IGRAs for Detection of *M. tuberculosis* Infection

World TB Day, 2011

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Disclaimers

- The views presented here are those of the speaker and not CDC.
- Off-label usage of the products under discussion may be broached during questions and answers, and will be verbally indicated as such.

Overview

- Need for testing
- Interferon Gamma Release Assays (IGRAs)
- FDA approved IGRAs
- Recommendations
- Controversies

Need for Testing

- 2008 Global Statistics
  - 9.4 million new cases
    - increasing
  - 1.8 million deaths
    - 5 thousand deaths / day
  - 2 billion infected
    - 1/3 of world population
- 2008 US Statistics
  - 12,898 new cases
    - declining
  - ~290 deaths
  - 12 million infected
    - 4 % of US population
Focus #1: Persons with Increased Prevalence of Infection with *M. tuberculosis*

- Close contacts of persons with TB disease
- Persons from areas with high incidence of TB
- Persons who visit areas with a high prevalence of TB, especially if visits are frequent or prolonged
- Residents and employees of high-risk congregate settings
- Health care workers who serve high-risk clients
- Populations defined locally as having an increased incidence of infection or disease due to TB
- Infants, children, and adolescents exposed to adults in high-risk categories

Focus #2: Persons at Increased Risk for Disease from *M. tuberculosis* if Infected

- Persons with HIV infection*
- Infants and children aged <5 years*
- Persons receiving immunosuppressive therapy*
- Persons recently infected with *M. tuberculosis* (within 2 yrs)
- Persons with history of untreated/inadequately treated TB
- Persons with silicosis, diabetes mellitus, chronic renal failure, leukemia, lymphoma, or cancer of the head, neck, or lung
- Persons with gastrectomy or jejunooileal bypass
- Persons who weight less than 90% of ideal body weight
- Populations defined locally as having an increased incidence of infection or disease due to *M. tuberculosis.*

* Indicates groups at increased risk of a poor outcome such as meningitis, disseminated disease, or death due to *M. tuberculosis.*

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**TST vs IGRA**


**Interferon Gamma (IFN-γ) Release Assays**

- Indirect tests for *M. tuberculosis* infection
- Do not differentiate latent infection from disease
- Three approved by FDA
  - “as an aid in the diagnosis of infection with *Mycobacterium tuberculosis*”
Types of IGRA

- Measure Δ IFN-γ concentration
  - Measure IFN-γ concentration by ELISA
  - Whole blood stimulated with & w/o antigen
  - e.g. QuantIFERON®-TB Gold
- Measure Δ # of cells releasing IFN-γ
  - Count cells by ELISpot
  - PBMCs* stimulated with & w/o antigens
  - e.g. T-Spot™.TB

*peripheral blood mononuclear cells

FDA Approved IGRAs

- QuantiFERON®-TB Gold (QFT-G)
  - FDA approved May 2005
- QuantiFERON®-TB Gold In-Tube (QFT-GIT)
  - FDA approved Oct 2007
- T-Spot®.TB (T-Spot)
  - FDA approved July 2008

Antigens

- M. tuberculosis antigens shared with NTM, & BCG
- Antigens specific to M. tuberculosis, c.g. ESAT-6 & CFP-10

(Antigen Specificity by Species)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>ESAT-6</th>
<th>CFP-10</th>
<th>ESAT-6</th>
<th>CFP-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. africanum</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. bovis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. avium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>M. branderi</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>M. cellulosum</td>
<td>-</td>
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<tr>
<td>M. cheloneae</td>
<td>-</td>
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<tr>
<td>M. fortum</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>M. gordonii</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>M. intracellulare</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. kansasii</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. malmoense</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. marinum</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. osnasioense</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>M. szulgai</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>M. terrae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. vaccae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>M. xenopi</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

Ganguly et al, 2008: 88, 510-517

QuantiFERON®-TB Gold (QFT-G)

**Stage 1 Whole Blood Culture**
- Draw blood into heparin
- Make 1 ml aliquots & add antigen
- Incubate overnight: INF-γ from sensitized lymphs

**Stage 2: Measure [IFN-γ] & Interpret**
- Harvest plasma from above settled cells
- Measure [IFN-γ] in ‘Sandwich’ ELISA
- Software calculates results and prints report

Antigens
- ESAT-6
- CFP-10
- Mitogen
- Control

**QFT-G Interpretation**

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>TB Response*</th>
<th>Nil</th>
<th>Mitogen - Nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥0.35 IU/ml</td>
<td>Any</td>
<td>Any</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;0.35 IU/ml</td>
<td>≥0.7</td>
<td>≥0.5</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&lt;0.35 IU/ml</td>
<td>≥0.7</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

TB Response* is the higher IFN-γ concentration resulting from stimulation by ESAT-6 or CFP-10 minus the IFN-γ concentration in plasma incubated with saline.

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**QuantiFERON®-TB Gold In-Tube (QFT-GIT)**

**Stage 1 Whole Blood Culture**
- 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**
- Collect plasma for ELISA
- Measure [IFN-γ] in ‘Sandwich’ ELISA
- Software calculates results and prints report

(QFT-GIT adds TB7.7 to ESAT-6 and CFP-10)

**QFT-GIT Interpretation**

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>TB Response</th>
<th>Nil</th>
<th>Mitogen - Nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥0.35 IU/ml and ≥25% of Nil</td>
<td>≥8.0</td>
<td>Any</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;0.35 IU/ml or &lt;25% of Nil</td>
<td>≥8.0</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&lt;0.35 IU/ml or &lt;25% of Nil</td>
<td>&lt;8.0</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

TB Response* is the IFN-γ concentration in plasma from blood stimulated with a single cocktail representing ESAT-6, CFP-10, and part of TB7.7, minus the IFN-γ concentration in plasma from unstimulated blood.
T-Spot.TB

- Collect blood
- Recover, wash, & count PBMCs
- Aliquot 250,000 PBMCs to 4 wells with anti-INF-γ
- Add media alone, ESAT-6, CFP-10 or PHA, & incubate
- Wash away cells
- Develop & count spots where cells produced IFN-γ

Sensitized T cell

INF-γ Antibody

INF-γ Captured

Detection Antibody

Chromogen

Spot

Media ESAT-6 CFP-10 Mitogen

T-Spot.TB Interpretation

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>TB Response</th>
<th>Nil</th>
<th>Mitogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥ 8 spots</td>
<td>≤ 10 spots</td>
<td>any</td>
</tr>
<tr>
<td>Borderline</td>
<td>5, 6, or 7 spots</td>
<td>≤ 10 spots</td>
<td>any</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 4 spots</td>
<td>≤ 10 spots</td>
<td>≥ 20 spots</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&lt; 5 spots</td>
<td>≤ 10 spots</td>
<td>&lt; 20 spots</td>
</tr>
</tbody>
</table>

TB Response* is the higher number of spots resulting from stimulation of PBMCs with two separate cocktails of peptides representing ESAT-6 or CFP-10, minus the number of spots resulting from incubation of PBMCs with saline.

IGRA Test Interpretation

- Based on IFN-γ response to TB antigens relative to nil value
- Interpretation criteria differ for different IGRA
- Unlike TST, not risk stratified (i.e., there are not multiple cutoffs for different risk groups)
- Still somewhat complicated

Differences in Currently Available IGRA

<table>
<thead>
<tr>
<th>Format</th>
<th>QFT-G</th>
<th>QFT-GIT</th>
<th>T-Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Tested</td>
<td>Whole Blood, by 12 hrs</td>
<td>Whole Blood, by 16 hrs; refrigerate plasma ≤ 28 days</td>
<td>PBMCs from whole blood by 30 hrs (Use T-Cell XTend if &gt; 8 hrs)</td>
</tr>
<tr>
<td>TB Antigen</td>
<td>ESAT-6 &amp; CFP-10 in separate wells</td>
<td>ESAT-6, CFP-10, &amp; TB 7.7 combined in blood draw tube</td>
<td>ESAT-6 &amp; CFP-10 in separate wells</td>
</tr>
<tr>
<td>Measured</td>
<td>Concentration of IFN-γ</td>
<td>Concentration of IFN-γ</td>
<td>Number spots (cells) producing IFN-γ</td>
</tr>
<tr>
<td>Results</td>
<td>Positive, negative, indeterminate</td>
<td>Positive, negative, indeterminate</td>
<td>Positive, negative, indeterminate, borderline</td>
</tr>
</tbody>
</table>
### IGRA vs. TST

<table>
<thead>
<tr>
<th>IGRA vs. TST</th>
<th>TST</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em></td>
<td><em>in vivo</em></td>
</tr>
<tr>
<td>Specific antigens</td>
<td>Less specific PPD</td>
</tr>
<tr>
<td>No boosting</td>
<td>Boosting</td>
</tr>
<tr>
<td>1 patient visit</td>
<td>2 patient visits</td>
</tr>
<tr>
<td>Results possible in 1 day</td>
<td>Results in 2 - 3 days</td>
</tr>
<tr>
<td>Stimulate within hours</td>
<td>Read in 48 - 72 hrs</td>
</tr>
<tr>
<td>Specialized lab test</td>
<td>Point-of-care test</td>
</tr>
</tbody>
</table>

### Evaluation of IGRAs

- **Problems:**
  - Lack of "gold standard" for TB infection
  - Inconsistent methods and test interpretation
- **Sensitivity**
  - Sensitivity = # positives / # culture (+) people tested
- **Specificity**
  - Specificity = # negative / # low-risk people tested
- **Agreement** with TST
- **Association** with exposure
- Forecast subsequent TB disease

### IGRA Sensitivity

- **80% in subjects with untreated, culture + TB**
  - Similar for different IGRAs
  - Ranges in studies from 57 to 100%
  - Similar to TST sensitivity
- **95% if either +IGRA or +TST**

### IGRA Sensitivity

- **Sensitivity in subjects with LTBI**
  - Extrapolated
  - Unable to accurately measure
- **Longitudinal studies are needed**
**IGRA Specificity**

- 99% in subjects at low risk for LTBI
  - Ranges from 89 to 99.6%
  - Similar to TST
    - 15 mm cutoff
  - But appears more specific than TST
    - 10 mm cutoff
  - after BCG vaccination
  - with NTM disease

**Agreement with TST**

- Poor agreement may be a good thing?
- Agreement varies widely
- Positive TST & Negative IGRA discordance
  - Associated with
    - BCG, NTM: suggests IGRA more specific?
    - TB Prevalence: suggests TST more sensitive?
- Negative TST & Positive IGRA discordance
  - Infrequent, and still unexplained

**Association with exposure**

- Recent exposure is better associated with IGRA results than with TST results
  - Fewer unexposed are IGRA+ than TST +
  - Similar number positive if exposed
- Long-term forecasting of TB disease?

Available evidence + Expert opinion = Guidelines
Recommendations (1)

- TST or IGRAs (QFT-G; QFT-GIT; T-Spot) should be used as aids to diagnose infection with *M. tuberculosis*.
  - using FDA approved test formats, and in compliance with CCLIA standards.
  - reporting both the qualitative interpretation and quantitative measurements.
  - arranging for IGRA testing prior to blood collection.
- As with the TST, IGRAs should not be used for testing persons with low risk of infection and low risk of disease due to *M. tuberculosis* (with exception for those likely to be at increased risk in the future).

Recommendations (2)

- IGRAs may be used in place of (and generally not in addition to) TST in all situations in which CDC recommends tuberculin skin testing (with noted preferences and special considerations).
  - Despite the indication of a preference, use of the alternative test (FDA-approved IGRA or TST) is acceptable medical and public health practice.
- Test selection should be based on the reasons for testing, test availability, and overall cost effectiveness of testing.

Recommendations (3)

- An IGRA is preferred for testing persons from groups that historically have poor rates of return for TST reading.
- An IGRA is preferred for testing persons who have received BCG (as a vaccine or for cancer therapy).
- TST is preferred for testing children younger than 5 years old.
Recommendations (4)

• IGRAs or TST (without preference) to test recent contacts of persons with infectious tuberculosis with special considerations for follow-up testing.
  – Negative results prior to 8 wks typically should be confirmed by repeating the test 8–10 weeks after the end of exposure
  – Repeating the same test minimizes misclassification due to test discordance
• IGRAs may be used in place of TST (without preference) for periodic screening that addresses occupational exposure (with special considerations regarding conversions and reversions because criteria for interpreting changes in IGRA results that identify new infection remain uncertain).

Recommendations (5)

• Sequential TST & IGRA may be useful if the initial test is negative and:
  – the risk of infection, the risk of progression, and the risk of a poor outcome are high (such as when persons with HIV infection, or children < 5 years old are at increased risk for M. tuberculosis infection), or
  – there is clinical suspicion for active tuberculosis (such as in persons with symptoms, signs, and/or radiographic evidence suggestive of active tuberculosis) and confirmation of M. tuberculosis infection is desired.

Recommendations (6)

• Sequential TST & IGRA may be useful if the initial test is positive and:
  – 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); or
  – in healthy persons who have a low risk of both infection and progression.

Recommendations (7)

• Repeating an IGRA or administering a TST may be useful when the initial IGRA result is indeterminate, borderline, or invalid, and a reason for testing persists.
• Each institution and TB control program should evaluate the availability, overall cost effectiveness, and benefits of IGRAs in prioritizing IGRA use in their own setting.
Recommendations (8)

- A diagnosis of *M. tuberculosis* infection, and decisions about medical or public health management should include epidemiological, historical, and other clinical information when using IGRA or TST results.

- Persons with a positive TST or IGRA result should be evaluated for:
  - likelihood of *M. tuberculosis* infection
  - risks of progression to tuberculosis disease if infected
  - symptoms and signs of tuberculosis disease.
  * With these risks, symptoms, or signs, additional evaluation is indicated.

Recommendations (9)

- A diagnosis of LTBI requires that tuberculosis disease be excluded by medical evaluation.

- In persons with symptoms, signs, or radiographic evidence of TB disease, and in those at high risk of progression to TB disease if infected, a positive result with either an IGRA or TST may be taken as evidence of *M. tuberculosis* infection.
  - However, negative IGRA or TST results are not sufficient to exclude infection in these persons.

Recommendations (10)

- In healthy persons who have a low likelihood both of *M. tuberculosis* infection and of progression to TB disease if infected, a single positive IGRA or TST result should not be taken as reliable evidence of *M. tuberculosis* infection.
  - Reevaluate to confirm lack of risk and consider repeat testing on a case-by-case basis; or
  - Alternatively, assume, without additional testing, that the initial result is falsely positive.

Recommendations (11)

- In persons with discordant test results (one positive and the other negative) decisions about medical or public health management requires individualized judgment in assessing:
  - the quality & magnitude of each result,
  - the probability of infection,
  - the risk of disease if infected, and
  - the risk of a poor outcome if disease occurs.
Need For Additional Research

- Further studies should be focused on determining the value and limitations of IGRAs in situations critical to TB control.
  - Are IGRAs better at predicting subsequent tuberculosis disease than TST?
  - Do IGRAs perform differently in children as compared to adults?
  - Discordance: why do simultaneously performed TST, QFT-GIT, QFT-G, and T-Spot results differ?

Controversies

- Pediatrics
- Boosting after tuberculin skin test
- Sequential testing and stability of results
- Longitudinal (predictive, prognostic) value
  - Can IGRAs forecast who will get TB?
  - Can they forecast who will not get TB?

Forecast Study Design

- Selected population
- Enrolled subjects
- Test for infection
  - Positive result
  - Negative result
- Follow-up period
  - TB disease
  - Disease free
  - TB disease
  - Disease free

Six Studies: Direct Comparison of IGRA with TST for Prognosis of TB Disease

<table>
<thead>
<tr>
<th>Author/IGRA</th>
<th>Place</th>
<th>Population</th>
<th>Number*</th>
<th>Mean Follow-up</th>
<th>IGRA Performance</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higuchi/QFT-G</td>
<td>Japan</td>
<td>School contacts</td>
<td>200</td>
<td>12 mo.</td>
<td>Non-inferior †</td>
<td>Little or no transmission</td>
</tr>
<tr>
<td>Leung/T-Spot:TB</td>
<td>Hong Kong</td>
<td>Silicosis patients</td>
<td>241</td>
<td>29 mo.</td>
<td>Superior</td>
<td>TB after neg. and high rates</td>
</tr>
<tr>
<td>Bakir/ELISpot</td>
<td>Turkey</td>
<td>Pediatric contacts</td>
<td>908</td>
<td>16 mo.</td>
<td>Non-inferior †</td>
<td>Explored combined tests</td>
</tr>
<tr>
<td>Harstad/QFT-GIT</td>
<td>Norway</td>
<td>Asylum seekers</td>
<td>820</td>
<td>23–32 mo.</td>
<td>Non-inferior †</td>
<td></td>
</tr>
<tr>
<td>Dell/QFT-GIT</td>
<td>Germany</td>
<td>Household contacts</td>
<td>953</td>
<td>42 mo.</td>
<td>Superior †</td>
<td>All cases in children found</td>
</tr>
</tbody>
</table>

* Recalculated for comparability † Greater efficiency with IGRA
References on Prognosis (p.1)


Reference on Prognosis (p.2)


Reference on Prognosis (p.3)


Acknowledgements

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