The Clinical Utility of IGRAs to Diagnose TB Infection in Pediatric Patients

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QIAGEN is pleased to welcome:

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Overview

• Background
• Case #1 (Dr. Cameron)
• QFT-Plus results: a systematic approach to interpretation (Dr. Kawamura)
• Case #2 (Dr. Cameron)
• QFT package insert update by QIAGEN (Parth Patel)

Summary

Q&A
Most M.TB is transmitted to children by adults (or adolescents)
A child newly diagnosed with TB is a sentinel event indicating recent transmission in a community.
Why does it matter?

- Risk of progression influenced by age
- Decades of potential benefit from treatment
- Excellent tolerability of TB medications

<table>
<thead>
<tr>
<th>Age at infection (y)</th>
<th>No disease (%)</th>
<th>Pulmonary TB (%)</th>
<th>CNS TB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>50</td>
<td>30-40</td>
<td>10-20</td>
</tr>
<tr>
<td>1-2</td>
<td>75-80</td>
<td>10-20</td>
<td>2.5</td>
</tr>
<tr>
<td>2-5</td>
<td>95</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>5-10</td>
<td>98</td>
<td>2</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>&gt;10</td>
<td>80-90</td>
<td>10-20</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

Cruz AT, Starke JR. Peds in Review 2010;31:13
Children with TB infection = reservoir for disease
Important TB Testing Considerations

• A child with suspected TB disease/at high risk of infection → disease
  • High sensitivity is imperative
  • Risk of missing a diagnosis is high
• TB screening in high TB-endemic setting or contact investigation
  • Goal, maximize PPV
• TBI screening in a low-incidence population
  • High specificity is imperative
  • Risk of overtreatment is high
## Comparison of Skin Test & IGRA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TST</th>
<th>IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigens studied</td>
<td>Many -PPD</td>
<td>ESAT-6, CFP-10, (TB-7.7)</td>
</tr>
<tr>
<td>Cross-reactivity with BCG</td>
<td>Yes</td>
<td>Unlikely</td>
</tr>
<tr>
<td>Cross-reactivity with NTM</td>
<td>Yes</td>
<td>Less Likely*</td>
</tr>
<tr>
<td>Estimated sensitivity, TB in non-US born children &gt; 2 yrs</td>
<td>50-75%</td>
<td>77-95%</td>
</tr>
<tr>
<td>Estimated specificity, TB in non-US born children &gt; 2 yrs</td>
<td>72-75%</td>
<td>90-93%</td>
</tr>
<tr>
<td>Distinguish TB infection vs. disease</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Boosting</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Patient visits required</td>
<td>Two</td>
<td>One</td>
</tr>
</tbody>
</table>

# AAP Red Book Recommendations

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>2015</th>
<th>2018</th>
<th>2021</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>≥ 5 years</td>
<td>≥ 2 years</td>
<td>TST recommended for &lt;2y, IGRA acceptable</td>
</tr>
<tr>
<td>Preferred test for BCG-immunized children</td>
<td>Yes</td>
<td>Unchanged</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Use in immunocompromised children</td>
<td>Cautiously</td>
<td>Unchanged</td>
<td>Unchanged</td>
</tr>
</tbody>
</table>

*States that some experts use down to 1 year of age; any negative result (IGRA or TST) should be interpreted cautiously in infants < 3 months of age*
Canadian Tuberculosis Standards 8th edition regarding children

- Either TST or IGRA in most situations, except:
  - IGRA preferred if:
    - BCG in infancy and age 2-10
    - BCG at an older age
    - Expertise to perform TST not available

https://www.tandfonline.com/doi/full/10.1080/24745332.2022.2036503
Clinical Case #1

- 9yo, Hispanic F
  - Immigrant from Colombia, BCG immunized
- Arrived in Houston, TX in January 2021
  - With mother and 4mo sibling
  - Father has lived in the US for >12 years
    - Does not recall TB testing, none recent
    - Asymptomatic
    - Mother had an IGRA (negative), CXR (normal)
- Sibling – negative PPD
- No additional TB risk factors
• Referred to TB clinic for evaluation of a “positive” QFT
  • Obtained during immigration process to the US
  • No quantitative value provided
Tuberculosis in Columbia

- 41 cases/100,000 (WHO)
  - “moderate”
- 21,000 cases in 2021
  - 1,100 MDR/RR (5%)
  - 2,450 TB associated deaths
  - 69% HIV negative
PMH

• Previously healthy
• No prior hospitalizations
• No regular medications
• Immunizations UTD (including BCG)
• NKMA
Physical Exam

• Well appearing
• No cervical adenopathy
• BCG scar over L deltoid
• Lungs clear, no axillary adenopathy
• Normal abdominal exam
• No rash
Prior evaluation

- Testing performed in Columbia prior to arrival
- Quantiferon “positive”
  - No quantitative value provided
• So what did we do?
Final diagnosis: No TB infection
Reflection

• Why was the QFT repeated?
• What made you confident that the US result was not a false negative?
• Did the QFT-Plus quantitative antigen and controls have any value?

Step 1: Systematically assessing both qualitative and quantitative results

- Review of control values
- Review of antigen tube results
QuantiFERON® TB Gold Plus

<table>
<thead>
<tr>
<th>Nil control</th>
<th>TB 1 Antigen</th>
<th>TB2 Antigen</th>
<th>Mitogen control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ and CD8+ T-Cells</td>
<td>None</td>
<td>CD4+ and CD8+ T-Cells</td>
<td>All</td>
</tr>
</tbody>
</table>

**Polypeptides**
- Long peptides (MHC class II)
- Additional short peptides (MHC class I)

**Antigens**
- ESAT-6
- CFP-10
- ESAT-6
- CFP-10

TB1 and TB2 results should be similar if no reactivity to CD8 antigens in the sample
QuantiFERON-TB Gold Plus (QFT-Plus) is a Qualitative test

<table>
<thead>
<tr>
<th>Nil (IU/ml)</th>
<th>TB1 minus Nil (IU/ml)</th>
<th>TB2 minus Nil (IU/ml)</th>
<th>Mitogen minus Nil (IU/ml)</th>
<th>QFT-Plus result</th>
<th>Report/Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤8.0</td>
<td>≥0.35 and ≥25% of Nil</td>
<td>Any</td>
<td>Any</td>
<td>Positive</td>
<td>M. tuberculosis infection likely</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>≥0.35 and ≥25% of Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.35 OR ≥0.35 and &lt;25% of Nil</td>
<td>≥0.5</td>
<td>Negative</td>
<td>M. tuberculosis infection NOT likely</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;8.0</td>
<td>Any</td>
<td></td>
<td>&lt;0.5</td>
<td>Indeterminate</td>
<td>Likelihood of M. tuberculosis infection cannot be determined</td>
</tr>
</tbody>
</table>

Positive results by TB1, TB2, or both are considered positive.

Step 1: Assessing both qualitative and quantitative results

Review positive and negative control quantitative values to:

• Assess the quality of the host response
• Assess potential technical error

What to expect in a healthy patient (not immunocompromised with NO risk of false negative or indeterminate results)

• Nil value close to zero
• Mitogen value near 10 IU/ml or above
Step 1: Assessing both qualitative and quantitative results

Review quantitative values of antigen (TB1 and TB2) to:

- Assess values in light of control results
- Assess potential technical error (eg. TB1>>>TB2)

What to expect?

- TB1 and TB2 values should be close in value or TB2>TB1 if CD8 antigen reactivity
Indeterminate QFT results – Causes

High Nil (＞8 IU/ml)

- May indicate:
  - Excessive levels of circulating IFN-γ (e.g., another infection)
  - Presence of heterophile antibody
  - Incorrect sample handling or processing

- High Nil indeterminates are rare
  - In clinical studies*, < 0.25% of subjects had IFN-γ＞8 IU/ml for Nil

Mitogen – Nil ＜0.5 IU/ml

- Biologic causes: see next slide
- Technical causes:
  - Incorrect sample handling
    - ＞16 hours from blood specimen draw to incubation
    - Transportation / incubation at incorrect temperature
  - Inadequate shaking of tubes - most common cause in manual handling
  - Overfilling of tubes
TST and IGRA test interpretation:
Risks for false-negative or indeterminate results

Host factors affecting TST and likely IGRAs

- HIV- low CD4, no ARVs
- Recent TB infection (<8 weeks)
- Infections (viral, fungal, bacterial)
- Other illness affecting lymphoid organs
- Recent live virus vaccination
- Immunosuppressive drugs
- Overwhelming TB
- Malnutrition
- Age (newborn, elderly)

If results are unexpectedly negative or indeterminate…ASK: Does my patient have any of these factors?
Step 2: Review of TB epidemiologic and progression risks
Population TB Exposure Risks warranting TB testing

- Recent contacts of a TB case
- Immigrants from high-prevalence countries (in Canada <50 cases/100,000 pop considered low incidence)
- Injection drug users
- Residents and employees of high-risk congregate settings (e.g., correctional facilities, nursing homes, homeless shelters, hospitals, and other health care facilities)
- Mycobacteriology laboratory personnel
- Children under 5 years of age, or children and adolescents exposed to adults in high-risk categories

Source: https://www.cdc.gov/tb/publications/factsheets/testing/skintestresults.htm
Estimated risk for TB relative to persons with no known risk factor

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>110~170 times</td>
</tr>
<tr>
<td>HIV infection</td>
<td>50~110</td>
</tr>
<tr>
<td>Solid Organ Transplant</td>
<td>20~74</td>
</tr>
<tr>
<td>Silicosis</td>
<td>30</td>
</tr>
<tr>
<td>Recent TB infection (&lt;2 years)</td>
<td>15</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>10~25</td>
</tr>
<tr>
<td>Carcinoma of head and neck</td>
<td>16</td>
</tr>
<tr>
<td>Abnormal chest radiograph with upper lobe fibro nodular disease typical of healed TB infection</td>
<td>6~19</td>
</tr>
<tr>
<td>TNF Alpha inhibitor therapy</td>
<td>1.7~9</td>
</tr>
<tr>
<td>Glucocorticoid therapy</td>
<td>4.9</td>
</tr>
<tr>
<td>Children less than 4 years old</td>
<td>2.2~5</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2~3.6</td>
</tr>
<tr>
<td>Underweight (BMI &lt;20)</td>
<td>2~3</td>
</tr>
<tr>
<td>Smoker (1 pack/ day)</td>
<td>2~3</td>
</tr>
<tr>
<td>Normal healthy individual</td>
<td>1</td>
</tr>
</tbody>
</table>

Source: Lobue and Menzies, Respirology 2010
Step 3: Putting it together: National guidelines on interpretation
National US recommendations on retesting based on risk

- Low to intermediate risk of progression from LTBI
  - No recommendation for repeat testing
  - Consider INFECTED if single test is positive

- Unlikely to be infected
  - If test positive, consider repeat or dual testing to maximize SPECIFICITY
  - A negative results from either test would be considered NEGATIVE
  - Considered infected only if BOTH tests are positive

- High risk of progression
  - If initial test result is negative, consider repeat or dual testing to maximize SENSITIVITY
  - A positive result from either/any test would be considered POSITIVE

Both TB infection tests may be used sequentially in the following situations:

- If either the TST or IGRA are negative, the other test may be used to increase sensitivity if the risk for infection is high, the risk for progression to tuberculosis disease is elevated, the risk for a poor outcome from tuberculosis disease is high and/or a person has conditions or habits that may reduce the sensitivity of the test.

- If the initial tuberculin skin test is positive, but the likelihood of tuberculosis infection is low, or risk of a false positive result due to BCG is high, then an IGRA may be used to increase specificity
US National guidance on test interpretation and clinical management depend on risk of TB infection or risk of progression and do not refer to quantitative values as low or high for TST, QFT or TSPOT.

Canadian National guidance do not refer to quantitative values as low or high for TST, QFT or TSPOT but unlike the US guidelines, both IGRA have borderline zones:

“When the initial IGRA result is borderline (equivalent to 5-7 spots with T-SPOT.TB or 0.2 to 1.0 IU/ml with QFT), the interferon-gamma release assay may be repeated or a tuberculin skin test used to help arrive at a diagnosis”
Clinical Case #2

• 2 mo, U.S. born, African-American, ex-full term M
• Asymptomatic
• Exposed to father - Smear positive and Xpert MTB PCR positive
  • Moved to the U.S. from Cameroon in early 2021
  • Household contact
Evaluation

• Well appearing
• Weight 6.4kg, 80%
• Normal exam
• Does a “wicked positive” QFT suggest disease over infection?
• Hyper-aerated lungs are clear.
• No lymphadenopathy identified.

Is the lung hyper-aeration concerning?
Admitted to TCH

• Work up initiated for disseminated TB
• First morning gastric aspirates x3 collected
  • Smear negative
  • MTB PCR negative
  • Cultures pending
Mediastinal, paratracheal and hilar lymph nodes

9 mm solid pulmonary nodule in LUL.
CNS Evaluation

• LP/CSF evaluation
  • WBC 16 (slightly elevated, L 72%)
  • RBC 2,000
  • Protein 99 (slightly elevated)
  • Glucose normal

• MRI brain w and w/o contrast
  • No evidence of TB meningitis

• Ophthalmology exam
  • Normal
Management

- PO levofloxacin, 10 mg/kg, BID (4/27/22 - present)
- PO isoniazid 150mg once daily (4/29/22 - present)
- PO linezolid 90 mg daily (4/29/22 - present)
- IV imipenem 100 mg q12 (4/29/22 - present)
  - +Augmentin (amoxicillin-clavulanate ES 40mg/kg dose) (4/29/22 - present)
Update: Source Case – Susceptibility

• Silent RIF mutation (molecular)
• DST – pan-susceptible
• RIPE therapy
Definitive Management/Clinical Course

- Gastric aspirate & CSF cultures – negative, final
- Treatment:
  - RIP+levofloxacin x2 months → INH/RIF
  - High dose RIF
- Repeat CXR @ 2 months – normal
- Normal growth & development while on treatment
- Treated for 6 months
Reflection

• Would you have done an additional test if the initial IGRA or TST was negative?
• What concerns are there for using IGRA in children <2?
• What made you confident that the result was not a false positive?
• Did the QFT-Plus quantitative antigen and controls values provide useful information?
• What role does QFT-plus play in contact investigation?

Study done in 16 sites across US
Interferon-g Release Assays in Children <15 Years of Age*

Largest prospective longitudinal pediatric study, compared predictive values of IGRAs vs. TST

- 3593 Children enrolled
- 92% of the children born outside US
- 25% (n = 900), <5 years, 6% (n= 219), <2 years

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Interferon-g Release Assays in Children <15 Years of Age* (2)

- Percentage of positive tests, by age group, for non-US-born children

Both IGRAs track appropriately with age; TST does not.
TST+/IGRA-discordance is highest in <5 age group (likely influenced by BCG vaccination)

### Performance of TST and IGRAs Based on Incident Cases

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST</td>
<td>50</td>
<td>73.6</td>
<td>0.2</td>
<td>99.9</td>
</tr>
<tr>
<td>QFT</td>
<td>75</td>
<td></td>
<td>90.1</td>
<td>100.0</td>
</tr>
<tr>
<td>T SPOT</td>
<td>50</td>
<td></td>
<td>92.8</td>
<td>99.9</td>
</tr>
</tbody>
</table>

NOTE: Cohort contain both treated and untreated children

KEY FINDING on SPECIFICITY

- Specificities of IGRAs were high (90-93% VS 73%) compared to TST
  - ~70% fewer positive IGRA results than TST results in Foreign-born children

INDETERMINATE RESULTS

- <1 % had an indeterminate QFT or invalid T-SPOT.TB result

KEY FINDINGS ON PREDICTING DISEASE and ACCURACY of NEGATIVE RESULTS

- Sensitivity of QFT for incident cases was highest among the 3 assays
- NPV of QFT was 100%
  - Of the 533 TST+/IGRAs- high-risk children, none developed disease including 54 children <2 years of age.

Authors conclusion: IGRAs can be used in children of all ages

Evolution of QFT over the past 20 years

1st generation QuantiFERON®-TB
- 2001: FDA approval
- Measured cell-mediated immunity to tuberculin purified protein derivative (PPD)
- Breakthrough: TST becomes a blood test

2nd generation QuantiFERON®-TB Gold (liquid antigen)
- 2004: FDA approval
- “Liquid antigen” version
- Antigens specific for *M. tuberculosis* with 99% specificity
- Clinical benchmark: No cross reactivity with BCG

3rd generation QuantiFERON®-TB Gold (QFT® in tube)
- 2007: FDA approval
- Logistical advantage – remote incubation
- Lab benchmark: Scalable and easily automated
- >1200 peer reviewed publications
- >30 million tests sold

4th generation QuantiFERON®-TB Gold Plus (QFT®-Plus)
- Q4 2014: CE-IVD
- 2017: FDA approved
- Addition of patented CD8 antigens – potential biomarker of intracellular TB burden
- New flexible blood draw options

Cautions removed by FDA and Canada Health
What were the cautions in the PI?

• The performance of the format of the QFT-Plus test has not been extensively evaluated with specimens from the following groups of individuals:

  • Individuals who have impaired or altered immune functions, such as those who have HIV infection or AIDS, those who have transplantation managed with immunosuppressive treatment or others who receive immunosuppressive drugs (e.g., corticosteroids, methotrexate, azathioprine, cancer chemotherapy), those who have other clinical conditions, such as diabetes, silicosis, chronic renal failure, and hematological disorders (e.g., leukemia and lymphomas), or those with other specific malignancies (e.g., carcinoma of the head or neck and lung)

• Individuals younger than age 17 years

• Pregnant women
Package Insert Cautions Removed

- The cautions were in place based on scientific evidence that was not available at the time of QFT-GIT approval in 2017
- Voluminous data was submitted to FDA and Health Canada (2021 and 2022)
- Systematic review/meta-analysis was performed for each study population to provide evidence for removal of cautions

Rigorous evidence review was required by FDA and Health Canada leading to caution removal
What does this mean?

• Cautions removed for use in the following populations:
  • Pediatrics
  • Pregnant patient
  • Immunocompromised patient

• QFT-Plus is the only IGRA assay with multiple generations and has the most evidence out of all the IGRA

• Regardless of the PI caution removal, QFT-Plus can and has been used in all patient populations
Summary

1. Clinicians and laboratories can use QFT-Plus with confidence in all populations as they did with TST, including children of all ages

2. The greatest advantage of QFT-Plus is its accuracy in BCG vaccinated children

3. In contact investigation, QFT’s operational advantages streamlines and simplifies TB testing for clinicians and patients

4. No test is perfect, but there will be less false positive and false negative QFTs compared to TST

- A clinician’s systematic approach of reviewing the QFT-Plus lab results will improve clinical interpretation of qualitative results

- Remember why your pediatric patient was tested and the consequences of having TB or developing TB:

- A patient’s TB risk, symptoms and vulnerability to bad outcomes are central to result interpretation and clinical management
Questions?

https://www.mummypages.co.uk/images/2115/105/5/0_2/questions.jpg
Head-to-head sensitivity comparison
Interferon-γ Release Assay Performance for Tuberculosis in Childhood*

Cohort: N=360  2010-2015 California TB registry data (≤18 years) with laboratory-confirmed TB. 95 had both TST and IGRA

<table>
<thead>
<tr>
<th>Age group</th>
<th>TST</th>
<th>QFT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-18yrs (n=69)</td>
<td>83%</td>
<td>96%</td>
<td>.01 significant</td>
</tr>
<tr>
<td>2-4 yrs (n=11)</td>
<td>91%</td>
<td>91%</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>&lt;2 yrs (n=15)</td>
<td>87%</td>
<td>80%</td>
<td>&gt;.99</td>
</tr>
</tbody>
</table>

Indeterminate results= 4%  Associated with being <1 year old and central nervous system disease

Conclusions on largest North American study comparing TST and QFT as diagnostic aids for active TB
• Similar sensitivity in children <5 yrs old
• Reduced sensitivity of both assays in children <2 yrs
• In children ≥5 years, IGRA has greater sensitivity than TST and should be considered the preferred immunodiagnostic test

Question & Answer