IGRA guidelines

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and
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• The speaker has no financial involvement with any organization or entity with a financial interest in the subject matter of materials discussed.

• This presentation is given without any financial rewards.
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For whom are these IGRA guidelines intended?

- These IGRA guidelines are intended for all healthcare workers concerned about diagnosing latent tuberculosis infection (LTBI).

- IGRAs should not replace the standard diagnostic methods (microbiology, molecular tests, clinical and radiological assessment) for diagnosing active TB.

- A negative IGRA does not rule out active TB.
What are IGRAs?
IGRA = Interferon-γ Release Assay

M. tuberculosis genome

Cole ST et al. Nature 1998; 393: 537-544
Behr MA et al. Science 1999; 284: 1520-1523

M. tuberculosis
H37Rv
4,411,529 bp

ESAT-6
CFP-10
RD-1
IGRAs are more specific for *M. tuberculosis* infection


<table>
<thead>
<tr>
<th>Strain tested</th>
<th>Antigens</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESAT-6</td>
<td>CFP 10</td>
<td></td>
</tr>
<tr>
<td><strong>Tuberculosis complex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M tuberculosis</em></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>M africanum</em></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>M bovis</em></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>
</tr>
<tr>
<td>tice</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>tokyo</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<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>
<tr>
<td><strong>Environmental strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M abcessus</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M avium</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M branderi</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M celatum</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M cheloneae</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M fortuitum</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M gordonii</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M intracellulare</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M kansasii</em></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>M malmoense</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M marinum</em></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>M oenavense</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M scrofulaceum</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M smegmatis</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M szulgai</em></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>M terrae</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M vaccae</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M xenopi</em></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Which IGRAs are available?

- **Measure $\Delta$ IFN-$\gamma$ concentration**
  - e.g. QuantiFERON®-TB Gold In-Tube
    - Whole Blood stimulated with TB antigens
    - Measure IFN-$\gamma$ by ELISA

- **Measure $\Delta$ # of cells releasing IFN-$\gamma$**
  - e.g. T SPOT. $TB^\text{®}$ (ELISpot)
    - PBMCs stimulated with TB antigens
    - Count spots

Indeterminate results: test vs. host failure

- **High background IFN-γ**
  - (abnormal negative control)
    - Concurrent illness
    - Mitogen put in wrong well (nil)
    - Defective tubes

- **Low mitogen**
  - (abnormal positive control)
    - Transient or chronic immune suppression
    - GFT-G or T-SPOT: no mitogen in control well
    - QFT-GIT: defective tubes, overfilling, inadequate shaking
IGRAs: time interval to conversion

- Interval for positive conversion following exposure to a patient with active TB is unclear
  - **TST**: 2-12 weeks → 8 weeks
  - **IGRA**:
    - NICE guidelines (UK): 6 weeks
    - CDC guidelines (USA): 8-10 weeks
    - ERS guidelines (EUR): 8 weeks

- Recent study:
  “IGRA conversion generally occurred 4-7 weeks after exposure, although it could be as late as 14-22 weeks!”

Erkens CGM et al. *ERJ* 2010; 36: 925-949

What are the (dis)advantages of IGRAs?

<table>
<thead>
<tr>
<th></th>
<th>TST</th>
<th>IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-reactivity with BCG</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cross-reactivity with NTM</td>
<td>Yes</td>
<td>Unlikely</td>
</tr>
<tr>
<td>Negative/positive control</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Reliability/reproducibility</td>
<td>Moderate &amp; variable</td>
<td>High</td>
</tr>
<tr>
<td>Boost effect</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Patient visits</td>
<td>Two</td>
<td>One</td>
</tr>
<tr>
<td>Trained personnel required</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Laboratory infrastructure required</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Time to obtain result</td>
<td>3days</td>
<td>1-2days</td>
</tr>
<tr>
<td>Material costs</td>
<td>Low</td>
<td>Moderate to high</td>
</tr>
</tbody>
</table>

Evaluation of IGRAs

Lack of “gold standard” for LTBI!

- **Sensitivity** → Compare to culture
  - Sensitivity: # positives/# culture (+) people tested

- **Specificity** → Subjects at low risk for LTBI
  - Specificity: # negative/# low-risk people tested

- Accuracy of IGRAs
- Agreement with TST
- Positive results vs. exposure
- Predicting TB disease
## Performance of IGRA test

### Sensitivity

<table>
<thead>
<tr>
<th>Series</th>
<th>Diagnostics</th>
<th>Subject</th>
<th>Studies n</th>
<th>Summary sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QFT-G</td>
<td>TB patients, adult</td>
<td>21</td>
<td>0.80 (0.78–0.82)</td>
</tr>
<tr>
<td>2</td>
<td>QFT-G-IT</td>
<td>TB patients, adult</td>
<td>6</td>
<td>0.74 (0.69–0.78)</td>
</tr>
<tr>
<td>3</td>
<td>QFT-G/G-IT</td>
<td>TB patients, child</td>
<td>9</td>
<td>0.82 (0.75–0.87)</td>
</tr>
<tr>
<td>4</td>
<td>QFT-G/G-IT, T.SPOT</td>
<td>HIV-infected TB patients</td>
<td>5</td>
<td>0.70 (0.60–0.79)</td>
</tr>
<tr>
<td>7</td>
<td>T.SPOT</td>
<td>TB patients</td>
<td>13</td>
<td>0.90 (0.86–0.93)</td>
</tr>
<tr>
<td>8</td>
<td>TST</td>
<td>Healthy subjects</td>
<td>20</td>
<td>0.77 (0.71–0.82)</td>
</tr>
</tbody>
</table>

### Specificity

<table>
<thead>
<tr>
<th>Series</th>
<th>Diagnostics</th>
<th>Subject</th>
<th>Studies n</th>
<th>Summary specificity (96% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QFT-G/G-IT</td>
<td>Healthy young adults</td>
<td>12</td>
<td>0.98 (0.97–0.99)</td>
</tr>
<tr>
<td>2</td>
<td>QFT-G/G-IT</td>
<td>Healthy young adults, BCG⁻</td>
<td>8</td>
<td>0.99 (0.98–1.00)</td>
</tr>
<tr>
<td>3</td>
<td>QFT-G/G-IT</td>
<td>Healthy young adults, BCG⁺</td>
<td>8</td>
<td>0.96 (0.94–0.98)</td>
</tr>
<tr>
<td>4</td>
<td>T.SPOT</td>
<td>Predominantly BCG vaccinated</td>
<td>8</td>
<td>0.93 (0.86–1.00)</td>
</tr>
<tr>
<td>5</td>
<td>TST</td>
<td>BCG not vaccinated</td>
<td>6</td>
<td>0.97 (0.95–0.99)</td>
</tr>
<tr>
<td>6</td>
<td>TST</td>
<td>BCG vaccinated</td>
<td>6</td>
<td>0.59 (0.46–0.73)</td>
</tr>
</tbody>
</table>

IGRAs:
negative predictive value

QFT-GIT pooled: 0.88

T-SPOT.TB pooled: 0.94

IGRAs:
NPV for progression to active TB

QFT-GIT pooled: 0.998
T-SPOT.TB pooled: 0.98

How should IGRAs be used in different population groups and settings?

1. Children
2. Immunocompromised patients
3. HIV-infected patients
4. Anti-TNF therapy patients
5. Contact tracing
6. Screening of occupational healthcare workers
7. High-incidence TB settings/populations
IGRAs:
clinical evidence base

Over 1,000 studies published
 Evidence published in all key clinical groups, including:
 TB suspects, healthcare workers, immunosuppressed (e.g. TNF-alpha, HIV, oncology, renal failure), contact tracing

BUT... still many areas of uncertainty!!
IGRAs should be used to detect/screen for latent tuberculosis (LTBI)

Always rule out active disease!!
(microbiology, molecular tests, clinical and radiological assessment)
Use of IGRAs in children

- **Children <5 years**: increased risk of infection and of developing active disease after exposure to contagious case
- **Children >5 years**: same immune response to TB infection as in healthy adults

- Available evidence is too scant to change current recommendations
- Essential to achieve highest sensitivity of detection when diagnosing LTBI, especially in children <5 years old

**TST** remains preferred test for detection of LTBI

**TST + IGRA** can increase sensitivity

When both tests are performed, treatment should be given in case of a positive result for either one of tests
Use of IGRAs in immunocompromised patients

- Primary vs. secondary immunodeficiency → **heterogeneous group** of patients
- TST: low sensitivity (→ cut-off 5mm should be used)
- **IGRAs have higher sensitivity** but is this high enough to rule out TB infection?
  → ‘probably’ YES in low-incidence settings/populations

<table>
<thead>
<tr>
<th>Two-step approach:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1/ TST</strong></td>
</tr>
<tr>
<td><em>positive</em> → IGRA only if BCG vaccinated, otherwise LTBI</td>
</tr>
<tr>
<td><em>negative</em> → IGRA</td>
</tr>
<tr>
<td><strong>2/ IGRA</strong></td>
</tr>
<tr>
<td><em>positive</em> → LTBI</td>
</tr>
<tr>
<td><em>negative</em> → most probably no LTBI (low-incidence setting)</td>
</tr>
</tbody>
</table>
Use of IGRAs in HIV-infected patients

- Upon diagnosis, all HIV-infected patients should undergo screening for latent TB!

- TST low sensitivity (and specificity) in HIV-patients!

- IGRA
  - High specificity
  - Sensitivity considerably higher compared to TST *but* ...
    - False-negative results!
    - More indeterminate results!! ~ CD4-cell count

<table>
<thead>
<tr>
<th>CD4 count (cells/µL)</th>
<th>Indeterminate results (%)</th>
<th>Total number of subjects tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;200</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>51-200</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td>≤50</td>
<td>30</td>
<td>109</td>
</tr>
</tbody>
</table>

Use of IGRAs in HIV-infected patients

- Upon diagnosis, all HIV-infected patients should undergo screening for latent TB!

- TST low sensitivity (and specificity) in HIV-patients!

1/ CD4 cells ≤500/ml → **IGRA**
   - IGRA positive: LTBI
   - IGRA negative: probably no LTBI

2/ CD4 cells >500/ml → **TST** (cut-off 5mm)
   - TST positive: LTBI (IGRA if BCG vaccinated)
   - TST negative → IGRA
Use of IGRAs in anti-TNF therapy patients

- Always rule out active TB (history, chest X-ray, sputum exam)!

- **TST negative** (<5 mm): no LTBI
  only if no immunocompromising conditions present and/or if no high-risk contact!

- **TST positive** (≥10 mm): LTBI (BCG vaccinated → IGRA)

- **TST intermediate** (5-9 mm) or negative with immunocompromising condition present and/or high-risk contact → IGRA

- IGRA
  *positive* → LTBI
  *negative* → most probably no LTBI (low-incidence setting)
Use of IGRAs in contact tracing

- **To test = to treat!**

- A negative test, performed within the pre-allergic phase (6-8 weeks, range 2-12 weeks), should be repeated 8-12 weeks after last potential contact.

  **Pre-allergic time period: IGRA = TST**

- Most country guidelines favor a **two-step approach** (positive TST → IGRA) to increase specificity

- **Belgium:**
  - low-incidence setting (<20/100,000)
  - low BCG vaccination status
  - IGRA: higher price, no reimbursement, not readily available everywhere
Use of TST and IGRA in contact tracing
(adults, children ≥5 yrs)

Rule out active TB!

- **TST**
  - NEGATIVE: NO LTBI
  - POSITIVE: IGRA
    - NEGATIVE: NO LTBI
    - POSITIVE: LTBI

(cut-off can be chosen low [5mm] to increase sensitivity)

(BCG vaccinated, ...)
Use of IGRAs in occupational HCW screening

• IGRAs have **some advantages**
  – higher specificity
  – no induction of booster effect

• **Lack of data on optimal cut-offs** for serial testing by IGRA

• **Unclear** interpretation and prognosis of IGRA conversions and reversions

1/ Pre-employment: **TST** (cf. contact tracing)

2/ No change in the strategy based on **TST** serial testing seems to be justified
Use of IGRAs in high-incidence populations

- Many people have LTBI
- High level of BCG-vaccination
- Increased exposure to NTM
- Increased exposure to \textit{M. leprae} homologues or IGRA-antigens

IGRAs have no added value to diagnose LTBI

Focus of prevention and control is to identify and treat active cases
What are the practical considerations of IGRAs in Belgium?
QuantiFERON®-TB Gold In-Tube (QFT-GIT)

Stage 1: Whole Blood Culture

- Collect 1mL of blood in 3 tubes
- Incubate at 37ºC for 16-24 hours.
- Centrifuge 5 minutes to separate plasma above gel

Stage 2: Measure [IFN-γ] & Interpret

- Collect 50 µL of plasma for ELISA
- Measure [IFN-γ] in ‘Sandwich’ ELISA
- Software calculates results and prints report

Cellestis. www.cellestis.com
QuantiFERON®-TB Gold In-Tube 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>≥ 0.5</td>
</tr>
<tr>
<td>Indeterminate</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></td>
<td>Any</td>
<td>&gt; 8.0</td>
<td>Any</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.
QuantiFERON®-TB Gold In-Tube
Practical considerations

- Turn-around time (TAT): 26h for single test (lab hands-on 50min)
- Cost: ~45€ (not reimbursed at the moment)
- **Advantages:**
  - Can be automated
  - Can be ‘batched’
  - No interobserver differences
  - More broadly available in Belgium
- **Disadvantages:**
  - Amount of Tcells tested is variable
  - Short time before incubation (16h)
  - Slightly less sensitive than T-SPOT
**T-SPOT. TB**

1. Collect blood sample, centrifuge to separate white blood cells which are washed and counted to maximise sensitivity.

2. Add WBCs [●] & specific TB antigens [●] to wells pre-coated with antibodies to IFN-γ [●] and incubate overnight (37°C, CO₂).

3. IFN-γ [●] is released from activated T cells. Wash wells, add secondary conjugated antibody [●]. Incubate for 1 hour.

4. Wash wells, add substrate and incubate for 7 minutes. Stop reaction with water. One spot [●] is the footprint of one activated T cell.

Panel A
- Nil Control
- ESAT-6 Panel A
- CFP 10 Panel B

Panel B
- Negative Result
- Positive Control
- Positive Result

OxfordImmunotec. www.oxfordimmunotec.com
## 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>≥ 6 spots</td>
<td>&lt; 10 spots</td>
<td>any</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 5 spots</td>
<td>&lt; 10 spots</td>
<td>&gt; 20 spots</td>
</tr>
<tr>
<td>Borderline</td>
<td>5, 6, or 7 spots</td>
<td>&lt; 10 spots</td>
<td>&gt; 20 spots</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>≤ 5 spots</td>
<td>&lt; 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.

Oxford Immunotec. www.oxfordimmunotec.com
T-SPOT. TB
Practical considerations

• Turn-around time (TAT): 24h for single test (lab hands-on 3-4h)
• Cost: ~60€ (not reimbursed at the moment)
• **Advantages:**
  – Amount of Tcells standardised (250,000/well)
  – Slightly more sensitive than QFT, especially in immunocompromised pts
  – Data about non-sanguinuous fluids… but not licenced!
• **Disadvantages:**
  – Cannot be automated, longer hands-on time
  – Cannot be ‘batched’
  – Inter-observer differences possible (in counting spots…)
  – Not available everywhere
Still an open question…

TST

IGRA