QuantiFERON TB Gold In-Tube testing in the Public Health Laboratory

“..the greatest needs in the United States are new diagnostic tools for the more accurate identification of individuals who are truly infected and who are also at risk of developing tuberculosis”

*US Institute of Medicine Report, “Ending Neglect”; 2000*

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Discussion Overview

• Implementation strategies and issues related to the use of the QuantiFERON Gold In-tube assay in Public Health Laboratories
• Logistical strategies related to specimen remote incubation and transport
• Assay implementation and performance
• Reporting criteria
• Testing sustainability within a public health laboratory setting
TB Screening Test Evolution

QTF-TB, 2001

TB Skin Test 1912

QTF-TB Gold 2005

QTF-TB Gold In-Tube 2007
QuantiFERON®-TB Gold In-tube (QTF-IT)

- Based on the quantitative measurement of IFN-γ secreted from stimulated T cells in human whole blood
  - T-cells reactive to *M. tuberculosis* - specific antigens are only present in those infected
  - Utilizes three peptides that simulate three proteins called ESAT-6, CFP-10 and **TB7.7(p4)**
    - absent from all *M.bovis* BCG strains and from most NTMs
- A two stage process:
  - Incubation of whole blood with TB-specific and immune function control antigens (mitogen/+ and nil/= assay controls)
  - Detection of IFN-γ using a rapid, single-step, sandwich ELISA
Stage 1. Specimen Collection (TRAINING)

Collect ~ 1 mL of blood in each tube per patient: Nil, TB antigen, Mitogen
* Tube volume must be between 0.8 to 1.2mL to avoid rejection.
* Tubes draw slowly. Use a purge tube for butterfly needles.

After collection, **vigorously mix** each tube for ~ 5 or 10 seconds to ensure adequate mixing of the blood/tube.
* Necessary for optimal test results.
Then..... Logistics

• Immediately ship to lab for incubation
  – Sample must be received within 16 hours of blood collection and incubated upright at 37°C for 16 to 24 hours.

  or

• Incubate at collection site within 16 hours of blood collection for 16-24 hours, then ship samples to lab within 3 days:
  – After incubation, samples are stable for up to 3 days at 2-27°C WITHOUT centrifugation
Stage 2

IFN-γ ELISA

Perform ELISA manually or using an automated system.

Centrifuge tubes at 2,000-3,000 x g for 15 minutes.

After centrifugation, plasma samples can be stored up to 8 weeks at 2° to 8° C or below -20° C for extended periods.

Measure OD to determine IFN-γ levels. Software calculates results and prints reports. LIS can be interfaced.
Result Reporting - Qual. vs Quan.

**Positive**

TB Ag – Nil ≥ 0.35 IU/ml and ≥ of 25% of Nil

Nil ≤ 8.0 IU/mL

Mitogen response: Any

**Negative**

TB Ag – Nil < 0.35 IU/mL

or

TB Ag- Nil ≥ 0.35 IU/mL and < 25% of Nil

Nil of ≤ 8.0 IU/mL

Mitogen - Nil response: ≥ 0.5 IU/ml

**Indeterminate**

Mitogen < 0.5 IU/mL (Low Mitogen)

or

Nil > 8.0 (High Nil)
QTF-IT Performance Survey

• 9 Public Health Laboratories surveyed

• Survey Criteria
  – Pre-Analytical Procedure
    • Specimen Incubation and Transport Practices
  – Analytical
    • Testing Process including method, platform and algorithm
  – Reporting
    • Qualitative vs Quantitative, interpretation, disclaimers/comments
Incubation Process

• 2 out of 9 survey sites allowed samples to be incubated by the PHL only
  – Removes external client incubation error
  – Requires delivery of samples to PHL within 16 hours of collection
    • ↑ in indeterminate results linked to ↑ hold time prior to incubation (Herrera et al.)

• 7 out of 9 survey sites allowed samples to be incubated either off-site or in-PHL
  – Clinics, laboratories (CLIA certification required), hospitals, Health Departments
Incubation Process

- In-Lab Tube Incubation Time Monitored
  - All 9 Labs

- Off-Site Tube Centrifugation Performed
  - All 7 Labs

- Off-Site Tube Incubation period monitored
  - All 7 Labs

- Incubator QC monitored off-Site
  - 2 Unsure
  - 5 Yes
Specimen Transport Mechanisms

- PHL Courier System: 7
  - Incubated Off-Site
  - Transport for specimens
- Private Courier: 1
  - Incubated In-Lab
  - Transport for samples
- Both Private and PHL Courier: 1
  - Incubated In-Lab
  - Transport for samples
  - Incubated Off-Site
  - Transport for specimens
Assay Implementation and Performance

- 7 labs perform method manually
- 2 labs perform method on automated platform
  - Assay manufacturer recommends Dynex DSX or DS2
    - Instrument software performs required calculations
    - LIMS Instrument interface issues
  - Bio-Rad Evolis is another platform option
    - Flex-E required for calculations
    - Instrument flags are not read by QTF software
Assay Quality Control

• All 9 labs cited the performance of quality control
  – Kit includes 4 standards that are assayed in duplicate or triplicate.
    • Must calculate the line of best fit, coefficient of variation between standard replicates, and the correlation of coefficient of the standard curve

  – 4 out of 9 labs included external quality control
    • Pooled patient sera
      – Mitogen, + TB antigen, Nil sample pools
    • Diluted Assay Standards
Data Reporting

• 4 labs released qualitative data only
  – Positive, Negative, Indeterminate

  – Comments include “Epidemiologic and clinical findings should be considered when interpreting QFT-G results and assessing the probability of TB infection and disease”
- TEST -               - RESULT -               - REFERENCE -

QUANTIFERON-TB GOLD    NEGATIVE           NEGATIVE
QUANTIFERON-TB GOLD

MYCOBACTERIUM TUBERCULOSIS INFECTION UNLIKELY, BUT CANNOT BE EXCLUDED
ESPECIALLY WHEN ANY ILLNESS IS CONSISTENT WITH TB DISEASE OR THE
LIKELIHOOD OF PROGRESSION TO DISEASE (E.G., BECAUSE OF
IMMUNOSUPPRESSION) IS INCREASED.

NOTE: DIAGNOSING OR EXCLUDING TUBERCULOSIS DISEASE, AND ASSESSING THE
PROBABILITY OF LTBI, REQUIRE A COMBINATION OF EPIDEMIOLOGICAL,
HISTORICAL, MEDICAL, AND DIAGNOSTIC FINDINGS THAT SHOULD BE TAKEN INTO
ACCOUNT WHEN INTERPRETING QUANTIFERON-TB GOLD RESULTS. SEE GENERAL
GUIDANCE ON THE DIAGNOSIS AND TREATMENT OF TB DISEASE AND LTBI
(http://WWW.CDC.GOV/NCHSTP/TB/).
5 labs released qualitative and quantitative data

- 4 labs provide IFN-γ value interpretations
  
  • $< 0.35$ IU/mL, Negative
    
    - Mtb infection is unlikely. Correlate with medical history as well as other clinical information.
  
  • $\geq 0.35$ IU/mL, Positive.
    
    - For a healthy person with a low likelihood of infection, a single positive result should not be taken as evidence of tuberculosis. Recommend repeat testing with a new sample or by using an alternative method if result not supported by clinical symptoms. Correlate with medical history as well as other clinical information.
  
  • Indeterminate
    
    - Uncertain likelihood of Mtb infection. Repeat testing in 4 to 6 weeks if clinically indicated. A high background or a lack of response to antigen stimulation prevent an interpretation related to TB to be made.
TEST REQUESTED: QUANTIFERON®-TB GOLD (IN TUBE) RESULTS (IU/ML)

<table>
<thead>
<tr>
<th>NIL IU/ML</th>
<th>TB – NIL IU/ML</th>
<th>MITOGEN – NIL IU/ML</th>
<th>RESULT</th>
<th>RESULT INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤8.0</td>
<td>≥0.35 AND ≥ 25% NIL VALUE</td>
<td>ANY</td>
<td>POSITIVE</td>
<td>M. TUBERCULOSIS INFECTION LIKELY</td>
</tr>
<tr>
<td>≤8.0</td>
<td>&lt; 0.35</td>
<td>≥ 0.5</td>
<td>NEGATIVE</td>
<td>M. TUBERCULOSIS INFECTION NOT LIKELY</td>
</tr>
<tr>
<td>≤8.0</td>
<td>≥ 0.35 AND &lt; 25% OF NIL VALUE</td>
<td>&lt;0.5</td>
<td>INDETERMINATE</td>
<td>INDETERMINATE FOR TB-ANTIGEN RESPONSIVENESS</td>
</tr>
<tr>
<td>&gt; 8.0</td>
<td>ANY</td>
<td>ANY</td>
<td>Indeterminate</td>
<td></td>
</tr>
</tbody>
</table>

THE PERFORMANCE OF QUANTIFERON®-TB GOLD (IN TUBE) HAS NOT BEEN DETERMINED IN CHILDREN YOUNGER THAN 17 YEARS OF AGE.
Quantiferon-TB Result Calculation in IU/mL
Nil 0.06  TB Ag 0.67  Mitogen >10
TB Ag - Nil = 0.61
Mitogen - Nil = >10

Criteria for Positive:
When NIL is <= 8.0 AND TB Ag minus Nil is >= 0.35 and >= 25% of Nil value

Criteria for Indeterminate:
When NIL is <= 8.0 AND TB Ag minus Nil is <0.35 or >= 0.35 and < 25% of Nil value, AND Mitogen minus Nil is < 0.5.

> When NIL is > 8.0

Interpretation of Quantiferon-TB Gold Test for Latent M. tuberculosis:

Positive:
Indicates specific T-cell response to antigens of M. tuberculosis. Followup evaluation and management should be the same as for a positive TB skin test. Note that M. kansasii, M. szulgai, and M. marinum infections may also exhibit a positive result.

Negative:
Patient is unlikely to have latent TB infection. Note that false negative results are possible in early disease or in patients with cancer or altered immune function. Repeat QFTB testing at 8-10 weeks after exposure is recommended for persons with recent exposure to an infected patient.

Indeterminate:
Cannot interpret results. T-cells were either stimulated in absence of antigen or failed to be stimulated by non-specific mitogen. If repeat testing is warranted, a new blood sample must be obtained.
- 6 out of 9 labs provided guidance regarding the cause of an indeterminate result on report.

- A high background interferon production (High Nil) does not allow an interpretation related to tuberculosis to be made.

- A lack of response to antigen stimulation (Low Mitogen) does not allow an interpretation related to tuberculosis to be made and may indicate immunosuppression.
Additional QTF-IT Practices

• 3 out of 9 labs utilize a LIMS interface.

• Common Assay Repeat Triggers
  – 3 labs repeat values close to the assay cut-off.
    • 0.35-0.70 for positives
    • 0.20 – 0.34 for negatives
  – 1 lab repeated samples based on patient’s previous QTF-IT result
  – Quality Control Failures
To Repeat or Not To Repeat

- 0 of the 9 labs routinely repeat positive specimens for result confirmation.

- Package Insert Statement
  - Where *M. TB* infection is not suspected, initially positive results can be confirmed by retesting the original plasma samples in duplicate. If repeat testing of one or both replicates is positive, the test result is considered positive.”

- Should all positive specimens be repeated in triplicate BEFORE result release?
From April 2009 until July 2010, MPHL repeated all initially positive QTF specimens in duplicate (if possible) for result verification.

- Results for 91 patients were compared
  - 80 patient results repeated upon duplicate testing
  - 11 patient results did not repeat
    - 9 repeated as negative and were released as indeterminate
      » 4 of the above 9 were QTF positive with repeat samples collected within 2-4 weeks
    - 2 repeated as indeterminate and were released as such
      » Both patients presented with negative repeat samples

Due to low sample volume, many samples could only be repeated once.

- Plug material can cause high repeat assay values
- 2 samples may have had tubes inverted upon repeat
QTF-IT Population Targets for PHLs

• Individuals who have received BCG vaccination
• Transient populations that will not return for TST reading
• Individual that present with Positive TST that do not have any additional clinical indications
• High Risk Populations- Known contacts
Sustainability in a PHL

• Client expansion
  – Pilot study to determine feasibility
  – Incubation and Transport are the primary barriers
  – QTF can replace TST for use in many populations

• Billing
  – Private Insurance, Medicaid, or Medicare

• Health Department Program Funding
  – Test costs versus clinician costs
  – Target certain populations through program initiatives
Questions?

Special Thanks

Dr. Angela Starks
Frances Tyrrell

MPHL Immunology Laboratory Staff
Joann Young
Shemeria Littleton-Wilson
Christian Nevarez
Romanda Redfield
Donna Hall