How to interpret the new QuantiFERON- TB Gold Plus test (QFT-Plus)

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UNSOM
Sierra NV Veterans Affairs Med Center
January 17, 2019
Tuberculin skin test (TST) vs. QuantiFERON-TB Gold Plus

- **Manual** placement, reading and data entry
- **Two patient visits** required, high no-show rate
- **Subjective** test
- **No internal controls**
- Poor surveillance tool
- In-vivo test – causes boosting that can affect future testing

- Highly specific, **not affected by BCG**
- Results with **one patient visit**
- **Objective** test
- **Electronic results** (straight to EMR)
- **Internal controls** and broader picture of the immune response
- Does not cause boosting

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**TST is an inefficient and subjective tool that is not patient or program-centered**
γ-interferon is an inflammatory messenger that causes the induration seen in a positive PPD and is measured by the QFT-Gold Plus test.
Up till now, PPD and γ-interferon tests did not differentiate between latent and active TB - this has been solely a clinical evaluation.
## Evolution of ELISA IGRA Technology

<table>
<thead>
<tr>
<th>Generation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st generation</td>
<td><strong>Quantiferon™-TB</strong></td>
</tr>
<tr>
<td>2001: FDA approval</td>
<td>Measured cell-mediated immunity to tuberculin purified protein derivative (PPD)</td>
</tr>
<tr>
<td>Breakthrough: TST becomes a blood test</td>
<td></td>
</tr>
<tr>
<td>2nd generation</td>
<td><strong>Quantiferon™-TB Gold</strong> (liquid antigen)</td>
</tr>
<tr>
<td>2004: FDA approval</td>
<td>&quot;Liquid antigen&quot; version</td>
</tr>
<tr>
<td>Antigens specific for <em>M. tuberculosis</em> with 99% specificity</td>
<td></td>
</tr>
<tr>
<td>Clinical benchmark: No cross-reactivity with BCG</td>
<td></td>
</tr>
<tr>
<td>3rd generation</td>
<td><strong>Quantiferon™-TB Gold</strong> (QFT® in tube)</td>
</tr>
<tr>
<td>2007: FDA approval</td>
<td>Logistical advantage – remote incubation</td>
</tr>
<tr>
<td>Lab benchmark: Scalable and easily automated</td>
<td></td>
</tr>
<tr>
<td>&gt;1200 peer reviewed publications</td>
<td></td>
</tr>
<tr>
<td>&gt;30 million tests sold</td>
<td></td>
</tr>
<tr>
<td>4th generation</td>
<td><strong>Quantiferon™-TB Gold Plus</strong> (QFT®-Plus)</td>
</tr>
<tr>
<td>Q4 2014: CE-IVD</td>
<td></td>
</tr>
<tr>
<td>2017: FDA approved</td>
<td>Addition of patented CD8 antigens – potential biomarker of intracellular TB burden</td>
</tr>
<tr>
<td>New flexible blood draw options</td>
<td></td>
</tr>
</tbody>
</table>
Up till now, only CD4 cells (Helper cells) were tested by $\gamma$-interferon tests. The new **QTF Gold Plus** test now looks at not only CD4, but also CD8 response after TB antigen exposure.

- CD4 (Helper) cells– if they recognize TB antigens– will secrete $\gamma$ interferon. This response **does not tell us anything about disease activity**– just a Yes or No answer. A positive response still needs a clinical evaluation to determine infection versus active disease.

- The new Quantiferon Gold Plus test for the first time also tests CD8 cell response to TB antigens– these killer cells **only get activated and secrete $\gamma$ interferon in the presence of recent infection or active disease**. Unlike the persistent positive CD4 cell response, if the infection is old and not active– CD8 cells shut off and do not secrete $\gamma$ interferon.
2 Antigen tubes of QFT-Plus allows the calculation of the CD8 response

TB2 Minus TB1 (i.e. CD8/CD4 – CD4)

TB2 – TB1 (Nil subtracted)
Surrogate for isolated CD8 response

Is this is recent infection?
Are they more likely to progress?

Does this information help inform clinical practice?

A more comprehensive picture of the immune response: Potential for additional valuable information for risk stratification?
Multicenter study of QuantiFERON-TB Gold Plus in patients with active tuberculosis

Horne D, Narita M et al, IJTLD, 22(6):617–621, 2018

QFT-GIT Sensitivity 94.3% (vs. 93.02%) (P=0.16)
• No statistical difference in sensitivity
• Agreement was 98.7%, Kappa 0.89 (CI .75–1.00)

Other findings:
• TB2 greater than TB1: 99/157 or 63%
• TB1 greater than TB2: 39/157 or 25%
• TB1 and TB2 equal: 16/157 or 10.2%
• Median difference between TB2 and TB1 (nil subtracted): 0.14 IU/ml
Summary- how to interpret the QFT-Plus test

- **The Nil control** - baseline γ-interferon level should be low, immune system not activated. Must be <8 to make test interpretable, usually near 0.

- **Mitogen control** - stimulates all CD4 cells to produce γ-interferon. Are they healthy enough to react, and was the test set up properly? Or are the cells either deteriorated or is the immune system so compromised there is no immune response? Need Mitogen-Nil value >0.5 to allow for proper test interpretation.

- **TB1 Antigen** - exposes CD4 (helper cells) to 2 proteins only found in TB and sees if immune system recognizes them and secretes γ-interferon. Similar to “TB test tube” result in prior Quantiferon Gold test (3rd generation test). **TB1-Nil >0.35 is interpreted as positive**.

- **TB2 Antigen** - now exposes not only CD4 cells but also CD8 (Killer cells) to proteins only found in TB. So we now not only see the CD4 cell response, but it also throws in CD8 response. CD8 response is usually only significant when recent exposure to TB or with active disease, and declines with treatment. **TB2- Nil >0.35 is interpreted as positive**. However, the greater the TB2- TB1 difference, reflecting CD8 activity (particularly if >0.6 iu)- the more likely new infection or active disease may be present.
The QFT-plus test is interpreted as “positive” even if only one of the two TB tubes are >0.35 IU. This increases sensitivity but in low risk patients this may be a false positive.

**QFT-Plus versus QFT test result interpretation**

<table>
<thead>
<tr>
<th>QuantiFERON-TB Gold In Tube</th>
<th>QuantiFERON-TB Gold Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test value</strong></td>
<td><strong>IU/ml</strong></td>
</tr>
<tr>
<td>TB Nil (IU/ml)</td>
<td>≥0.35</td>
</tr>
<tr>
<td>TB2 Nil (IU/ml)</td>
<td>≥0.35</td>
</tr>
<tr>
<td>Both TB1 &amp; TB2 Nil (IU/ml)</td>
<td>≥0.35</td>
</tr>
</tbody>
</table>

**QFT-Plus:** Positive results by TB1, TB2, or both are considered positive. No change in cut-point or definition of indeterminate results
- Doubles the information from antigen tubes per test
- May be clinically useful when needing maximum sensitivity, as well as specificity
1. **Review control values to:**
   - Assess the quality of the host response
   - Assess potential technical error

   **What to expect** in a healthy patient with NO risk of false negative or indeterminate?
   - Nil value close to zero
   - Mitogen value near 10 IU/ml or above

2. **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
**WARNING:** A negative QFT-Plus results or lack of a CD8 response **does not rule out** TB disease, subclinical TB or TB that may progress

**However,** the **presence and strength** of the CD8 response may help clinical assessment of patients with risk of disease, new infection or progression of TB
Scenario 1: In a S. American country, a rheumatologist has 3+ smear positive active cavitary tuberculosis and coughing for 2 months

She takes the contact investigation upon herself. Her strain is pan-susceptible

She has 2 daughters (age 1 and 3), a baby sitter and husband in her home and visits with her sister frequently.

QFT-Plus is performed
Scenario 1: In a S. American country, a rheumatologist has active cavitary tuberculosis and coughing for 2 months

She has 2 daughters (age 1 and 3), a baby sitter and husband in her home and visits with her sister frequently.

QFT-Plus is performed 6/18
   Husband: Positive
   Babysitter: Positive
   Sister: Positive

   2 daughters: Negative
   Babysitter’s 6 yr old child: Negative
   (occasional contact)

CXRs performed on all household contacts: NORMAL

**Isoniazid started** on husband, babysitter, sister and 2 children (All asymptomatic with normal examinations)

Is primary prophylaxis of the QFT-negative daughters warranted?
TB EXPOSURE SCENARIO: CONTACT INVESTIGATION

Scenario 1: Examining the QFT results
Initial QFT June 2018 and repeated Sept 2018

Daughter age 3

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gama-Interferon</td>
<td>Negativo (Nil)</td>
</tr>
<tr>
<td>Quantiferon TB Gold Plus: Nil</td>
<td>0.06 Ul/L</td>
</tr>
<tr>
<td>Antiguo TB1 menos Nil</td>
<td>0.02 Ul/L</td>
</tr>
<tr>
<td>Antiguo TB2 menos Nil</td>
<td>0.02 Ul/L</td>
</tr>
<tr>
<td>Mitogeno menos Nil</td>
<td>Superior a 10.00 Ul/L</td>
</tr>
</tbody>
</table>

Check list:
- Control values
- Consistency of TB1 and TB2
- Change in values between tests?
- Conversion? Weak or strong?
Scenario 1: Examining the QFT results
Initial QFT June 2018 and repeated Sept 2018

Check list:
- Control values
- Consistency of TB1 and TB2
- Change in values between tests?

Findings:
- Nil closer to zero than 8.0 IU/ml
- Healthy mitogen responses
- Missing: 3 of 4 TB1 and TB2 values

Diagnosis: LTBI unlikely
Clinical management:
1. Obtain all TB1 and TB2 quantitative values
2. If values close to zero, stop INH
3. If values close to cut point consider continuing INH? ---conservative approach in setting with known transmission and highly vulnerable contact

Documented transmission hence, important to ensure thorough assessment
Scenario 2:
New QFT-plus discordant positive, then negative ....What is going on?

42 y/o Montana-born RN gets annual HCW screening
No travel or TB exposure
no meds, or medical problems
normal exam
negative TST in the past

<table>
<thead>
<tr>
<th>Year</th>
<th>Ag-nil IU/ml</th>
<th>QFT-GIT Ag tube</th>
<th>QFT-Plus TB1</th>
<th>QFT-Plus TB2</th>
<th>Result reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2017</td>
<td>0.45</td>
<td>0.01</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2017</td>
<td>0.03</td>
<td>0.00</td>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Was ATS guidelines followed?
Does a CXR need to be done?
Goal: Minimize non-reproducible positive results in serial testing of low-risk populations by testing hypothesis of using a conservative definition of QFT-Plus test conversion (TB1 & TB2 concordant positive)

Method: Prospective testing of 989 HCWs with QFT and QFT-Plus. LTBI risk obtained through questionnaire. 626 HCWs without any risks

Results:
• QFT - QFT-Plus agreement: 95.6% (95% CI, 94.3-96.9) Kappa= 0.57
• QFT/QFT-Plus discordant results (84.8%) fell within the range of 0.2-0.7 IU/ml
• Positive rate = 2.1% QFT and 3.0% for QFT-Plus
• **Conservative definition of positive for QFT-Plus 1.0% (CI, 0.2-1.7) , P = 0.0002**
• Follow-up testing: 90.9% (10/11 QFT-Plus) had a negative QFT result.

Conclusions:
• QFT-Plus had high degree of agreement with QFT
• Conservative interpretation of QFT-Plus identified nearly all non-reproducible positive results

**Interpretation?**

<table>
<thead>
<tr>
<th>NAME</th>
<th>VALUE</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantiFERON Incubation</td>
<td>Incubation performed.</td>
<td></td>
</tr>
<tr>
<td>QuantiFERON-TB Gold Plus</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>QuantiFERON Criteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The QuantiFERON-TB Gold Plus result is determined by subtracting the Nil value from either TB antigen (Ag) tube. The mitogen tube serves as a control for the test.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QuantiFERON TB1 Ag Value</td>
<td>0.59</td>
<td>(IU/mL)</td>
</tr>
<tr>
<td>QuantiFERON TB2 Ag Value</td>
<td>1.09</td>
<td>(IU/mL)</td>
</tr>
<tr>
<td>QuantiFERON Nil Value</td>
<td>0.08</td>
<td>(IU/mL)</td>
</tr>
<tr>
<td>QuantiFERON Mitogen Value</td>
<td>&gt;10.00</td>
<td>(IU/mL)</td>
</tr>
</tbody>
</table>

Clinical Information: SRC:UR

PERFORMING LAB: LabCorp Phoenix, 5005 S 40th Street Ste 1200, Phoenix, Phone - 8007889743, Director - MD
PERFORMING LAB: LabCorp Seattle, 550 17th Avenue Ste 300, Seattle, Phone - 2068617000, Director - MD
PERFORMING LAB: LabCorp Burlington, 1447 York Court, Burlington, Phone - 8007624344, Director - MD

Clinical Information: SRC:UR
HCW with a false positive QFT-plus on routine yearly testing. Previously neg. QFT-Gold and repeat QFT-Plus was also negative.

<table>
<thead>
<tr>
<th>Report Released Date/Time: Aug 08, 2018@09:31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provider: ACCINELLI, DIANA M</td>
</tr>
<tr>
<td>Specimen: BLOOD, SEND 0805 40</td>
</tr>
<tr>
<td>Specimen Collection Date: Aug 05, 2018@15:45</td>
</tr>
<tr>
<td>Test name</td>
</tr>
<tr>
<td>QUANTIFERON-TB</td>
</tr>
<tr>
<td>Eval: A negative Quantiferon-TB result does not preclude the possibility of</td>
</tr>
<tr>
<td>Eval: M. tuberculosis infection or tuberculosis disease: false negative results</td>
</tr>
<tr>
<td>Eval: can be due to stage of infections, co-morbid conditions which affect</td>
</tr>
<tr>
<td>Eval: immune functions, or other individual immunological factors.</td>
</tr>
<tr>
<td>Eval: A positive Quantiferon-TB result might not indicate infection with</td>
</tr>
<tr>
<td>Eval: M. tuberculosis. Reactivity to protein present in other mycobacteria may</td>
</tr>
<tr>
<td>Eval: cause false positive responses.</td>
</tr>
<tr>
<td>Eval: A positive Quantiferon-TB result should be followed by further medical</td>
</tr>
<tr>
<td>Eval: and diagnostic evaluation for tuberculosis disease (e.g., AFB smear and</td>
</tr>
<tr>
<td>Eval: culture. chest X-Ray).</td>
</tr>
</tbody>
</table>

**Comment:**
QF Nil = 0.08
QF TB AG1 = 0.2
QF TB AG2 = 0.49
QF Mitogen = >10
QF TB AG1-nil = 0.12
QF TB AG2-nil = 0.41
QF MITOGEN-NIL >10

Test performed at Nevada State Health Laboratory, Reno, NV.

Performing Lab Sites
[654] IOANNIS A. LOUGARIS VAMC [CLIA# 2900988228]
975 KIRMAN AVE, RENO, NV 89502-0993
Any concerns?

**Order**

[Image of a medical order form]

**View SmartLink Info**

Quantiferon Gold Plus TB (4 tube) (Order #185455845) on 12/16/18

**Component Results**

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
<th>Ref Range &amp; Units</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT NIL</td>
<td>0.53</td>
<td>IU/mL</td>
<td>Final</td>
</tr>
<tr>
<td>QFT TB1 - NIL</td>
<td>0.38</td>
<td>&lt;=0.34 IU/mL</td>
<td>Final</td>
</tr>
<tr>
<td>QFT TB2 - NIL</td>
<td>0.25</td>
<td>&lt;=0.34 IU/mL</td>
<td>Final</td>
</tr>
<tr>
<td>QFT Mitogen - NIL</td>
<td>&gt;10.00</td>
<td>IU/mL</td>
<td>Final</td>
</tr>
<tr>
<td>QFT Gold Plus</td>
<td>Positive!</td>
<td>Negative</td>
<td>Final</td>
</tr>
</tbody>
</table>

**Comment:**

POSITIVE: M. tuberculosis infection likely. The predictive value of a positive QUANTIFERON result in diagnosing M. tuberculosis infection depends on the probability of infection, which is assessed by historical, epidemiological and other findings. A positive QUANTIFERON-Plus result should be followed by further medical/diagnostic evaluation for active M. tuberculosis infection.

The TB1-NIL tube specifically detects CD4+ lymphocyte activity stimulated by peptide antigens that simulate mycobacterial
Is this LabCorp interpretation correct?

**QuantIFERON-TB Gold Plus**

**NAME**

- **F QuantIFERON Incubation**
  - Incubated, testing to follow.
- **F QuantIFERON-TB Gold Plus**
  - Positive
- **F QuantIFERON Criteria**
  - The QuantIFERON-TB Gold Plus result is determined by subtracting the Nil value from either TB antigen (Ag) tube. The mitogen tube serves as a control for the test.
- **F QuantIFERON TB1 Ag Value** 0.04 (IU/mL)
- **F QuantIFERON TB2 Ag Value** 8.29 (IU/mL)
- **F QuantIFERON Nil Value** 0.05 (IU/mL)
- **F QuantIFERON Mitogen Value** 0.05 (IU/mL)

**Clinical Information:**

PERFORMING LAB: LabCorp Phoenix, 5005 S 40th Street Ste 1200, Phoenix, Phone - 8007680743, Director - MDCollum

PERFORMING LAB: LabCorp Seattle, 550 17th Avenue Ste 300, Seattle, Phone - 2068617000, Director - MDTowneill

PERFORMING LAB: LabCorp Burlington, 1447 York Court, Burlington, Phone - 8007624344, Director - MDHancock

**Clinical Information:**

Nawara, Christopher M, 10/27/1969
Is this Renown lab interpretation correct?

<table>
<thead>
<tr>
<th>Component</th>
<th>Your Value</th>
<th>Standard Range</th>
<th>Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT NIL</td>
<td>&gt;10.00 IU/mL</td>
<td>IU/mL</td>
<td></td>
</tr>
<tr>
<td>QFT TB1 - NIL</td>
<td>0.30 IU/mL</td>
<td>&lt;=0.34 IU/mL</td>
<td></td>
</tr>
<tr>
<td>QFT TB2 - NIL</td>
<td>-0.15 IU/mL</td>
<td>&lt;=0.34 IU/mL</td>
<td></td>
</tr>
<tr>
<td>QFT Mitogen - NIL</td>
<td>-0.15 IU/mL</td>
<td>IU/mL</td>
<td></td>
</tr>
<tr>
<td>QFT Gold Plus</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

NEGATIVE: M. tuberculosis infection NOT likely. A negative result must be considered with the individual's medical and historical data relevant to the probability of M. tuberculosis infection and potential risk of progression to tuberculosis disease, particularly for individuals with impaired immune function. Negative predictive values are likely to be low for persons suspected to have M. tuberculosis disease and should not be relied on to exclude disease.
Why factors may cause an equivocal result?

**Order**

View SmartLink Info
Quantiferon Gold Plus TB (4 tube) (Order #1849555826) on 12/9/18

**Component Results**

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT NIL</td>
<td>0.31</td>
</tr>
<tr>
<td>QFT TB1 - NIL</td>
<td>0.07</td>
</tr>
<tr>
<td>QFT TB2 - NIL</td>
<td>0.00</td>
</tr>
<tr>
<td>QFT Mitogen - NIL</td>
<td>0.00</td>
</tr>
<tr>
<td>QFT Gold Plus</td>
<td><strong>Equivocal</strong></td>
</tr>
</tbody>
</table>

**Comment:**
EQUIVOCAL: Results are indeterminate for TB antigen responsiveness. Indeterminate results are uncommon and may be related to the immune status of the individual being tested, but may also be related to a number of technical factors. Clinicians can choose to redraw a specimen or perform other procedures as appropriate.

**Lab Information**

Lab
Hyperspace - UNR ME... Cline, Kevin E.
Highlights of the New QFT- Plus Test

- This new test allows blood to be drawn in only one tube, and the test doesn’t have to be rushed to lab same day
- CD4 cells recognize TB, **whether or not disease is active or old**
- The TB1 tube tests CD4 activity only- a positive result tells you the patient has been exposed to Tuberculosis- but tells you nothing about Latent vs Active infection
- CD8 cells are immune cells that are only active in new or active infections, and turn off in old or inactive infection (latent TB)
- In Tube 2, CD4 cells are now tested with CD8 cells- giving you some idea if this TB infection is actually recent or active. The greater the difference between TB2 and TB1 (reflecting activated CD8 cells), the more likely this is possible.
- Be careful – look at control values (nil and mitogen) first to be sure test is interpretable, then look at TB1 and TB2 tubes
- Low risk patients- if only one of the 2 TB tubes are positive- worth repeating test as likely a false positive result. Maybe these patients shouldn’t be tested in first place
Some of these slides are taken from this excellent presentation available online.

What’s the ‘Plus’ in QuantiFERON-TB Gold Plus? 
A clinical update of next generation TB testing

L. Masae Kawamura, M.D.
Senior Director, Medical and Scientific Affairs, TB Diagnostics, Global, QIAGEN