Interferon Gamma Release Assays for the Laboratory Detection of *Mycobacterium tuberculosis* Infection

Dave Warshauer, Ph.D., D(ABMM)
Deputy Director
Communicable Disease Division
WSLH
Phone (608) 265-9115
E-mail warshadm@slh.wisc.edu

Objectives

- Describe the FDA approved interferon gamma release assays (IGRAs) for the laboratory detection of *Mycobacterium tuberculosis* infection.
- Describe the CDC recommendations for the use of IGRAs.
- Describe potential problems with IGRAs and future areas for research.

Historical Perspective of LTBI

- 1890 Robert Koch produced "tuberculin"
  - Broth culture filtrate that he thought might cure TB
  - Observed a local reaction at site of inoculation in a TB patient, but no such reaction in non-TB patients
  - Foundation for use of tuberculin for TB diagnosis
- 1908 Charles Mantoux described intradermal injection with a controlled dose of Koch's tuberculin
- 1934 PPD (purified protein derivative) of "old tuberculin" was developed using a precipitate of filtrates from heat treated cultures of *M. tb*.
  - Neither pure nor specific for *M. tb*
- 2001 First FDA approved IGRA

Mantoux Test

- Based on
  - Measurement of the induration (mm)
  - Risk of being infected with TB and/or progression to disease if infected

TST Interpretation

- Based on
  - Measurement of the induration (mm)
  - Risk of being infected with TB and/or progression to disease if infected
Cut points for interpretation

<table>
<thead>
<tr>
<th>&gt;=5 mm positive in</th>
<th>&gt;=10 mm in</th>
<th>&gt;=15 mm in</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive pts</td>
<td>Recent immigrants</td>
<td>Persons with no known risk factors for TB</td>
</tr>
<tr>
<td>Recent contacts of TB case patients</td>
<td>Injection drug users</td>
<td>Persons at low risk for TB who are tested at start of employment</td>
</tr>
<tr>
<td>Organ transplants and other immunosuppressed</td>
<td>Residents of high-risk congregate settings: prisons, LTCE, etc.</td>
<td>Mycobacteriology lab personnel</td>
</tr>
<tr>
<td></td>
<td>Children &lt;4 yrs</td>
<td></td>
</tr>
</tbody>
</table>

TST Shortcomings

False-Positive Reactions
- Infections with NTMs
- BCG Vaccination
- Incorrect administration
- Incorrect interpretation
- Incorrect antigen used

False-Negative Reactions
- Cutaneous anergy
- Recent TB infection (within 8-10 weeks)
- Very old TB infection
- Very young age (<6mo)
- Recent live-virus vaccination
- Recent viral illness
- Overwhelming TB disease
- Incorrect administration
- Incorrect interpretation

Next Generation

- 2001 QuantiFERON-TB (QFT)
  - The first blood based test for detection of LTBI
  - First generation test using PPD and an M. avian antigen
- 2005 QFT-TB Gold
- 2007 QFT-GIT
- 2008 T-Spot.TB

Interferon Gamma Release Assays (IGRAs)

- Blood tests for detecting M. tuberculosis infection
  - Measure cell mediated immunity to M. tb
  - Sensitized white blood cells release IFN-gamma in response to contact with TB antigens
    - ESAT-6
    - CFP 10
    - TB7.7 (QFT-GIT)
  - Do not differentiate latent infection from active disease

No Cross-reactivity to BCG and Most NTMs

<table>
<thead>
<tr>
<th>Complex</th>
<th>Tuberculosis</th>
<th>Antigens</th>
<th>Status</th>
<th>Environmental</th>
<th>Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESAT-6</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>CFP 10</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>TB7.7</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>VFA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VFA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>VFA</td>
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<td>VFA</td>
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<tr>
<td>VFA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
T-SPOT. TB Test Kit

- 96-well format
  - Twelve, 8-well strips
  - 4 wells used per patient; 24 patients per kit
  - Positive and Negative control for each patient test
- Utilizes standard blood collection tubes

Performing the T-SPOT. TB Test

- Peripheral blood mononuclear cells (PBMCs) are separated from whole blood and washed
- Removes any source of background interference
- Washed PBMCs are counted to ensure a standardized number of cells are added to the assay
- Blood must be processed within 8 hours of collection
- Xtend® reagent increases time to 32 hours

The Science behind T-SPOT™

Collect white cells using BD CPT tube or Ficoll extraction

Interpretation of Results

TABLE 3. Interpretation criteria for the T-SPOT.TB Test (T Spot)

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Nil*</th>
<th>TH Response†</th>
<th>Mitogen‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive§</td>
<td>≥10 spots</td>
<td>5 spots</td>
<td>Any</td>
</tr>
<tr>
<td>Baseline**</td>
<td>≤5 spots</td>
<td>5, 6, or 2 spots</td>
<td>Any</td>
</tr>
<tr>
<td>Negative††</td>
<td>&lt;2 spots</td>
<td>&lt;2 spots</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Panel A

Nil Control

Positive Control

ESAT-4

CFP 10

Panel B

Negative

Positive

QuantiFERON®-TB Gold In-Tube

Stage 1 – Blood Stimulation and Harvesting

- QFT Collection tubes
- After blood collection, QuantiFERON® blood tubes (nil, TB Ag, PHA) mix thoroughly by shaking.
Stage 1 – Blood Stimulation and Harvesting

- As soon as possible, and within 16 hours of collection, incubate tubes upright at 37°C for 16-24 hours.
- After incubation can hold blood tubes up to 3 days at 2-27°C
  – If separate plasma, up to 28 days at 2-8°C

Stage 1 – Blood Stimulation and Harvesting

- Centrifuge tubes at 2000 – 3000 g (RCF) for 15 minutes to separate plasma.

Stage 2 – Human IFN-γ ELISA

- Add conjugate to each well, then add plasma or standards.
- Shake plate and incubate for 120 minutes at room temperature.
  * Can be automated

Stage 2 – Human IFN-γ ELISA

- Wash plate 6 times. Add substrate.
- Incubate for 30 minutes at room temperature.
  * Can be automated
Stage 2 – Human IFN-γ ELISA

- Calculate results using QuantiFERON® Analysis Software.
- >0.35 IU gamma interferon considered positive

Performance Characteristics
No good Gold Standard for LTBI

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity*</th>
<th>Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantiFERON Gold in-Tube</td>
<td>70-84%</td>
<td>96% (BCG Vac) 99% (non-BCG)</td>
</tr>
<tr>
<td>T-SPOT.TB</td>
<td>88-90%</td>
<td>86-93%</td>
</tr>
<tr>
<td>TST</td>
<td>70-77%</td>
<td>59% (BCG Vac included) 97% (BCG Vac excluded)</td>
</tr>
</tbody>
</table>

*Compared to active TB

CDC Guidelines for the use of IGRAs

- MMWR June 25, 2010 59:RR-5
- TSTs and IGRAs should be used as aids in diagnosing infection with M. tb
  - Surveillance purposes
  - To identify persons likely to benefit from treatment
- As with TST, IGRAs should not be used for testing person who have a low risk for both infection and progression to active TB if infected
- An IGRA may be used in place of (but not in addition to) a TST in all situations in which CDC recommends TST --- with preferences and special considerations

CDC Guidelines (Cont.)

- IGRAs FDA-Approved as in vitro diagnostic aids for detection of Mtb infection
  - Including active disease and LTBI
  - Used in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations

CDC Guidelines (Cont.)

- QFT-GIT, T-Spot and TST results may not be interchangeable
  - Measure different aspects of the immune response
  - Use different antigens
  - Use different interpretation criteria
- Therefore, different tests can yield different results!
**CDC Guidelines (Cont.)**

- **Situations in which IGRA preferred**
  - For persons who have received BCG
  - IGRA preferred for persons/groups that have low rates of returning to have TST read.

- **Situations in which TST preferred**
  - For children <5 years
  - IGRA in conjunction with TST advocated by some experts

**CDC Guidelines (Cont.)**

- **Situations in which testing with both TST and IGRA may be considered**
  - When risk for infection, progression, and poor outcome is increased
  - When clinical suspicion exists for active TB and confirmation is desired.
    - Positive second test increases detection sensitivity, but multiple negative results cannot exclude TB infection
  - When initial IGRA is indeterminate, borderline or invalid, repeat IGRA or perform TST

**CDC Reporting Recommendations**

- Qualitative test interpretation
  - Positive
  - Negative
  - Borderline (T-Spot.TB)
  - Indeterminate

- Quantitative assay measurements
  - IU/ml
  - Number of spots

**Serial Testing of HCWs**

- **Cleveland Clinic Study**
  - HCWs who underwent preemployment QFT-GIT testing 2007-2010
  - 7,374 IGRAIs performed
  - 486 (6.6%) positive at baseline
  - 305 (4.1%) indeterminate
  - 6583 (89.3%) negative
    - 2.8% (52/1,857) identified as converters
      - 71% with values ≤1 IU/ml
      - None with active TB or part of outbreak investigation
    - Previous annual conversion rate of 0.09% using TST

- Quantitative interferon-γ release assay results to T-cell assay converters (n = 52):
Cleveland Protocol

- If new converter
  - Repeat IGRA, chest x-ray, ID evaluation
  - If no identifiable risk factors, consider treatment if >1 IU/ml
    - 15/52 would have been considered converters (0.8% vs 2.8%)
    - May reduce unnecessary treatment

Study Conclusions

- Conversions/reversions tend to occur around the cut-off
  - Did see reversions in HCWs with values >3 IU/ml
- Studies needed to establish new cutoffs for conversions in serial testing of HCWs

Effects of Incubation Delays on QFT-GIT Results

  - Compared results when blood incubated immediately after collection to results after 6- and 12-hour delays
  - 128 HCWs from Stanford Hosp and Clin
Positive-to-Negative Reversions

- 19% (5/26) with 6-hr delay
- 22% (5/23) with 12-hr delay
- Underscore importance of preanalytical practices

Effect of Delayed Incubation on QGT-GIT
Whitworth, WC, et al. Presented at 8th National Conference on Laboratory Aspects of Tuberculosis, Atlanta, June 2012

- <1hr delay to incubation
  - 25% (37/148) positive

- 11-12 hr delay to incubation
  - 20.9% (31/148) positive
  - p = .03

Effect of Incubation Duration on QFT-GIT
Whitworth, WC, et al. Presented at 8th National Conference on Laboratory Aspects of Tuberculosis, Atlanta, June 2012

- 23-24 hr incubation
  - 25.8% (39/151) positive

- 16-17 hr incubation
  - 23.2% (35/151) positive
  - p = 0.08

Effect of Incubation Temperature on QFT-IT
Whitworth, WC, et al. Presented at 8th National Conference on Laboratory Aspects of Tuberculosis, Atlanta, June 2012

- Incubation at 37C
  - 20.6% (21/102) positive
- Incubation at 35C
  - 20.6% (21/102) positive
- No impact on result interpretation, but significant differences in the qualitative IGRA results

Wrap up
Advantages of IGRAs

- Require a single patient visit
- Not subject to reader bias
- Use defined TB antigens
- Not affected by prior BCG vaccination
- Controlled laboratory based test
- Objective result
- No possibility of adverse reactions in hypersensitive individuals
- Do not boost responses upon subsequent testing

Disadvantages of IGRAs

- Blood must be processed within 8-32 hours after collection
- Possibility of errors in collection or transport of samples
- Lab variability and errors in performance or interpretation of the test
- Limited data on use in certain populations
  - Immunocompromised
  - Patients on immunosuppressive drugs
  - Patients with hematological disorders, diabetes, malignancies
- Limited data on the use of IGRAs to determine risk for developing TB disease

Logistical and Economic Laboratory Issues

- Processing of blood within required time frame
- Test verification more difficult
- Need sufficient test volume to make economically feasible
- Transfer of cost from employee health or TB Program to the laboratory

Outstanding Issues

- Reproducibility in the “real world”
  - Impact of time from blood draw to incubation
  - Impact of incubation time
  - Impact of time of day blood drawn
  - Impact of how collection tubes handled
  - Impact of technical variations on the test performance
- Serial testing of HCWs
  - The “wobble” phenomenon
  - Do we need a gray zone and/or different cutoff values?
- Which test is the best predictor of progression to active TB?

Thank You!

Questions are guaranteed in life; Answers aren’t.