Contact Investigation
San Antonio, Texas
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New Tools in the Diagnosis of Tuberculosis
Mary Long, MSPH
July 19, 2007

New Tools in the Diagnosis of Tuberculosis

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Heartland National TB Center
July 19, 2007
Objective

• Describe new diagnostic tools for tuberculosis:
  – Rapid Detection Methods
  – QuantiFERON-Gold
  – Elispot or T-Spot TB Assay
  – Genotyping

Laboratory Background

• CDC Lab Performance Criteria, 1995
  – Smear (AFB) results within 24 hours
  – Identification of TB within 10-21 days
  – Drug susceptibility results in 15-30 days
Laboratory Background

• CDC Recommendations July 2000
  – Nucleic Acid Amplification Test (NAAT) on first sputum and on first smear-positive specimen
  – Rapid detection in 85-90% of patients
  – Rapid confirmation of smear positive patients
  – Detection of 75% smear-negative TB disease cases

Rapid Detection Methods
Nucleic Acid Amplification Test (NAA) or Direct Amplified Nucleic Acid Test (DAT)

• Direct test on patient specimens for M. tuberculosis
  – M. tuberculosis direct test-MTD (Gen-Probe)
    • Performed on smear + or – specimens
    • Sensitivity 91-95%; specificity >99%
  – Amplicor TB test (Roche)
    • Performed on smear positive specimens only
    • Sensitivity 80-92%; specificity >99%
Rapid Detection Methods
MTD (Gen-Probe) or Amplicor

- Both utilize PCR technology to amplify rRNA specific to *M. tb*.
- Turn around time is 4-6.5 hours
- **FDA approved on respiratory specimens only**

Rapid Detection Methods
NAA/DAT Advantages

- Rapid detection on direct specimens
- Affects treatment decisions (TB or NTM)
- Reduces health costs
  - Isolation
  - Contact Investigations
Rapid Detection Methods

NAA/DAT Disadvantages

• Tests only for *M. tuberculosis*
• Expensive
• Complex test
• Approved only on respiratory specimens (some use in CSF)
• Contaminated specimens pose problems
• Cannot be used to monitor therapy

QuantiFERON-Gold

QFT-G

• Diagnostic aide for *M. tuberculosis* infection, whether TB disease or latent TB infection
QuantiFERON-Gold

**QFT-G**

- *In-vitro* enzyme immunoassay that utilizes a peptide cocktail simulating ESAT6 and CFP10 proteins to stimulate white blood cells to secrete interferon-gamma
  - ESAT6 is early secretory antigen
  - CFP10 is culture filtrate protein 10

**QuantiFERON-Gold**

**QFT-G**

- ESAT6 and CFP10 are found in:
  - *M. tuberculosis*
  - *M. kansasii, marinum, szulgai*
  - *NOT BCG and all sub strains*
  - *NOT* in more than 20 other mycobacteria tested
QuantiFERON-Gold
QFT-G

- Needs viable white blood cells
- Uses heparinized (lithium only) whole blood (minimum of 5 ml needed)
- Specimen needs to be in lab within 12 hours of draw (transport at room temp)
- Total laboratory test time 16-24 hours
QuantiFERON-Gold

QFT-G

• Each specimen is run with the ESAT6 and CP10 AND
  – Phytohemaglutin (M) – tests to see if you have viable cells that are producing interferon-γ
  – Saline (0) – negative control to measure background level of interferon-γ

Interpretation of QFT-G* results form IFN-γ concentrations in test samples

<table>
<thead>
<tr>
<th>ESAT6-nil* or CFP10-nil* or both</th>
<th>Nil</th>
<th>Mitogen-nil**</th>
<th>QFT-G Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.35 IU/ml* and &gt;50% above nil</td>
<td>Any</td>
<td>Any</td>
<td>Positive</td>
<td>M. tuberculosis infection likely</td>
</tr>
<tr>
<td>&lt;0.35 IU/ml</td>
<td>≤0.7</td>
<td>≥0.5</td>
<td>Negative</td>
<td>M. tuberculosis infection unlikely but cannot be excluded, especially when illness is consistent with TB disease and likelihood of progression to TB disease is increased</td>
</tr>
<tr>
<td>&lt;0.35 IU/ml</td>
<td>Any</td>
<td>&lt;0.5</td>
<td>Indeterminate</td>
<td>QFT-G results cannot be interpreted as a result of low mitogen response</td>
</tr>
<tr>
<td>≤50% above nil</td>
<td>&gt;0.7</td>
<td>Any</td>
<td>Indeterminate</td>
<td>QFT-G results cannot be interpreted as a result of high background response</td>
</tr>
</tbody>
</table>

* QuantiFERON®-TB Gold test
1 Interferon-gamma
* The IFN-γ concentration in the blood incubated with a mixture of synthetic peptides simulating early secretory antigenic target-6 (ESAT6) minus the IFN-γ concentration in blood incubated with saline.
* The IFN-γ concentration in the blood incubated with a mixture of synthetic peptides simulating culture filtrate protein-10 (CFP10) minus the IFN-γ concentration in blood incubated with saline.
* IFN-γ concentration in the blood incubated with mitogen minus the IFN-γ concentration in blood incubated with saline.
** International units per mL.
QuantiFERON-Gold

• **Advantages**
  – Requires single patient visit to draw blood
  – Results can be ready in 24 hours
  – Uniform results (no reader bias as with TST)
  – Not affected by prior BCG vaccination
  – No booster phenomenon

• **Disadvantages**
  – Not readily available, very few labs routinely offer test
  – Cost and timing make it difficult for routine, wide-spread use
  – Labs need to batch specimens so turn around time is usually longer than 24 hours
QuantiFERON-Gold

• More Disadvantages
  – Blood must be processed within 12 hours after collection (need viable white blood cells)
  – Limited data on use of test in children younger than 17 years
  – Limited data on use of test on immunocompromised people

QuantiFERON-Gold

• CDC recommends QFT-G to be used in all circumstances that the TST would currently be used – especially contact investigations
• Use IN PLACE OF, not in addition to the TST
QuantiFERON-Gold

• A positive QFT-G should elicit the same evaluation and patient management as a positive skin test

• Negative QFT-G in persons recently exposed should be confirmed with a repeat QFT-G in 8-10 weeks post-exposure

QuantiFERON-Gold

• A negative QFT-G alone should not be used to exclude *M. tuberculosis* in:
  – immunocompromised adults
  – children <5 years
  – patients ready to start treatment with TNF-alpha inhibitors

*Medically evaluate all; QFT-G or TST alone is not enough!*
QuantiFERON-Gold Modifications

- QuantiFERON-Gold in a Tube (QFT-GIT) is a new version of this test that cuts the need for immediate transportation of the blood specimen to the lab. It is in the investigative stage.

Elispot or T-spot TB Assay

- In-vitro enzyme immunoassay that utilizes same antigens (ESAT6 and CFP10) as QFT-G
- Done in microtiter plate coated with interferon-gamma
- Utilizes white blood cells from harvested from whole blood
- Incubate test overnight
T-Spot TB Assay

Add WBCs to wells coated with anti-IFN-gamma antibodies

IFN-gamma binds to antibodies

Each spot represents one IFN-gamma-producing cell

Add second antibody and substrate, which gives color change

T-Spot TB Assay Sample  Positive Result

Negative control

ESAT-6

CFP10

Mitogen

> 5 spots with either antigen is considered positive
More data in children and HIV+ persons; probably more sensitive than QFT-G

High frequency of indeterminate results in immunocompromised; not fully tested in children

Cost not yet determined

<table>
<thead>
<tr>
<th>QFT- GOLD</th>
<th>T-SPOT TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA-approved</td>
<td>Under review by FDA</td>
</tr>
<tr>
<td>Logistically simpler; no WBC separation</td>
<td>Requires cell separation</td>
</tr>
<tr>
<td>High frequency of indeterminate results in immunocompromised; not fully tested in children</td>
<td>More data in children and HIV+ persons; probably more sensitive than QFT-G</td>
</tr>
<tr>
<td>Cost to laboratory is $30+ per test</td>
<td>Cost not yet determined</td>
</tr>
</tbody>
</table>

Genotyping

- DNA testing (PCR technique) done on the patient’s laboratory-grown *M. tuberculosis* isolate
- There is diversity in the strains of *M. tuberculosis* due to low levels of spontaneous DNA mutations
- This diversity can be a means to further identify specific strains of *M. tuberculosis* = fingerprint
- Very specialized testing, only done in 2 labs in the US (California & Michigan), turn around time usually 10 working days
Genotyping

2 tests (PCR technique) done on all submitted specimens

• Type 1: Spoligotype - Spacer oligonucleotide typing
  – Detects variability in the direct repeat region of M. tuberculosis DNA
  – Reported as a 15 digit numerical identification

• Type 2: MIRU - Mycobacterial Interspersed Repetitive Units
  – Reported as a 12 digit number where each digit represents the number of repeats (1-9, 10=a, 11=b, 12=c)

Genotyping

Third technique done on some isolates

• RFLP (Restriction Fragment Length Polymorphisms); results on agarose gel
  – Requires more isolate material
  – Cannot be done on nonviable cultures; must “grow” isolate to perform test
  – Laborious, delays reporting
  – Visual comparison of results; therefore hard to compare isolates not tested together
  – No numerical result
Sample Genotyping Results

Spoligotype: 777377347760400
MIRU designation:14322404354b

Genotyping Reporting

Both spoligotype and MIRU results are reported for an isolate. A PCR cluster designation (e.g. XY002) is given when isolates from the same TB control program have identical spoligotypes and MIRU types.
Genotyping Reporting

If RFLP is done, results will be compared to the PCR cluster label (e.g. XY002). Isolates with the same PCR label may have different RFLP results; they are then not considered to be “identical” and NOT from the same cluster.

Genotyping

• Detects genetically identical strains of TB
• Reinforces chain of transmission – can confirm clusters determined during contact investigations
• Can help guide investigation and lead to case findings
• Helpful in outbreaks to determine if you have a common source of transmission
• Can help detect lab errors (not M. tuberculosis or is a contaminated isolate)
Acknowledgements

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• CDC Guidelines for Using the QuantiFERON-Gold TB Test for Detecting Mycobacterium tuberculosis Infection, United States: MMWR December 16, 2005, Vol. 54, No RR-15
• CDC Guide to the Application of Genotyping to Tuberculosis Prevention and Control: June 2004

Questions?

Thank you!