Interferon-Gamma Release Assays (IGRAs)
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
Interferon-Gamma Release Assays (IGRAs)

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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)

www.cellestis.com
**Original QuantiFERON-TB (QFT) versus TST**

<table>
<thead>
<tr>
<th>QFT</th>
<th>TST</th>
</tr>
</thead>
<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*

- 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)

Genetic Region of Difference 1 (RD-1)
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>
<thead>
<tr>
<th>Tuberculosis complex</th>
<th>ESAT</th>
<th>CFP</th>
<th>Environmental strains</th>
<th>Antigens</th>
<th>ESAT</th>
<th>CFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>M tuberculosis</td>
<td>+</td>
<td>+</td>
<td>M abcessus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M africanum</td>
<td>+</td>
<td>+</td>
<td>M avium</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M bovis</td>
<td>+</td>
<td>+</td>
<td>M branderi</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>BCG substrain</td>
<td>-</td>
<td>-</td>
<td>M cellatum</td>
<td>-</td>
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<td>-</td>
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<td>gothenburg</td>
<td>-</td>
<td>-</td>
<td>M chelonae</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>moreau</td>
<td>-</td>
<td>-</td>
<td>M fortitum</td>
<td>-</td>
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<td>tice</td>
<td>-</td>
<td>-</td>
<td>M gordonii</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>tokyo</td>
<td>-</td>
<td>-</td>
<td>M intracellular</td>
<td>-</td>
<td>-</td>
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<tr>
<td>danish</td>
<td>-</td>
<td>-</td>
<td>M kansas</td>
<td>+</td>
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<tr>
<td>glaixo</td>
<td>-</td>
<td>-</td>
<td>M malmoense</td>
<td>-</td>
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<tr>
<td>montreal</td>
<td>-</td>
<td>-</td>
<td>M marinum</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>pasteur</td>
<td>-</td>
<td>-</td>
<td>M oenavense</td>
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<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M scrofulaceum</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M smeagmatis</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M szulga</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M terrae</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M xenopi</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
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
- Centrifuge 5 minutes to separate plasma above gel
- Incubate at 37ºC for 16-24 hours

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 unlikely</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 likely</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>
</tbody>
</table>

---

**Patient: PATIENT.TEST QT3**  
Act #:L000000000034

**Order Doc: PUBLIC HEALTH LABORATORY EAST**  
Specimen: 0933:F100003R  COMP Collected: 09/01/05-1240 Received: 09/06/05-1353

**Test** | **Result** | **Flag** | **Reference**
---|------------|---------|----------------|
PH QUANT TB | 0.160 | | IU/ML |
PH QUANT ESAT-6 | 0.164 | | IU/ML |
PH QUANT CFP-10 | 0.203 | | IU/ML |
PH QUANT MIT | 5.356 | | IU/ML |
PH QT ESAT-6-NIL | 0.007 | | IU/ML |
PH QT CFP10-NIL | 0.043 | | IU/ML |
PH QT MIT-NIL | 9.036 | | IU/ML |
PH QUANT INTERF | NEGATIVE | | L | NEGATIVE |

*NO ESAT-6 OR CFP-10 RESPONSIVENESS DETECTED.*

*M. tuberculosis INFECTION UNLIKELY, BUT CANNOT BE EXCLUDED ESPECIALY WHEN:*
1. ANY ILLNESS IS CONSISTENT WITH TB DISEASE.
2. LIKELIHOOD OF PROGRESSION TO DISEASE (e.g., DUE TO IMMUNOSUPPRESSION) IS INCREASED.
QuantiFERON-TB Gold

**Table 2. Test Sensitivity and Specificity for CFP-10 and ESAT-6 at Various Cutoffs in Whole-Blood IFN-γ Assay**

<table>
<thead>
<tr>
<th>Cutoff, IFN-γ (IU/ml)</th>
<th>CFP-10</th>
<th>ESAT-6</th>
<th>CFP-10 and/or ESAT-6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity (%)</strong></td>
<td><strong>Sensitivity (%)</strong></td>
<td><strong>Specificity (%)</strong></td>
<td><strong>Sensitivity (%)</strong></td>
</tr>
<tr>
<td>0.05</td>
<td>92.5</td>
<td>81.4</td>
<td>94.8</td>
</tr>
<tr>
<td>0.10</td>
<td>94.4</td>
<td>77.1</td>
<td>96.2</td>
</tr>
<tr>
<td>0.15</td>
<td>95.8</td>
<td>72.9</td>
<td>97.6</td>
</tr>
<tr>
<td>0.20</td>
<td>96.7</td>
<td>71.2</td>
<td>99.1</td>
</tr>
<tr>
<td>0.25</td>
<td>97.2</td>
<td>67.8</td>
<td>99.1</td>
</tr>
<tr>
<td>0.30</td>
<td>97.7</td>
<td>66.9</td>
<td>90.1</td>
</tr>
<tr>
<td>0.35</td>
<td>98.6</td>
<td>65.3</td>
<td>99.5</td>
</tr>
<tr>
<td>0.40</td>
<td>98.6</td>
<td>61.9</td>
<td>99.3</td>
</tr>
<tr>
<td>0.45</td>
<td>98.6</td>
<td>60.2</td>
<td>100.0</td>
</tr>
<tr>
<td>0.50</td>
<td>99.1</td>
<td>60.2</td>
<td>100.0</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 (0.33) is in boldface.

Mori et al. 2004 Am. J. Respir Crit Care Med. 170: 59-64
### T-Spot.TB

#### 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><em>M. tuberculosis</em> 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 <em>M. tuberculosis</em> infection</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 10 spots</td>
<td>≤ 4 spots</td>
<td></td>
<td><em>M. tuberculosis</em> infection unlikely</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&gt; 10 ≤ 10 spots</td>
<td>Any</td>
<td>Any</td>
<td>&lt; 5 spots</td>
</tr>
</tbody>
</table>
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
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. Monk, M.D.,
Margaret J. Villar, M.D.
Division of Tuberculosis Elimination
National Center for HIV, STD, and TB Prevention

2003

2005
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
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.

Periodic Screening (e.g., Healthcare Workers)

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*

---

• **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

Potential sources of variability and their impact on results in IGRAs

<table>
<thead>
<tr>
<th>Source of variability</th>
<th>Impact on assay</th>
<th>QFT</th>
<th>T-SPOT.TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of blood draw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of blood draw</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Storage temperature</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Specimen quality</td>
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</tr>
</tbody>
</table>

* According to the manufacturer, delay after plasma separation contributes to IGRAs variability.

**TABLE 1 Potential sources of variability and their impact on results in IGRAs**

Potential sources of variability and their impact on results in IGRAs

- Analytical sources
- Immunological sources
Movement Toward a Change in IGRA Criteria for ‘Positive’

- 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
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 IGRAs in prioritizing IGRA use in their setting
Pearls for TST vs. IGRAs

• Discordance between the TST and IGRAs 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!

• IGRAs 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?