TB Intensive
Tyler, Texas
June 2-4, 2010
IGRA: Diagnosing TB in the Twenty-First Century with Case Studies
Peter Barnes, MD
June 4, 2010

Interferon Gamma Releasing Assays: Diagnosing TB in the Twenty-First Century
Peter Barnes, MD
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TOPICS

- Use of interferon-gamma release assays (IGRAs)
- Diagnosis of latent tuberculosis infection (LTBI)
- Diagnosis of active tuberculosis

LATENT TUBERCULOSIS INFECTION

- Infection without disease
- The source of most TB cases in the United States
- Accurate identification of LTBI is critical for targeted therapy to prevent TB
- Diagnosis of LTBI depends on the tuberculin skin test
TUBERCULIN SKIN TEST

- Injection of tuberculin, which contains hundreds of antigens for T-cells
- Requires two visits
- Requires skilled personnel for placement and interpretation

TUBERCULIN SKIN TEST

- Inter-observer variability
- Exposure to BCG or to nontuberculous mycobacteria can sensitize T-cells to PPD because of shared antigens
- Complex guidelines to define LTBI
SKIN TESTING LEAST RELIABLE IN HIGH-RISK GROUPS

- Homeless, drug users less likely to return for reading
- False-positive tests in BCG-vaccinated foreign-born
- False-negative tests in immunocompromised patients

BLOOD TESTS FOR LTBI

- Quantiferon TB-GOLD (Cellestis)
- Quantiferon TB-Gold IT (Cellestis)
- T-SPOT TB (Oxford Immunotec)
**M. TUBERCULOSIS-SPECIFIC ANTIGENS**

- Sequencing the TB genome has revealed two antigens, ESAT-6 (early secreted antigenic target 6 kD protein) and CFP10 (culture filtrate protein 10)
  - Not present in BCG
  - Not present in most environmental mycobacteria (except *M. kansasii*, *M. szulgai*, *M. marinum*)

**PRINCIPLES**

- Memory T-cells from persons with LTBI expand and produce IFN-gamma in response to ESAT-6 and CFP10
- Detects IFN-gamma produced by *M. tuberculosis*-specific memory T-cells
QuantiFERON-TB Gold (ELISA)

Obtain blood

Transfer blood to wells and add antigens

Culture overnight. TB-infected individuals secrete IFN-γ

Harvest supernatants and perform ELISA

Wash, add substrate, incubate 30 min

Measure OD and determine IFN-γ levels

QuantiFERON-TB Gold In Tube

Stage One – Blood Incubation and Harvesting

IFN-γ stable refrigerated for at least 8 weeks.


2. Centrifuge tubes for 5 minutes.

Stage Two – Human IFN-γ ELISA

3. Add plasma and conjugate to ELISA plate. Incubate for 120 mins.

4. Wash and add substrate. Read absorbance after 30 min.
ADVANTAGES OF QFT-GOLD-IT

- T-cells rapidly exposed to antigen
- Additional antigen (TB 7.7)
- May be more sensitive than QFT-GOLD test
- More convenient for laboratory

INTERPRETATION OF QFN-TB GOLD TEST

- Nil well < 0.7 IU
- Mitogen well > 0.5 IU
- ESAT-6 or CFP10 minus Nil well > 0.35 IU
### Test Results

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Flag</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM Quant TB</td>
<td>0.160</td>
<td>5</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>PM Quant NIL</td>
<td>0.164</td>
<td>5</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>PM Quant 2R-4</td>
<td>0.164</td>
<td>5</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>PM Quant CF9-10</td>
<td>0.203</td>
<td>5</td>
<td>NEGATIVE</td>
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<tr>
<td>PM CF9-10</td>
<td>0.256</td>
<td>5</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>PM QT 2R75-95</td>
<td>0.197</td>
<td>5</td>
<td>NEGATIVE</td>
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<tr>
<td>PM QT 95-95</td>
<td>0.193</td>
<td>5</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>PM QT 5-5</td>
<td>0.194</td>
<td>5</td>
<td>NEGATIVE</td>
</tr>
</tbody>
</table>

### Notes:
- **PM Quant TB** is less than or equal to 0.150, indicating no evidence of TB disease.
- **PM Quant NIL** is less than or equal to 0.200, indicating no evidence of TB disease.
- **PM Quant 2R-4** is less than or equal to 0.200, indicating no evidence of TB disease.
- **PM Quant CF9-10** is less than or equal to 0.200, indicating no evidence of TB disease.
- **PM CF9-10** is less than or equal to 0.200, indicating no evidence of TB disease.
- **PM QT 2R75-95** is less than or equal to 0.200, indicating no evidence of TB disease.
- **PM QT 95-95** is less than or equal to 0.200, indicating no evidence of TB disease.
- **PM QT 5-5** is less than or equal to 0.200, indicating no evidence of TB disease.

### Additional Notes:
- PM Quant TB is lower than 0.150, indicating no evidence of TB disease.
- PM Quant NIL is lower than 0.200, indicating no evidence of TB disease.
- PM Quant 2R-4 is lower than 0.200, indicating no evidence of TB disease.
- PM Quant CF9-10 is lower than 0.200, indicating no evidence of TB disease.
- PM CF9-10 is lower than 0.200, indicating no evidence of TB disease.
- PM QT 2R75-95 is lower than 0.200, indicating no evidence of TB disease.
- PM QT 95-95 is lower than 0.200, indicating no evidence of TB disease.
- PM QT 5-5 is lower than 0.200, indicating no evidence of TB disease.

### Reference:
**T-SPOT TB (ELISPOT)**

Obtain blood, isolate cells, place in wells containing no antigen, ESAT-6, CFP10 or mitogen control

Add cells to wells coated with anti-IFN-gamma antibodies

IFN-gamma binds to antibodies

Each spot represents one IFN-gamma-producing cell

Add second antibody and substrate, which gives color change

**INTERPRETATION OF T-SPOT TB TEST**

- Six or more spots in the ESAT-6 or CFP10 well, compared to the negative control well
- Indeterminate if mitogen well shows <20 spots and ESAT-6 and CFP10 wells are negative
T-SPOT TB TEST
SAMPLE RESULT

- Negative control
- ESAT-6
- CFP10
- Mitogen

QUANTIFERON-TB GOLD VERSUS T-SPOT

- Quantiferon measures production of IFN-gamma to TB-specific antigens
- T-spot measures the number of IFN-gamma-producing cells
- Quantiferon is logistically simpler for lab
- T-spot may be more sensitive
INTERPRETING IGRA RESULTS

• Results should not be reported as positive or negative

• Always obtain values (IU for Quantiferon, number of spots for T-spot)

• Values near the cut-off are most subject to change and may be false-positive or false-negative

INDETERMINATE IGRA RESULTS

Pattern of results can provide useful information

1) Poor response to mitogen that resolves with repeat assay
   - Delayed specimen processing
   - Technical errors

2) Persistent poor response to mitogen
   - Anergy from immunosuppression
   - May occur in healthy persons

3) High background
   - Often persistent, reasons unclear
   - IGRA not useful
INDETERMINATE IGRA RESULTS

- More common in immunocompromised (HIV infection, cancer, extremes of age)
- QFT-Gold more often indeterminate than T-SPOT in immunocompromised
- T-SPOT corrects for reduced T-cells by placing defined number of cells per well

IGRAS

- Require one visit
- Less operator-dependent
- More objective, laboratory-based
- No boosting by skin test
- More specific than testing with PPD
T-cells Exposed to Antigen

- Effectors
- Effector Memory (rapid responders)
- Central Memory (slow responders)

Detected by IGRA

Detected by TST

**IMMUNE RESPONSE TO M. TUBERCULOSIS ANTIGENS**

1. In the absence of antigen, effector memory cells decrease in number, whereas central memory cells do not. Therefore, the TST may detect remote exposure better.

2. Exposure to NTM may skew immune response toward cross-reactive antigens in TB. This can result in a positive TST and negative IGRA.
DIAGNOSTIC USES OF IGRAS

- Diagnosis of LTBI
- Diagnosis of active tuberculosis

DIAGNOSIS OF LTBI

- IGRAs more specific in BCG-vaccinated
- IGRA results correlate better with exposure than TST results
- Comparative sensitivity unclear
- Some TST+ persons with LTBI are IGRA-, perhaps because the number of effector memory cells decline with time after infection
DIAGNOSIS OF TUBERCULOSIS

- QFT 64-89% sensitive
- T-SPOT 83-96% sensitive
- T-SPOT may be more sensitive, especially in immunocompromised
- Only helpful if prevalence of LTBI is low
- Has not been compared with nucleic acid amplification tests

CHILDREN

- High susceptibility to tuberculosis
- Increased frequency of false-negative TSTs, especially in those <5 years old
- Tuberculosis more difficult to diagnose than in adults
- Treatment of LTBI is a high priority
IGRAS IN CHILDREN

- In children with TB, 65-100% IGRA+, 60-80% TST+
- T-SPOT more sensitive in HIV+, malnourished, those <2 years old
- IGRAs do not separate TB and LTBI
- T-SPOT may be more sensitive than TST in infants with LTBI

IMMUNOSUPPRESSED PATIENTS

- TST has low sensitivity
- IGRAs are probably more sensitive than TST for tuberculosis and LTBI
- IGRAs may be useful to screen patients prior to immunosuppressive therapy
CHOOSING BETWEEN IGRAS

- All are FDA-approved
- Comparable sensitivity and specificity in most situations
- QFT-Gold logistically simpler than T-SPOT
- T-SPOT probably more sensitive in children and immunocompromised
- Medicare reimbursement for IGRAs is approximately $80

SHOULD IGRAS BE USED NOW?

- Specificity better, and sensitivity equal or better than TST
- Logistically simpler than TST
- Billed cost of IGRAs greater than TST, but personnel costs, cost of errors reduced
- I would favor widespread usage for diagnosis of LTBI but not TB
CDC GUIDELINES

- QFT- Gold IT may be substituted for the TST for most purposes. Presumably this is also true for T-SPOT TB.
- Caution in children and immunocompromised

EFFECT OF TST ON IGRA RESULTS

- Some studies show that TST can "boost" IGRA results, and convert negative tests to positive ones
- Boosting is more likely in those with positive TST and IGRA results
- Results around the cut-off values are most likely to change from negative to positive
- The effect of the TST is not seen after 3 days but is seen after 7 days
IMPLICATIONS FOR PERFORMANCE OF IGRAS

• If TST and IGRA are planned, perform IGRA when TST is placed or read, but not more than 3 days after TST placement
• Always obtain the actual value of the IGRA (IU for QFN-TB Gold, number of spots for T-SPOT), not just a positive or negative result

USING IGRAS

• If cost is not a major issue, I favor using IGRAs to diagnose LTBI in most settings, and using both IGRAs and the TST in severely immunocompromised
USING IGRAS

- Immunocompromised persons
  - IGRA for LTBI in less immunocompromised
  - IGRA and TST for LTBI in severely immunocompromised
- Pre-immunosuppression
  - IGRA and TST for LTBI

USING IGRAS

- Immunocompetent persons ≥ 5 years
  - Use IGRA instead of TST to detect LTBI
  - IGRA is an adjunct for diagnosis of TB
- Children < 5 years old
  - Use both IGRA and TST for diagnosis of LTBI and tuberculosis
**USING IGRAS**

- If cost prohibits use of IGRAs in most persons, reserve for
  - BCG-vaccinated persons
  - severely immunocompromised
  - prior to immunosuppressive therapy

**SUMMARY**

- TST has problems with logistics, specificity and complexity of interpretation
- IGRAs utilize *M. tuberculosis*-specific antigens and provide logistic advantages over TST
- IGRAs detect effector memory cells that produce IFN-gamma
SUMMARY

- Quantiferon-TB Gold/IT detects IFN-gamma by ELISA
- T-SPOT TB detects IFN-gamma-producing cells
- Indeterminate IGRAs can provide useful information
- IGRAs not very useful for diagnosis of TB

DIAGNOSIS OF LTBI

- IGRAs more specific than TST in BCG-vaccinated
- IGRAs may be more sensitive than TST for recent infection, but may be less sensitive for remote infection
- All IGRAs FDA-approved
DIAGNOSIS OF LTBI

• QFT-Gold/IT easier for laboratories
• T-SPOT may be more sensitive in children and immunocompromised
• IGRAs can be used instead of TST in most situations
• Consider both IGRA and TST in severely immunocompromised and children < 5 years old

SUMMARY

• If cost prevents universal IGRA use, reserve for BCG-vaccinated, severely immunocompromised and those about to receive immunosuppression
• The TST can affect the IGRA result. If performing both tests, do IGRA ≤3 days after placement of TST
• Obtain absolute IGRA values, as those near the cutoff may be false-positive or false-negative