Interferon-γ Release Assays (IGRAs)

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Lisa Y. 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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I have no conflicts of interests to disclose
Overview

• Development of interferon-gamma release assays (IGRAs)

• FDA-approved IGRAs

• Current recommended use: CDC/ATS/IDSA recommendations

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
- >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 Versus In-vitro Assays


IFN-γ release assays (IGRAs)

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

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

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>Antigens</th>
<th>Environmental strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESAT</td>
<td>CFP</td>
</tr>
<tr>
<td>M tuberculosis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>M africanum</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>M bovis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>BCG substrain</td>
<td></td>
<td></td>
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<tr>
<td>gotthenburg</td>
<td></td>
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<tr>
<td>moreau</td>
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<td>tice</td>
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<td>tokyo</td>
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<td>danish</td>
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<td>glaxo</td>
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<td>montreal</td>
<td></td>
<td></td>
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<tr>
<td>pasteur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M abcessus</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M avrum</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M branderi</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M celatum</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M chelonae</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M fortuitum</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M gordonii</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M intracellulare</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M kansasii</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M malmoense</td>
<td>-</td>
<td></td>
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<tr>
<td>M marinum</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M mcnarvaise</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M scrofulaceum</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M smegmatis</td>
<td>-</td>
<td></td>
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<tr>
<td>M szulgai</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M terrae</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M xenopi</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

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**

- **Mtb**
  - Nil
  - PHA
  - 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**

- **Mtb**
  - Nil
  - PHA
  - 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*
QFT-GIT calculation.....

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

Saline  ESAT-6  CFP-10  PHA

IFN-γ Antibody
Sensitized T cell
IFN-γ Captured
Detection Antibody
Chromogen Spot

****
What Result is Considered Positive?

- Depends on the test
- Based on calculation of IFN-\(\gamma\) response to TB antigens relative to IFN-\(\gamma\) response to nil
- Unlike TST, not risk stratified (i.e., there are not multiple cutoffs for different risk groups)
- Still somewhat complicated
  - Software 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>
### Test Results

**Run Date:** 09/06/05  
**Run Time:** 1414  
**Public Health Laboratory of East Texas  
11937 U.S. Highway 271  
Tyler, TX 75708  
Dr. David Lakey

**Patient:** PATIENT.TEST QTB3  
**Act #:** L000000000234

**Order Doc:** PUBLIC HEALTH LABORATORY, EAST  
**Specimen:** 0903:FL00003R  
**Comp Collected:** 09/03/05-1240  
**Received:** 09/06/05-1353  

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Flag</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH QUANT TUB</td>
<td>0.160</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT ESAT-6</td>
<td>0.164</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT CFP-10</td>
<td>0.293</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT MIT</td>
<td>9.256</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT ESAT-6-NIL</td>
<td>0.397</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT CFP-10-NIL</td>
<td>0.343</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT MIT-NIL</td>
<td>9.096</td>
<td>IU/ML</td>
<td></td>
</tr>
</tbody>
</table>

**PH QUANT INTERP:** NEGATIVE

- **NO ESAT-6 OR CFP-10 RESPONSIVENESS DETECTED.**
- **M. TUBERCULOSIS INFECTION UNLIKELY, BUT CANNOT BE EXCLUDED ESPECIALLY WHEN:**
  - 1. ANY ILLNESS IS CONSISTENT WITH TB DISEASE.
  - 2. LIKELIHOOD OF PROGRESSION TO DISEASE (E.g., DUE TO IMMUNOSUPPRESSION) IS INCREASED.

---

### Test Results

**Run Date:** 09/06/05  
**Run Time:** 1618  
**Public Health Laboratory of East Texas  
11937 U.S. Highway 271  
Tyler, TX 75708  
Dr. David Lakey

**Patient:** PATIENT.TEST QTB1  
**Act #:** L00000006016  

**Order Doc:** PUBLIC HEALTH LABORATORY, EAST  
**Specimen:** 0903:FL66601R  
**Comp Collected:** 09/03/05-1280  
**Received:** 09/06/05-1352

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Flag</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH QUANT TUB</td>
<td>6.122</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT ESAT-6</td>
<td>2.687</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT CFP-10</td>
<td>2.762</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QUANT MIT</td>
<td>32.662</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT ESAT-6-NIL</td>
<td>2.565</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT CFP-10-NIL</td>
<td>2.680</td>
<td>IU/ML</td>
<td></td>
</tr>
<tr>
<td>PH QT MIT-NIL</td>
<td>32.539</td>
<td>IU/ML</td>
<td></td>
</tr>
</tbody>
</table>

**PH QUANT INTERP:** POSITIVE

- **ESAT-6 AND/OR CFP-10 RESPONSIVENESS DETECTED.**
- **M. TUBERCULOSIS INFECTION LIKELY.**

**NOTE:** QUANTIFERON TB GOLD IS AN INDIRECT TEST FOR
QuantiFERON-TB Gold

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</td>
<td>≥ 8 spots</td>
<td>Any</td>
<td>M. tuberculosis infection likely</td>
</tr>
<tr>
<td>Borderline</td>
<td>≤ 10</td>
<td>5, 6, or 7</td>
<td>Any</td>
<td>Uncertain likelihood of M. tuberculosis infection</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 10</td>
<td>≤ 4 spots</td>
<td></td>
<td>M Tb infection unlikely</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&gt; 10</td>
<td>Any</td>
<td>Any</td>
<td>Uncertain likelihood of M. tuberculosis infection</td>
</tr>
</tbody>
</table>

*Nil* indicates no response.

Mitogen++ indicates any mitogen was used.

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.
Interpretation of Results

Nil Control

ESAT-6 Panel A

CFP 10 Panel B

Negative Result

Positive Control

Positive Result

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

Reproducibility and Variability

- **At least 4 sources of variability**
  - Type of measurement (ELISA or ELISPOT)
  - Reproducibility of complex biological reaction
  - Natural variability of immune responses
  - Variability introduced during test performance or manufacturing

- **QFT 11% variance overall**
  - 8% between first/second testing of same specimen

- **T-spot variance**
  - 4% (with robust response)
  - 22% (near the cut point)
Boosting and Special Considerations

- Boosting by prior TST has been observed in as little as 3 days post-TST and may wane over several months.

- If both tests are to be used, do the IGRA first.

- Because the IGRA is a functional assessment of viable lymphocytes, special attention should be paid to technical aspects of the test (how blood is drawn/stored, etc.)

(New) ATS/CDC/IDSA Guidelines
Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children

Lewinsohn et al. CID. 2016
Recommendations

• **Question 1:**
  Should an IGRA or a TST be performed in individuals 5 years or older who are likely to be infected with *Mtb*, who have a low or intermediate risk of disease progression, and in whom it has been decided that testing for LTBI is warranted?

• **Recommendation 1a:**
  We recommend performing an IGRA rather than a TST in individuals 5 years or older who meet the following criteria:
  1. are likely to be infected with *Mtb*,
  2. have a low or intermediate risk of disease progression,
  3. it has been decided that testing for LTBI is warranted, and
  4. either have a history of BCG vaccination or are unlikely to return to have their TST read (strong recommendation, moderate-quality evidence).
Recommendations

• Question 1:
  Should an IGRA or a TST be performed in individuals 5 years or older who are likely to be infected with *Mtb*, who have a low or intermediate risk of disease progression, and in whom it has been decided that testing for LTBI is warranted?

• Recommendation 1b:
  We recommend performing an IGRA rather than a TST in individuals 5 years or older who meet the following criteria:
  (1) are likely to be infected with *Mtb*,
  (2) have a low or intermediate risk of disease progression,
  (3) it has been decided that testing for LTBI is warranted, and
  (4) either have a history of BCG vaccination or are unlikely to return to have their TST read (conditional recommendation, moderate-quality evidence).

Recommendations

• Question 2:
  Should an IGRA or a TST be performed in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been decided that testing for LTBI is warranted?

• Recommendation 2:
  There are insufficient data to recommend a preference for either a TST or an IGRA as the first-line diagnostic test in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been determined that diagnostic testing for LTBI is warranted.
Recommendations

• **Question 2:**
  Should an IGRA or a TST be performed in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been decided that testing for LTBI is warranted?

• **Recommendation 2:**
  There are insufficient data to recommend a preference for either a TST or an IGRA as the first-line diagnostic test in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been determined that diagnostic testing for LTBI is warranted.
Recommendations

• Question 3:
  Should an IGRA or a TST be performed in individuals 5 years or older who are unlikely to be infected with *Mtb*, but in whom it has been decided that testing for LTBI is warranted?

• Recommendation 3a:
  We suggest performing an IGRA instead of a TST (conditional recommendation, low-quality evidence).

• Recommendation 3b:
  We suggest a second diagnostic test if the initial test is positive (conditional recommendation, very low-quality evidence).

Remarks: The confirmatory test may be either an IGRA or a TST. When such testing is performed, the person is considered infected only if both tests are positive.
Recommendations

• Question 4:
   Should an IGRA or a TST be performed in healthy children <5 years of age in whom it has been decided that testing for LTBI is warranted?

• Recommendation:
   We suggest performing a TST rather than an IGRA in healthy children <5 years of age for whom it has been decided that diagnostic testing for LTBI is warranted
   (conditional recommendation, very low-quality evidence).

‘Remarks’ Regarding Preference

• A TST is an acceptable alternative in settings where an IGRA is unavailable, too costly, or too burdensome.

• Note that this statement is repeated after every recommendation of the IGRA over the TST
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

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!

- The tests are not perfect. They provide one piece of your whole picture when assessing a patient, not the ‘answer’.

- No test (TST or IGRA) overrides clinical, epidemiologic or historical data
Questions?

I just want a test that gives me the answer.

- TB doc in Texas