Interferon Gamma Release Assays (IGRAs)

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Tuberculosis (TB)

- Caused by *Mycobacterium tuberculosis*
- Leading cause of infectious disease mortality globally
- Two forms:
  - Latent TB infection (LTBI)
  - Active TB disease
- Detection and treatment of LTBI is critical for TB control

Image: James Archer, CDC Public Health Image Library (PHIL)
Tests for TB Infection

- Two types of tests available to detect TB infection
  - Tuberculin skin test (TST)
  - Interferon gamma release assays (IGRAs)
- Both are indirect tests for *M. tuberculosis* that measure cellular immune response to mycobacterial protein antigens
- If testing positive, active TB must be ruled out (no gold standard diagnostic test for LTBI)
Tuberculin Skin Test (TST)

• Before 2001, the only commercially available immunologic test for TB infection in the United States (US)
• In the US, performed by the Mantoux method
• Limitations:
  • False positive results may occur due to BCG vaccination or nontuberculous mycobacterium (NTM) infection
  • Patients must return to a healthcare provider for test reading
  • Inter- and intrareader variability

Photograph of positive tuberculin skin test. Image credit: David Kopanoff, CDC Public Health Image Library (PHIL)
Interferon Gamma Release Assays (IGRAs)

- Developed to overcome TST limitations
- *In vitro* blood tests
- Recommended by CDC as a *diagnostic aid* for TB infection
- First FDA-approved in 2001
- Two available types in the US:
  - QuantiFERON Gold Plus (Qiagen)
  - T-SPOT. *TB*® (Oxford Immunotec Ltd)
Basis of IGRAs

1. APC

2. APC

3. APC - T cell

4. IFN-γ - T cell
IGRAs: Basic Procedure

1. Blood collection from patient
2. Exposure of T cells in blood to *M. tuberculosis*-derived antigens (ESAT-6, CFP10)
3. Measurement of IFN-gamma
4. Patient sample results compared with positive and negative control values
QuantiFERON Gold Plus

1. Blood collection, tube shaking
2. Incubation
3. Plasma removal
4. Add plasma, conjugate
5. Incubation
6. Measure optical density (OD)
QuantiFERON Gold Plus

1. Blood collection, tube shaking
   - Incubation
   - Plasma removal

4. Add plasma, conjugate
   - Shake plate (mix)

2. Incubation
   - Centrifugation

5. Incubation
   - Wash
   - Enzyme substrate
   - Incubate
   - Enzyme stop

3. Plasma removal
   - Measure optical density (OD)

T-SPOT. TB®

1. Blood collection
   - Centrifugation

4. Add samples, antigens, and controls to T-SPOT plate
   - Incubate
   - Wash

2. PBMC transfer
   - Centrifuge/wash
   - Count PBMCs

5. Add conjugate
   - Add substrate

3. Prepare PBMC suspension
   - Centrifuge/wash

6. Count spots
IGRA Result Possibilities

**QuantiFERON Gold Plus**
- Positive
- Negative
- Indeterminate

**T-SPOT. TB®**
- Positive
- Negative
- Borderline
IGRA Performance Characteristics

**Sensitivity** 80-90%+
- most studies conducted among patients with *culture-confirmed* active TB (biased)
- lower among patients with immunosuppression (HIV, immunosuppressive therapy), children < 2 years old

**Specificity** *generally* 95%+ for LTBI in settings with low TB incidence
- studies conducted among low-risk individuals with no known risk factors
IGRA Strengths & Limitations

**Strengths**

- No cross-reactivity with BCG or *most* non-tuberculous mycobacteria
- Results in 24-48 hours
- Only one patient visit needed for result
- No boosting effect

**Limitations**

- Pre-analytic sources of variability
- Lower sensitivity in immunosuppressed, young children (↑ false negatives)
- Can’t distinguish LTBI from active TB
- Cross-reactivity with *M. marinum*, *M. szulgai*, *M. flavescens*, and *M. kansasii*
IGRAs (and TSTs) should be used as aids in diagnosing *M. tuberculosis* infection; can be used for surveillance or to identify persons likely to benefit from treatment:

- people at increased risk for TB: contacts of active TB patients, people from areas w/ high incidence of TB, etc.
- people at increased risk for progression if infected: immunosuppressed patients, children < 5 yrs, etc.

IGRAs (and TSTs) should NOT be used for
- testing persons with low risk for infection and progression to active TB if infected.
- monitoring anti-TB treatment response
### CDC 2010 IGRA Testing Guidelines (cont.)

[https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm](https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm)

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<tr>
<th>IGRA preferred</th>
<th>TST preferred</th>
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<td>• Groups with low rates of return for TST read (e.g. homeless persons, drug-users)</td>
<td>• Children aged &lt; 5 years old</td>
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<td>• Persons who have received BCG</td>
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**Either test acceptable**

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<td>• Recent contacts of active TB patients</td>
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<td>• Periodic screening of persons at risk for occupational exposure (e.g. HCWs)</td>
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**Consider both tests**

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<td>• Initial test negative, but high suspicion for active TB, or risk for infection/progression increased</td>
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<tr>
<td>• Initial test positive, but risk for infection/progression low</td>
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In Summary...

- Interferon gamma assays (IGRAs) are a diagnostic aid for TB infection
- Detect IFN-gamma release from T cells in response to TB-derived antigens
- Sensitivity and specificity generally high but can be lower among some patient groups (interpret results in context)
- Keep in mind strengths and limitations
- CDC guidelines for IGRA use: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5905a1.htm
References

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