Latent TB Infection; Tuberculin Skin Testing (TST) & Interferon-γ Release Assays (IGRA)

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Chicago Tuberculosis Intensive, April 2015
Disclosures

• None
Diagnosing Latent TB Infection (LTBI)

**Multiple components:**

- **TST or IGRA**
  - Sensitivity and specificity *not perfect*

- **Patient history**
  - Need to ask about TB symptoms, contacts, co-morbidities

- **Chest X-ray**
  - Need to look at film (don’t rely on radiologist report)

- **Physical examination**
  - Lymph nodes, abdominal masses, etc
  - Exam findings may be quite subtle
Background Principle of testing: “Targeted TB Testing”

- A TB control strategy that is used to identify, evaluate, and treat persons who are:
  - At high risk for having latent tuberculosis infection (LTBI) or -
  - At high risk for developing active TB disease once infected with *M. tuberculosis*.

- All testing activities should be accompanied by a plan for appropriate follow-up medical evaluation and treatment.
Background Principle of testing:
“Targeted TB Testing”

• **Basic principle:** Plan of care made *before* TST/IGRA testing performed:
  • “A decision to test with TST or IGRA is a decision to treat”
  • or *at least* “strongly consider” treatment

• Undifferentiated population screening not recommended

MMWR June 09, 2000 / 49(RR06);1-54
Who to test for TB infection?
Patients at Increased Risk for acquiring TB infection

- Close contacts of persons known or suspected to have active tuberculosis
- Foreign-born persons from areas that have a high incidence of active tuberculosis
- Visitors of TB endemic countries, especially if visits are frequent or prolonged
- Residents and employees of congregate settings whose clients are at increased risk for active tuberculosis (e.g., correctional facilities, long-term care facilities, and homeless shelters)
- Health-care workers who serve patients who are at increased risk for active tuberculosis
- Populations defined locally as having an increased incidence of latent *M. tuberculosis* infection or active tuberculosis, possibly including medically underserved, low-income populations, or persons who abuse drugs or alcohol
- Infants, children, and adolescents exposed to adults who are at increased risk for latent *M. tuberculosis* infection or active tuberculosis

MMWR 2000;49(No. RR-6)
Patients at Increased Risk of Progression from LTBI to Active TB disease

**Persons at Increased Risk**

- Persons infected with HIV;
- Children younger than 5 years of age;
- Persons who were recently infected with *M. tuberculosis* (within the past 2 years);
- Persons with a history of untreated or inadequately treated TB disease, including persons with fibrotic changes on chest radiograph consistent with prior TB disease;
- Persons who are receiving immunosuppressive therapy such as tumor necrosis factor-alpha (TNF) antagonists, systemic corticosteroids equivalent to/greater than 15 mg of prednisone per day, or immunosuppressive drug therapy following organ transplantation;
- Persons with silicosis, diabetes mellitus, chronic renal failure, leukemia, or cancer of the head, neck, or lung;
- Persons who have had a gastrectomy or jejunoileal bypass;
- Persons who weigh less than 90% of their ideal body weight;
- Cigarette smokers and persons who abuse drugs and/or alcohol; and
- Populations defined locally as having an increased incidence of disease due to *M. tuberculosis*, including medically underserved, low-income populations.

CDC 2013: Core Curriculum on Tuberculosis
Risk of Developing TB disease

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Risk of Developing TB</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB infection and no risk factors</td>
<td></td>
<td>For people with TB infection, <strong>no risk factors</strong>, and no treatment, the risk is about 5% in the first 2 years after infection and about 10% over a lifetime.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>About 10% over a lifetime</td>
</tr>
<tr>
<td>TB infection and diabetes</td>
<td></td>
<td>For people with TB infection and <strong>diabetes</strong>, and with no treatment, the risk is three times as high, or about 30% over a lifetime.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>About 30% over a lifetime</td>
</tr>
<tr>
<td>TB infection and HIV infection</td>
<td></td>
<td>For people with TB infection and <strong>untreated HIV infection</strong> and with no LTBI treatment, the risk is about 7% to 10% PER YEAR, a very high risk over a lifetime.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>About 7% to 10% PER YEAR</td>
</tr>
</tbody>
</table>

CDC 2013: Core Curriculum on Tuberculosis
Mantoux Tuberculosis Skin Test

- Tubersol® and Aplisol® are the two commercially available tuberculin products.

- The tuberculin is administered using a single-dose disposable tuberculin syringe with a 27-gauge needle.

- In the US, the Mantoux tuberculin skin test consists of a 0.1 ml intradermal injection containing 5 tuberculin units of purified protein derivative (PPD) solution.
  - TST reading should be conducted within 48 to 72 hours of administration.
Purified-protein derivative (PPD) tuberculin products

- **Tubersol** – by Sanofi Pasteur Limited
  - 2013 tubersol shortage

- **Aplisol** – by JHP Pharmaceuticals, LLC
  - Limited supplies

- Comparative performances
  - Near equal in sensitivity and specificity
  - ? Of slightly lower specificity possible with Aplisol product – unclear.
    - CDC recommendations to PH programs to use consistently one product

In Vivo and In Vitro Diagnostic Tests

Presentation of mycobacterial antigens

Antigen presenting cell

Memory T-cell

IFN-γ

TNF-α

IL-8, etc.

Mantoux Tuberculosis Skin Test

Remember:
Don’t “read what’s red” -
Rather “feel what’s real”
TST interpretation (Review)

≥ 5 mm induration = “Positive” result

Highest risk patients for progression to active TB

- Human immunodeficiency virus (HIV)-positive persons
- Recent contacts of TB case patients
- Persons with fibrotic changes on chest radiograph consistent with prior TB
- Patients with organ transplants and other immunosuppressive conditions
  - e.g. Patients receiving ≥ 15 mg/d of prednisone for ≥ 1 month
  - Risk of TB in patients with corticosteroids increases with higher dose and longer duration.)
TST interpretation (Review)

\[ \geq 10 \text{ mm induration} = \text{“Positive” result} \]

- Recent immigrants (i.e., within past 5 years) from TB endemic countries
- Injection drug users
- Residents and employees of select congregate settings – Examples:
  - Prisons and jails, nursing homes and other long-term care facilities
  - Health care employees - hospitals and other health care facilities
  - Homeless shelters
- Mycobacteriology laboratory personnel
- Persons with immunomodulatory medical conditions – Examples:
  - Silicosis, diabetes mellitus, chronic renal failure, hematologic malignancies (e.g., leukemias and lymphomas), other specific malignancies (e.g., carcinoma of the head, neck, or lung), weight loss >10% of ideal body weight, gastrectomy, and jejunointestinal bypass
- Children < 4 years of age, or infants, children and adolescents exposed to adults at high-risk
TST interpretation (Review)
> 15 mm induration = “Positive” result

• Persons with no known risk factors for TB
  • Raises question ‘why was a TST performed?’
  • Further exploration of patient’s risk factors and exposure history warranted
  • Ensure results of TST are indeed accurate
### Factors that may affect the TST

<table>
<thead>
<tr>
<th>Type of Reaction</th>
<th>Possible Cause</th>
</tr>
</thead>
</table>
| **False-positive** | • Nontuberculous mycobacteria  
                       • BCG vaccination  
                       • Problems with TST administration |
| **False-negative** | • Anergy  
                       • Viral, bacterial, fungal coinfection  
                       • Recent TB infection  
                       • Very young age; advanced age  
                       • Live-virus vaccination  
                       • Overwhelming TB disease  
                       • Renal failure/disease  
                       • Lymphoid disease  
                       • Low protein states  
                       • Immunosuppressive drugs  
                       • Problems with TST administration |
Problems in assessing TST and IGRA accuracy:

- **NO “Gold Standard”** to confirm LTBI or culture-neg. TB diagnosis
  - No confirmatory testing available for LTBI or culture (-) TB
  - TST and IGRA indirectly measure immunologic response rather than MTB

- Approximations of assay sensitivity and specificity made by testing populations with known characteristics – e.g.:
  - Culture positive TB (assay sensitivity)
  - Populations very unlikely to have TB (e.g. non-traveling farmers from Iowa)
TB infection to disease – “Ideal role” of immunologic biomarkers

Positive marker with infection (with or w/o clinical disease)

- Immunocompetent human host with viable Latent TB Infection
- TST(+)/IGRA(+)
- Ideal Biomarker(+)
- ~10%

Active TB Disease

- Higher quantitative marker with progressive TB disease
- TST(+/-)/IGRA(+/-)
- Ideal Biomarker(+)
- No LTBI Tx
- ~90%

Non-reactivatable “LTBI”

- Elimination of marker with resolution of infection (no viable bacilli)
- TST(+)/IGRA(+)
- Ideal Biomarker(-)
- ~90%
LTBI and treatment – “Ideal role” of immunologic biomarkers

Viable LTBI

TST(+) / IGRA(+)  
Ideal Biomarker(+)

Non-viable “LTBI”

TST(+) / IGRA(+)  
Ideal Biomarker(-)

Non-reactivatable “LTBI”

TST(+) / IGRA(+)  
Ideal Biomarker(+)  
Disappearance of biomarker with resolution of infection

LTBI Tx

LTBI Tx
Interferon Gamma Release Assays (IGRA) methodology:

- Measure IFN-γ release after exposure of either whole blood or peripheral blood mononuclear cells (PBMCs) to select antigens encoded within the regions of difference-1 & 11
- A region of the MTB genome absent in all BCG strains and in most NTM

- **Whole blood** → QuantiFERON-TB Gold In-Tube (QFT-GIT), Cellestis, Carnegie, Australia

- **PBMCs** → T-SPOT. TB (TSPOT), Oxford Immunotec, Abingdon, UK

Basic Principle behind IGRAs

• T-cell lymphocytes release IFN-γ, an immune cytokine in response to specific antigens, including those from TB infection

• 3 synthetic TB-specific antigens identified:
  - RD1: ESAT-6, CFP-10
  - RD11: TB7.7

• IFN-γ is stable and measurable in the plasma
ESAT-6 and CFP-10

• Region of Difference 1 (RD1)
  • Gene region lost from *M. bovis* as it is processed to the BCG vaccine

• 9 proteins are made by this genomic area including ESAT-6 and CFP 10

• T-cells of patient with a MTB infection respond to these proteins by secreting IFN-γ and other agents

• Since these RD1 proteins are not found in BCG or most NTM, patients with previous BCG vaccination or most NTM infections will not have the T-cell response to produce IFN-γ when exposed to ESAT-6 or CFP10 antigens
### Interferon-gamma Release Assays (IGRAs)
**ESAT-6 and CFP-10 Antigens**

<table>
<thead>
<tr>
<th>MTB Complex</th>
<th>Antigens</th>
<th>NTM species</th>
<th>Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESAT</td>
<td>CFP</td>
<td>ESAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CFP</td>
</tr>
<tr>
<td><strong>M tuberculosis</strong></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>M africanum</strong></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>M bovis</strong></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>BCG substrain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gothenburg</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>moreau</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>tice</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>tokyo</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>danish</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>glaxo</td>
<td>-</td>
<td>-</td>
<td></td>
</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>

**NTM species**
- M abcessus complex
- M avium
- M branderi
- M celatum
- M chelone
- M fortuitum complex
- M gordonii
- M intracellulare
- M kansasii
- M malmoense
- M marinum
- M oenavense
- M scrofulaceum
- M smegmatis
- M szulgai
- M terrae
- M vaccae
- M xenopi

**BCG = bacille Calmette-Guérin**
Advantages of IGRAs

• Requires a single patient visit to conduct the test.

• Results can be available within 24 hours.

• Does not boost responses measured by subsequent tests.

• Prior BCG vaccination does not cause a false-positive IGRA test result.

• ‘Relative’ objectivity in assay interpretation
  • Exceptions exist
Disadvantages of IGRAs

• Blood samples must be processed within 8-30 hours after collection while white blood cells are still viable.

• Errors in collecting or transporting blood specimens or in running and interpreting the assay can decrease the accuracy of IGRAs.

• Limited data on the use of IGRAs to predict who will progress to TB disease in the future.

• Limited data on the use of IGRAs for:
  • Children younger than 5 years of age;
  • Persons recently exposed to *M. tuberculosis*;
  • Immunocompromised persons; and
  • Serial testing.

• Tests may be expensive.
### Comparing TST and IGRA platforms

<table>
<thead>
<tr>
<th><strong>TST</strong></th>
<th><strong>IGRA</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculin is injected under the skin and produces a delayed-type</td>
<td>Blood is drawn for testing; test measures the immune response to the TB bacteria in whole blood</td>
</tr>
<tr>
<td>hypersensitivity reaction if the person has been infected with *M.</td>
<td></td>
</tr>
<tr>
<td><em>tuberculosis</em></td>
<td></td>
</tr>
<tr>
<td>Requires two or more patient visits to conduct the test</td>
<td>Requires one patient visit to conduct the test</td>
</tr>
<tr>
<td>Results are available 48 to 72 hours later</td>
<td>Results can be available in 24 hours (depending on the batching of specimens by the laboratory and transport)</td>
</tr>
<tr>
<td>Can cause booster phenomenon</td>
<td>Does not cause booster phenomenon</td>
</tr>
<tr>
<td>Reading by HCW may be subjective</td>
<td>Laboratory test not affected by HCW perception or bias</td>
</tr>
<tr>
<td>BCG vaccination can cause false-positive result</td>
<td>BCG vaccination does not cause false-positive result and infection with most nontuberculous mycobacteria does not cause false-positive result</td>
</tr>
<tr>
<td>A negative reaction to the test does not exclude the diagnosis of</td>
<td>A negative reaction to the test does not exclude the diagnosis of LTBI or TB disease</td>
</tr>
<tr>
<td>LTBI or TB disease</td>
<td></td>
</tr>
</tbody>
</table>
Currently Available IGRAs

- **QuantiFERON®** (Cellestis)
  - 2\textsuperscript{nd} generation QFT®-Gold (QFT-G) FDA approved May 2005
  - 3\textsuperscript{rd} generation QFT®-Gold In-Tube (QFT-IT) FDA approved October 2007

- **T-SPOT.®TB** (Oxford Immunotec)
  - Evolved from Elispot
  - FDA approved July 2008
Differences in currently available IGRA testing platforms:

<table>
<thead>
<tr>
<th></th>
<th>QFT–GIT</th>
<th>T–Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Process</strong></td>
<td>Process whole blood within 16 hours</td>
<td>Process peripheral blood mononuclear cells (PBMCs) within 8 hours, or if T-Cell Xtend® is used, within 30 hours.</td>
</tr>
<tr>
<td><strong>M. tuberculosis Antigen</strong></td>
<td>Single mixture of synthetic peptides representing ESAT-6, CFP-10 and TB7.7</td>
<td>Separate mixtures of synthetic peptides representing ESAT–6 and CFP-10</td>
</tr>
<tr>
<td><strong>Measurement</strong></td>
<td>IFN-g concentration</td>
<td>Number of IFN-g producing cells (spots)</td>
</tr>
<tr>
<td><strong>Possible Results</strong></td>
<td>Positive, negative, indeterminate</td>
<td>Positive, negative, indeterminate, borderline</td>
</tr>
</tbody>
</table>

http://www.cdc.gov/tb/publications/factsheets/testing/IGRA.pdf
QuantiFERON-TB IT methodology

1. Collect 1mL of blood into Nil, Antigen and Mitogen tubes. Shake well. Incubate tubes at 37°C for 16-24 hrs.

2. Centrifuge tubes for 15 minutes. Harvested plasma is stable refrigerated for at least 4 weeks.

3. Add conjugate, plasma samples and standards to ELISA. Incubate for 120 minutes at room temperature.

4. Wash and add substrate. Read absorbance after 30 minutes.

5. Software calculates results and prints reports.

http://www.cellestis.com
QuantiFERON-TB Gold

Courtesy of Mayo Medical Laboratories, 2013
QuantiFERON-TB tubes & roles

TB Antigen tube
Assesses IFN-γ response to highly-specific TB antigens.

Mitogen tube (Positive control)
Can be useful to indicate
- Patient’s immune status
- Correct blood handling and incubation
Note: a low-mitogen result, in conjunction with a negative TB result, is classified as an “indeterminate”.

Nil tube (Negative control)
Adjusts for background noise.

http://www.cellestis.com
IFN-γ measurement by QuantiFERON-TB GIT

IFN-γ released in response to ESAT-6, CFP-10 or TB7.7 stimulation

• Calculated as the difference in antigen-stimulated IFN-γ production in blood \emph{minus} the IFN-γ concentration in blood incubated with saline (e.g. Nil; negative control)

\[ \text{TB Ag} - \text{Nil} = \text{measured result} \]
QuantiFERON-TB Gold In-Tube (QFN-GIT) Interpretation Criteria

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>TB specific antigen response (IU/mL)*</th>
<th>Nil control (IU/mL)</th>
<th>Mitogen control (IU/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥ 0.35 (and ≥ 25% of Nil)</td>
<td>≤ 8.0</td>
<td>any</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; 0.35 OR ≥ 0.35 and &lt; 25% of Nil</td>
<td>≤ 8.0</td>
<td>≥ 0.5</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&lt; 0.35 OR ≥ 0.35 and &lt; 25% of Nil</td>
<td>≤ 8.0</td>
<td>&lt; 0.5</td>
</tr>
</tbody>
</table>

*Corrected for Nil response.

http://www.cellestis.com
QuantiFERON-TB Gold Interpretation

**INTERPRETATION**

**VALIDATION**

**Mitogen**
- Nil < 0.50 IU/mL
- and/or Nil > 8.0 IU/mL

**TB Antigen**
- Nil ≥ 0.35 IU/mL

**INDETERMINATE**

**Nil ≤ 8.0 IU/mL**

**NEGATIVE**

**POSITIVE**
T. Spot TB procedural steps

Methodology rationale:

- Removes background interferon gamma to maximize sensitivity
- Utilizes a standard number of PBMCs to correct for a patient’s immune status

1. Collect the blood sample. At the lab, PBMCs are separated from whole blood, washed, counted and inoculated into 4 separate microtiter wells.

2. PBMCs and specific TB antigens are added to wells pre-coated with antibodies to IFN-γ and incubated 16 to 20 hours at 37°C, CO2.

3. IFN-γ is released from activated T cells and captured. Wash wells, add secondary conjugated antibody. Incubate for one hour.

4. Wells are washed. A substrate is added which produces spots where interferon gamma was secreted by T cells. Spots are counted.

http://www.oxfordimmunotec.com
The T-SPOT. TB test requires four wells to be used for each patient sample.

- **Nil Control**
- **Panel A (ESAT-6)**
- **Panel B (CFP10)**
- **Positive Control**

More detailed information can be found on the Oxford Immunotec website: [http://www.oxfordimmunotec.com](http://www.oxfordimmunotec.com)
Select procedural differences b/w QFN GIT and T.Spot TB

<table>
<thead>
<tr>
<th></th>
<th>T-SPOT. TB Test</th>
<th>In-Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard blood collection tubes are</td>
<td>Yes</td>
<td>No; requires three specialized tubes drawn in specific order.</td>
</tr>
<tr>
<td>used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood collection tube is filled</td>
<td>Yes</td>
<td>No; specialized tubes must be filled between 0.8mL and 1.2mL or within</td>
</tr>
<tr>
<td>using standard phlebotomy practices</td>
<td></td>
<td>the black mark on the side of the tube label. Over- or under-filling of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>the tubes outside the range may lead to erroneous results.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phlebotomist gently inverts blood</td>
<td>Yes</td>
<td>No; once filled, each tube must be mixed by shaking 10 times. Over-</td>
</tr>
<tr>
<td>tube after drawing specimen,</td>
<td></td>
<td>energetic shaking should be avoided to minimize erroneous results due</td>
</tr>
<tr>
<td>consistent with standard phlebotomy</td>
<td></td>
<td>to gel dislodgement.</td>
</tr>
<tr>
<td>practices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A borderline zone is used, consistent</td>
<td>Yes</td>
<td>No; “Although not included in FDA-approved interpretation criteria for</td>
</tr>
<tr>
<td>with the recommendations of the 2010</td>
<td></td>
<td>QFT-GIT...an appropriate borderline category for QFT-GIT might increase its</td>
</tr>
<tr>
<td>CDC Guidelines</td>
<td></td>
<td>accuracy...”</td>
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</tbody>
</table>

http://www.oxfordimmunotec.com
<table>
<thead>
<tr>
<th></th>
<th>Tuberculin skin test</th>
<th>IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigens</strong></td>
<td>PPD</td>
<td>Peptides from CFP-10 and ESAT-6</td>
</tr>
<tr>
<td><strong>Test substrate</strong></td>
<td>Skin</td>
<td>PBMC</td>
</tr>
<tr>
<td><strong>Time required for result</strong></td>
<td>48-72 h</td>
<td>20 h incubation and 3 h for the final results</td>
</tr>
<tr>
<td><strong>Cells involved</strong></td>
<td>Neutrophils, memory CD4 T-cells and CD8 T-cells; cells need to home and transmigrate out of capillaries into the skin</td>
<td>CD4 T-cells in the wells*</td>
</tr>
<tr>
<td><strong>Cytokines involved</strong></td>
<td>IFN-γ, TNF-α, TNF-β</td>
<td>IFN-γ</td>
</tr>
<tr>
<td><strong>Read-out</strong></td>
<td>Measure of diameter of dermal induration, transverse to long axis of arm</td>
<td>Enumeration of IFN-γ spots</td>
</tr>
<tr>
<td><strong>Read-out units</strong></td>
<td>Millimeters</td>
<td>IFN-γ spot forming cells</td>
</tr>
<tr>
<td><strong>Outcome measure</strong></td>
<td>Level of induration</td>
<td>Number of IFN-γ producing T-cells</td>
</tr>
<tr>
<td><strong>Effect of treatment on test response</strong></td>
<td>No effect, unless given soon after exposure</td>
<td>Decline in response with treatment at the population level but wide interindividual variation in rate of decline</td>
</tr>
</tbody>
</table>

**Predominant CD4+ T cells**
CD45 RO memory phenotype (long-lived central memory cells?)

48-h PPD APC-processing: MHC class II restricted?

**Predominant CD4+ T cells**
CD45 RA-CCR7- effector memory phenotype (Recent encountered antigen in-vivo)

20h peptide APC processing or presenting?
TST vs. IGRAs: *Sensitivity*

<table>
<thead>
<tr>
<th></th>
<th># Studies</th>
<th>Sensitivity (95% CI)</th>
<th>Chi-Square for heterogeneity (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TST</strong></td>
<td>14</td>
<td>0.71 (0.65-0.74)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Size of reaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mm</td>
<td>9</td>
<td>0.74 (0.66-0.82)</td>
<td>0.001</td>
</tr>
<tr>
<td>10 mm</td>
<td>4</td>
<td>0.72 (0.50-0.95)</td>
<td>0.01</td>
</tr>
<tr>
<td>15 mm</td>
<td>1</td>
<td>0.40 (0.25-0.56)</td>
<td>-</td>
</tr>
<tr>
<td><strong>QuantiFERON-TB Gold™</strong></td>
<td>13</td>
<td>0.76 (0.70-0.83)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Elispot™ or T-SPOT.TB™</strong></td>
<td>12</td>
<td>0.88 (0.81-0.95)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### TST vs. IGRAs Specificity

<table>
<thead>
<tr>
<th></th>
<th># Studies</th>
<th>Specificity (95% CI)</th>
<th>Chi-Square for heterogeneity (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TST</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.66 (0.46-0.86)</td>
<td>0.001</td>
</tr>
<tr>
<td>Not BCG vaccinated</td>
<td>3</td>
<td>0.98 (0.96-1.00)</td>
<td>NS</td>
</tr>
<tr>
<td>BCG Vaccinated</td>
<td>5</td>
<td>0.56 (0.34-0.78)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥ 10 mm</td>
<td>6</td>
<td>0.58 (0.37-0.79)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥ 15 mm</td>
<td>3</td>
<td>0.87 (0.70-1.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>QuantiFERON-TB Gold™</td>
<td>9</td>
<td>0.97 (0.95-0.99)</td>
<td>0.01</td>
</tr>
<tr>
<td>Not BCG vaccinated</td>
<td>2</td>
<td>1.0 (0.94-1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>BCG Vaccinated</td>
<td>7</td>
<td>0.96 (0.94-0.99)</td>
<td>0.01</td>
</tr>
<tr>
<td>Elispot™ or T-SPOT.TB™</td>
<td>4</td>
<td>0.92 (0.88-0.95)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

IGRA’s vs. TST

- IGRAs do not require a second visit
- Mitogen-negative “indeterminate” results better to detect anergic cases
- IGRAs do not trigger an amnesic response (i.e., boosting effect)
- IGRAs correlate well with TST to detect LTBI in contact investigations (Brock I, et al AJRCCM 2004, Ewer K et al. Lancet 2003)
- IGRAs are more specific in BCG vaccinated patients for the diagnosis of LTBI (Ferrara G, et al. Lancet 2006)
IGRA limitations

• Peripheral blood T-cell transcriptional cytokine signatures and functional T-cell profile can be substantially different in LTBI and active TB\textsuperscript{1,2}

• TST+/IGRA- results in LTBI are probably not always related to false+ TST results
  • Discordant TST+/IGRA- cases can show RD1-peptide T-cell activation using assays with longer stimulation time\textsuperscript{3,4}
  • Moderate concordance ($\kappa = 0.69$) between 2 commercial IGRA\textsuperscript{s}\textsuperscript{5}
  • IGRA results variability over time\textsuperscript{6}

(1) Berry MP, *Nature* 2010
(2) Sutherland JS et al. *Eur J Immunol* 2009
(4) Butera O, at el. *BMC Infect Dis* 2009
(6) van Zyl-Smith RN, *Am J Respir Crit Care Med* 2009
IGRA testing in U.S. Health Care Workers (HCWs) – challenges:

• Principle: Despite using a test with a specificity approaching 99%, when the prevalence of MTB infection is \(< 1\%\), the majority of positive (IGRA) results will be \textit{false positives}\textsuperscript{1}
  
  • Prevalence of active TB in the US has declined from 6.2 cases per 100,000 persons in 1998 to \textbf{3.2 in 2012}\textsuperscript{2}
  
  • Previous studies using TST for HCW annual screening reported \(<1.0-1.2\%\) conversion rates\textsuperscript{3}

\textit{How to interpreted ‘low risk’ HCWs with new (+) IGRA result??}

2. CDC. Reported Tuberculosis in the United States, 2012; aval. October 2013
Is the QGF-GIT TB ag-nil threshold of 0.35 IU/mL acceptable?

• Balance between IGRA assay sensitivity and specificity
  • PPV very unclear
  • Variability in results from serial testing
• Should a ‘borderline’ zone with broader threshold-ranges be considered?
• When should QFN testing be repeated?
IGRAs in Health Care Workers (HCWs)

• In a recent meta-analysis (N=6605) \(^1\)
  • Overall QFN and TSPOT:
    • Reversions: 22.1 to 71.4%
    • Conversion: 0.7 to 14.4%
  • QFN in low-intermediate TB incidence settings:
    • Conversion 5.1% (95%CI 4.5 - 5.7)
    • Increase likelihood with QFN between 0.2 – 0.7 IU/uL
  • With the use of a “borderline zone” (0.2-0.7):\(^2\)
    • Conversion rate: 11%\(\rightarrow\)3.6%
    • Reversion rate: 22.1%\(\rightarrow\)5.2%

• Consider retesting pts who fall into “borderline zone”
• It is unclear the risk of TB over time by using this approach

HCW QFN-GIT reversion rates in 3 U.S. centers

VA Hosp. Palo Alto, CA
Univ. of Chicago, IL
Cleveland Clinic, OH

<table>
<thead>
<tr>
<th>Test results</th>
<th>VAPAHCS</th>
<th>UIC</th>
<th>CC</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeat positive result</td>
<td>113</td>
<td>338</td>
<td>25</td>
<td>476</td>
</tr>
<tr>
<td>Reversion</td>
<td>73</td>
<td>275</td>
<td>38</td>
<td>386</td>
</tr>
<tr>
<td>Total (n)</td>
<td>186</td>
<td>613</td>
<td>63</td>
<td>862</td>
</tr>
</tbody>
</table>

At least 2 QFT tests required, one of which was a (+) result and followed by either a (+) or (-) QFN result

A TB ag-nil cut-off value of 1.11 value was most significant in predicting reversions

HCW QFN-GIT reversion rates in 3 US centers using different TB ag-nil cut-off values

Using a TB ag-nil cut-off value of 1.11 IU/mL

Exploratory group
575 HCWs
300/575 (52%) reversions

≥ 1.11

TBag-nil

< 1.11

275 HCWs
TBag-nil ≥ 1.11
75/275 (27%) reversions

≥ 2.17

TBag-nil

< 2.17

176 HCWs
TBag-nil ≥ 2.17
32/176 (18%) reversions

99 HCWs
TBag-nil < 2.17
43/99 (43%) reversions

300 HCWs
TBag-nil < 1.11
225/300 (75%) reversions

≥ 0.72

TBag-nil

< 0.72

96 HCWs
TBag-nil ≥ 0.72
62/96 (65%) reversions

204 HCWs
TBag-nil < 0.72
163/204 (80%) reversions

Figure 1: Receiver operating characteristic (ROC) decision tree identifying statistically significant TBag-nil (in IU/mL) separation points which predict those HCWs with a positive TB test result at time one who retest negative at time two. Logistic regression analysis on a separate Confirmatory sample of 287 HCWs validated all 3 separation points at 0.72, 1.11, and 2.17 IU/mL and remained statistically significant for all subgroups by chi-square (P < 0.001). 1 Kappa = 0.48, chi-square = 131.0, P < 0.001, 2 Kappa = 0.16, chi-square = 8.2, P < 0.01, 3 Kappa = 0.27, chi-square = 20.4, P < 0.001.

Fluctuations in IGRA results – multiple possible causes:

- Biologic / host variability
  - Natural CD4 cell variations – daily
  - Other stimuli of T-cells – concurrent infections, vaccinations, etc.
  - Concurrent medications

- IGRA testing methodology
  - Shaking of tubes after blood collection
  - Time to incubation
  - ELISA between-run variability
  - Manufacturing related (tubes)
Future IGRA considerations with “low-risk” groups

- Adopting an “intermediate zone”
  - 0.2 – 0.7 IU/mL
  - 0.35 – 1.0 IU/mL
  - 0.35 – 2.0 IU/mL

- Cautious interpretation when QFN values within the ‘intermediate zone’ for lower risk people
  - Consider new TB infection risk factors
  - Consider repeat testing depending upon host factors

Boosting T-cell IGRA responses after TST

- QFN or T.Spot assays may be misleading if performed > 3 days after TST
  - Higher rates of ‘boosted’ IFN-γ or spots detected beyond 3 days
- TST-boosting of IGRAs more common in baseline IGRA (+) pts (‘sensitized’ pts)
  - Much less common to occur in baseline TST or IGRA neg. (-) pts.

Same boosting study showed high variability in IGRA assay results

**TABLE 4. CHARACTERISTICS OF INDIVIDUALS WHO WERE QUANTIFERON-TB GOLD IN-TUBE OR T-SPOT.TB NEGATIVE, OR WITH BORDERLINE RESULTS, WHO DISPLAYED BOOSTING OF THEIR RESPONSES AFTER TUBERCULIN SKIN TEST ADMINISTRATION**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Demographics</th>
<th>Test Format</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4 (Day 0)</th>
<th>Day +3</th>
<th>Day +7</th>
<th>Day +28</th>
<th>Day +84</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female, 48 years, nonsmoker, TST = 15.5 mm; high risk</td>
<td>ESAT-6 (SFU/well)</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>24</td>
<td>33</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CFP-10 (SFU/well)</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>14</td>
<td>5</td>
<td>25</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN-γ (IU/ml)</td>
<td>3.29</td>
<td>5.59</td>
<td>2.89</td>
<td>3.09</td>
<td>2.48</td>
<td>9.72</td>
<td>12.29</td>
<td>11.72</td>
</tr>
<tr>
<td>9</td>
<td>Female, 45 years, nonsmoker, TST = 19 mm; medium risk</td>
<td>ESAT-6 (SFU/well)</td>
<td>NA</td>
<td>1</td>
<td>NA</td>
<td>2</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CFP-10 (SFU/well)</td>
<td>NA</td>
<td>3</td>
<td>NA</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN-γ (IU/ml)</td>
<td>NA</td>
<td>0.19</td>
<td>0.16</td>
<td>0.26</td>
<td>0.18</td>
<td>5.30</td>
<td>1.59</td>
<td>4.20</td>
</tr>
<tr>
<td>12</td>
<td>Female, 31 years, smoker, TST = 19 mm; medium risk</td>
<td>ESAT-6 (SFU/well)</td>
<td>1</td>
<td>3</td>
<td>NA</td>
<td>1</td>
<td>3</td>
<td>32</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CFP-10 (SFU/well)</td>
<td>6</td>
<td>3</td>
<td>NA</td>
<td>4</td>
<td>5</td>
<td>12</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN-γ (IU/ml)</td>
<td>1.64</td>
<td>0.95</td>
<td>0.99</td>
<td>0.66</td>
<td>1.10</td>
<td>7.20</td>
<td>11.04</td>
<td>5.12</td>
</tr>
</tbody>
</table>

**TABLE 5. COMPARISON OF QUANTIFERON-TB GOLD IN-TUBE AND T-SPOT.TB VARIABILITY, BORDERLINE ZONES, AND PROPOSED THRESHOLDS FOR CONVERSION**

<table>
<thead>
<tr>
<th></th>
<th>QuantIFERON-TB Gold In-Tube</th>
<th>T-SPOT.TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer-defined assay cut-point</td>
<td>&gt;0.35 IU/ml</td>
<td>≥6 spots*</td>
</tr>
<tr>
<td>Within-subject short-term variability</td>
<td>±80% of IFN-γ response</td>
<td>±3 spots</td>
</tr>
<tr>
<td>Borderline or uncertainty zone</td>
<td>0.2–0.7 IU/ml</td>
<td>4–8 spots (inclusive)</td>
</tr>
<tr>
<td>Proposed conversion threshold</td>
<td>Increase from &lt;0.35 to &gt;0.7 IU/ml</td>
<td>Increase from &lt;6 to &gt;9 spots (inclusive)</td>
</tr>
</tbody>
</table>

* The U.S. Food and Drug Administration borderline zone for result interpretation includes values of 5, 6, and 7 spots.
Proposed Assessment of Combined IGRA and TST for suspected LTBI

Careful Assessment of risk factors for TB infection and TB progression

- TST+/IGRA+
- TST+/IGRA- (*)
- TST-/IGRA+ (*)
- TST-/IGRA-

Repeat IGRA at 3 mo?

- IGRA+
- IGRA-

LTBI
False+ TST due to BCG?
IGRA level? Poss LTBI
False+ IGRA? or Reversion?
No LTBI (**)

(*) Either TST+ or IGRA+ test can be significant in immunosuppression
(**) TST-/IGRA- does not rule out LTBI in immunosuppression

Adapted from Lalvani A, Pareek M Brit Med Bull 2010
TST or IGRA use in Children?

• Challenges with IGRA usage in young children:
  • Phlebotomy can be more difficult
  • Microbiologic confirmation more difficult in children (more paucibacillary disease)
  • Possible age-related immunologic variances
    • Much higher risk for HIV and/or TB disease progression in children < 1 yo (irrespective of CD4 counts)

• TST use can be problematic with recent BCG vaccination
TST or IGRA use in Children?

- High variation between TST, T.Spot TB and QFN-GIT in children\(^1\)
  - Possibly decreased IFN-\(\gamma\) production in young children
- Neither TST or IGRA have shown superiority in the diagnosis of LTBI or active TB in children
  - IGRA can be useful as an adjunctive test to TST\(^2\)
- Use of combination testing in children can increase sensitivity of TB Infection\(^3\)
  - TST, IFN-\(\gamma\) production, IP-10

CDC Updated Guidelines (2010)

- TST or IGRA can be used for surveillance or for treatment decisions in pts at risk of TB infection or progression to active TB
- IGRAs should be performed according approved protocols with quantitative and qualitative reporting
- High rate of false +IGRA if prevalence of TB infection <1%
- Test selection should be made based on clinical reason and context and availability, cost and effectiveness of testing
- An IGRA can be used in place (but typically not in addition to) a TST in all situations that a TST is recommended to diagnose TB infection, with select considerations (next slide)
  - Despite select preferences for a testing platform, use of an alternative FDA approved test (IGRA or TST) is acceptable medical & public health practice

Mazurek G et al. MMWR June 25, 2010/Vol 59/No. RR-5
CDC Updated Guidelines (2010) – Cont’d

• IGRA preferred, but TST is acceptable:
  • Low likelihood to return for TST reading (e.g. homeless, drug abusers)
  • Prior BCG vaccination (improve acceptance of LTBI Tx)

• TST preferred, but IGRA is acceptable:
  • Children < 5 yo

• Either TST or IGRA may be used without preference
  • Recent TB contacts with active case (Repeat testing 8-10 wks after end of exposure if negative test)
  • Periodic screening of HCW

• Both TST and IGRA can be considered:
  • High risk of TB infection and progression, and risk of poor outcome
    • HIV
    • Children < 5 yo
  • Investigation of active TB (?)
  • To enhance compliance to LTBI Tx (?)

Mazurek G et al. MMWR  June 25, 2010/Vol 59/No. RR-5
Conclusions

- Despite recent progress (i.e. IGRA), immunodiagnosis of LTBI remains challenging and areas of controversy exists.
- IGRAAs are more specific than TST, but a TST+/IGRA- probably does not always represent a false negative TST.
- Both TST and IGRAAs cannot differentiate between active TB, subclinical TB, LTBI and host-cleared TB infections.
- Serial testing with IGRA is associated with a high rate of conversions and reversions that can be reduced in HCW with the use of a “gray zone” but long-term data is needed.
- New diagnostics needed to not only more accurately detect T-cell activity against MTB, but also identify pts. with LTBI with viable MTB (at risk of reactivation).
- IGRA testing has low positive predictive value (long-term risk of developing active TB with a (+) IGRA is low.)
Final points of consideration:

- TST and IGRAs identify *an adaptive immune response* against MTB
  - *Not directly* detecting MTB infection
- The % of persons without treatment who truly remain infected with MTB after TST or IGRA conversions *is unknown*
- Unclear how long adaptive immune responses *persist (+)* in a patient in the absence of live/viable MTB

The End

• More information on a challenging topic usually raises *more questions*

……more to come!

• Thank you for your attention