IGRA

- Discordance between the TST and IGRA has been measured up to 20% in patients known to be infected with Mtb. Don’t order both tests, pick the right test to start with!
- IGRA shine when used in BCG-vaccinated populations (increased specificity)
- NO study has shown the IGRA to be ‘better’ in US-born (or non-BCG-vaccinated) individuals. The TST can be used AND trusted in this population
- No test (TST or IGRA) overrides clinical, epidemiologic or historical data
CDC Recommendations

- A diagnosis of *M. tuberculosis* infection, and the decisions about medical or public health management should include epidemiological, historical, and other clinical information when using IGRA or TST results
  - Decisions should not be based on IGRA or TST results alone

- Particularly relevant for managing discordant test results (e.g., TST+/QFT-)

Questions?