NOLA TB TIMES

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TB Diagnostics—A Special Issue on TB Diagnosis 101

Happy New Year and Happy Mardi Gras to everyone!

We already have seven presumptive cases of tuberculosis (TB) referred to us in Region 1. The question of TB diagnostics always comes up as the diagnosis is not clear cut in many situations. One disadvantage of conventional TB diagnosis is the wait time of up to eight weeks for culture confirmation, followed by another 2 to 4 weeks for drug susceptibility testing. Here at the Office of Public Health, TB lab specimen processing does not occur in-house, further delaying the diagnosis. During this delay, patients may receive empiric anti-TB treatment. However, without DST results to tailor therapy, patients with drug-resistant disease may receive suboptimal regimens that exacerbate resistance. In addition, adolescents and adults with inadequately treated or untreated TB may continue to spread infection. Diagnostic delay also postpones contact investigation, which identifies other TB-infected individuals. So in this first issue of the year, we will try to tackle the TB diagnostics. Please review the references provided on the last page of the newsletter contents to explore this on your own as well.

Sincerely,
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Diagnostic Discrepancy Data

Worldwide, 37% of total TB cases (estimated 9.6 million) went undiagnosed or were not reported. Of the 480,000 multi-drug resistant TB only 25% were detected and reported. The number of co-infected with HIV and TB was one-third of the estimated 1.2 million in 2014. The numbers in pediatrics are similarly disappointing. Recent studies estimate that less than two-thirds of tuberculosis (TB) cases occurring in childhood (0-14 years) are detected worldwide. Due to paucibacillary disease in pediatrics, having culture as the only reference standard available, makes it even more difficult to test new diagnostics in children.

Comprehensive Approach

Diagnosis of TB starts with having a high index of suspicion of TB. Prolonged cough for more than 3 weeks, night sweats, unexplained weight loss, chills, fevers are suspicious for pulmonary TB. Extra-pulmonary TB may present with the symptoms of involved organ system along with systemic symptoms. A thorough history and physical exam would further lead in the right direction. Selection of the most suitable tests for detection of MTB infection should be based on the reasons and the context for testing, test availability, and overall cost effectiveness of testing.
Understanding the Basics

Chiang et al. explain the basics in the following terms. In general, to diagnose an infection, a test can detect either the pathogen or the host response to the pathogen. However, TB diagnosis is complicated by the need to distinguish between disease and latent TB infection (LTBI). Currently available tests cannot discriminate between the two. Pathogen-based diagnostics are divided into genotypic tests, which detect nucleic acid fragments from *Mycobacterium* (MTB), and phenotypic tests, which detect whole microbes or their components. The sensitivity of pathogen-based methods depends on bacterial burden.

Drug Susceptibility Testing (DST) methods are characterized in 2 ways: first, as indirect or direct, and second, as phenotypic or genotypic. Direct tests are performed on the patient specimen, whereas indirect tests are performed on culture isolates of MTB. Phenotypic DST evaluates the strain’s growth or metabolic activity in the drug’s presence, whereas genotypic DST detects resistance-conferring mutations. The two may not always correlate.

Detection of TB Infection

Currently, there are two methods available for the detection of *M. tuberculosis* infection in the United States. The test are: Mantoux tuberculin skin test (TST, Tubersol PPD, Sanofi Pasteur Ltd., Toronto, Ontario, Canada); and interferon-gamma release assays (IGRAs): QuantiFERON-TB Gold In-Tube test (QFT, Cellestis/Qiagen, Carnegie, Australia) and T-SPOT® TB test (T-SPOT, Oxford Immunotec, Abingdon, UK).
<table>
<thead>
<tr>
<th>Images</th>
<th><img src="image1" alt="TST" /></th>
<th><img src="image2" alt="QFT" /></th>
<th><img src="image3" alt="T-SPOT" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Technique</td>
<td>Skin test-0.1 ml of PPD injected intradermally on volar surface of arm; read in 48-72 hours for induration</td>
<td>Blood draw; 1 mL of whole blood in each of 3 provided tubes</td>
<td>Blood draw; 2-6 mL of whole blood depending on age, in 1 green top tube</td>
</tr>
<tr>
<td>Procedure</td>
<td>Conglomerate of over 200 TB antigens (including ones in BCG vaccine) to elicit a Type IV hypersensitivity response; 5mm, 10 mm, 15 mm are used depending on risk factors</td>
<td>Enzyme linked immunosorbent assay (ELISA) measures interferon-γ (IFN-γ) secreted by the patient’s T cells on stimulation with 3 MTB antigens: ESAT6, CFP10, and TB7.7. (+) and (-) controls; if either fail, an indeterminate is reported</td>
<td>Enzyme-linked immunosorbent spot (ELISPOT) is used to quantify the T lymphocytes that produce IFN-γ after incubation with ESAT6 and CFP10. (+) and (-) controls; if either fail, an invalid is reported; a borderline value is also available</td>
</tr>
<tr>
<td>FDA-approval</td>
<td>1940’s</td>
<td>2005, QFT-GIT in 2007</td>
<td>2008</td>
</tr>
<tr>
<td>How long</td>
<td>Has to be read in 48-72 hours</td>
<td>Has to be processed in 12-16 hours</td>
<td>Has to be processed in 32 hours</td>
</tr>
<tr>
<td>Where</td>
<td>Commercially available</td>
<td>Commercially available</td>
<td>Available through Oxford Immunotec Memphis, TN</td>
</tr>
<tr>
<td>Cost</td>
<td>$20 per vial</td>
<td>$50-$100*</td>
<td>$50-$100*</td>
</tr>
<tr>
<td>BCG cross-reactivity</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cross-reactivity with NTM</td>
<td>Yes</td>
<td><em>M. marinum, M. kansasii, M. szulgai, and M. flavescent</em></td>
<td><em>M. marinum, M. kansasii, M. szulgai, and M. flavescent</em></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>63%-100%</td>
<td>56% - 93%</td>
<td>50%-100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>9%-100%</td>
<td>99%-100%</td>
<td>85%-100%</td>
</tr>
<tr>
<td>Estimated specificity in BCG-unvaccinated children</td>
<td>95%-100%</td>
<td></td>
<td>90%-95%</td>
</tr>
<tr>
<td>Estimated specificity in BCG-vaccinated children</td>
<td>49%-65%</td>
<td>89%-100%</td>
<td></td>
</tr>
<tr>
<td>Estimated sensitivity (confirmed TB disease)</td>
<td>75%-85%</td>
<td>80%-85%</td>
<td></td>
</tr>
<tr>
<td>Estimated sensitivity (clinical TB disease)</td>
<td>50%-70%</td>
<td>60%-80%</td>
<td></td>
</tr>
</tbody>
</table>

*May depend on the supplier, cost for both may vary if offered in bulk to an institution;  
*See Ref 4 Tables 4-7;  
*Ref 5, Table 1
Chest Radiographs

1. Radiographic abnormalities are often seen in the apical and posterior segments of the upper lobe or in the superior segments of the lower lobe
2. May occur anywhere and differ in size, shape, density and cavitation
3. Children may have minimal abnormality; may see lymphadenopathy in lateral films
4. Atypical patterns in HIV-infected patients, less cavitary disease if CD4 counts are low; CXR may even be normal
5. Mixed nodular and fibrotic lesions may progress to disease quickly
6. Discrete, calcified granulomas less likely to progress

Detection of the Pathogen

- Requires collection of appropriate specimen (sputum, urine, or CSF)
- Sputum collected 3 times, 8-24 hours apart, at least one of them being an early morning specimen; yield increases with multiple collections
- Sputum should be done for TB disease at any body-site
- Sputum should be sent for acid fast bacilli (AFB) smear and culture
- Collected via coughing, induction of sputum, bronchoscopy or gastric aspiration
- In extrapulmonary TB, specimen placed in formalin is not viable for culturing

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Recommendations

Center for Disease Control and Prevention

1. IGRA preferred in population who may not return for follow up and BCG-Vaccinated individuals
2. TST preferred in children < 5 years
3. Either okay in contact investigation and in employment setting
4. Both can be considered in immunocompromised individuals, if further evidence for treatment is needed, or if the test is inconclusive

American Academy of Pediatrics

1. In children < 5 years of age, TST is preferred
2. In children > 5 years of age with a prior BCG vaccine, IGRA is preferred and children unlikely to return for a TST reading
3. Consider both in cases as above

World Health Organization

1. Exclusive use of the TST due to insufficient data in resource-limited setting, cost, and complexity of IGRA assays
Acid Fast Bacilli (AFB) Smear Classification

- Stained and acid-washed smears examined microscopically (Tubercle bacilli in red)
- 5,000 to 10,000 bacilli per milliliter of specimen needed to allow the detection of bacteria in stained smears
- 10 to 100 bacilli needed for a positive culture

Two procedures are used:
1. Direct Microscopy: Carbol-fuchsin methods which include the Ziehl-Neelsen (ZN) and Kinyoun methods
2. Fluorescent Microscopy (FM): Aluramine-O or auramine-rhodamine dyes

- Negative smears do not exclude TB disease; and other bacteria can be AFB positive as well
- Results available within 24 hours
- Smear results are reported as number of AFB observed per 1000x magnification
  1. 4+ = > 9 per field
  2. 3+ = 1-9 per field
  3. 2+ = 1-9 per 10 fields
  4. 1+ = 1-9 per 100 fields
  5. +/- = 1-2 per 300 fields

- The greater the number, the more infectious the patient
- 3 sputum specimens in trained hand with ZN has sensitivity of 30% - 80%; specificity about 97%
- FM about 10% more sensitive than ZN but less specific 90% - 97%
- FM is more expensive than ZN

Drug Susceptibility Testing (DST)

- The proportion method is the most common method
- Performed on Lowenstein-Jensen (LJ) agar
- To perform the test, a critical concentration of the drug—the amount that inhibits wild-type organisms but not resistant mutants—is placed in the medium. If the proportion of resistant bacilli exceeds 1%, the strain is considered resistant. DST is routinely performed with more than one critical concentration for isoniazid. Higher doses of the drug may overcome low-level resistance
- The proportion method lacks interpretations for ethambutol, pyrazinamide, and second-line drugs
- In the MODS assay some wells contain antibiotics; the appearance of cords in antibiotic containing wells indicates resistance
- The median time to TB detection was 7 - 10 days for the MODS assay compared with 24 - 32 days for LJ culture
- Line probe assay via PCR and sequencing of resistance genes are becoming more common
Culture Methods

- Considered the gold standard; lower limit of detection of 10 bacilli per milliliter of sputum
- Solid (3 - 6 weeks) and liquid broth based (4 - 14 days) culture methods available
- Broth-based methods include BACTEC, MGIT, VersaTREK, and MBBACT
- In adults, the first culture detects 85% of cases, the second culture adds a further 12%\(^6\)
- Non-commercial media include thin layer agar (TLA) and egg-based Lowenstein-Jensen (LJ); these have a 93% sensitivity
- Cultures are monitored for 8 weeks
- The most common automated liquid culture method includes Mycobacteria Growth Indicator Tube (MGIT), which contains Middlebrook 7H9 broth; monitoring can be done automatically using a BACTEC™ MGIT™ 960 system\(^8\)
- Liquid culture methods have a higher sensitivity, but the bottles are more expensive and the contamination rate is higher
- Microscopic Observation Drug-Sensitivity (MODS) assay sensitivity is reported to be 92% - 97.5%; faster and cheaper than liquid culture
- The MODS assay uses inverted light microscopy to visualize cord formation in wells with Middlebrook 7H9 broth-based medium and examined daily
- Document culture conversion by 2 negative cultures, a month apart each

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Jeffrey Cirillo, Ph.D.
Professor
Director of CAPRI
Texas A&M Health Science Center
Nucleic Acid Amplification Tests (NAATs)

- These test amplify MTB-specific nucleic acid sequences, allowing direct detection of MTB in clinical specimens
- In-house and standardized commercial assays are available
- In smear positive adults, the pooled sensitivity and specificity is higher than 95%\(^6\)
- In smear negative patients, the pooled estimate is close to 66%\(^6\)
- Current FDA-approved NAATs in USA include AMTD Test (Gene-Probe, Inc.), BD ProbeTec ET Direct TB Assay (Becton-Dickinson), Amplicor MTB PCR Test (Roche), SNAP MTB complex (Syngene, Inc.), Accuprobe MTB complex test (Gene-Probe, Inc.), Rapid Diagnostic system for MTB (Gene-Probe, Inc.), Rapid Identification test for MTB complex (Gene-Probe, Inc.), and Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA, USA)\(^{10,11}\)
- Xpert assay is the first to be automated and standardized
- Reports results in 2 hours; direct detection of MTB as well as rifampin resistance
- Xpert has overall specificity (for MTB identification) of 98%; sensitivity of 98% in smear positive case and 68% in smear negative culture positive case\(^6\); the sensitivity is 80% in HIV positive patients (95% CI, 67% - 88%)
- In pediatric patients, reported specificity is 98% for sputum/gastric lavage; pooled sensitivity is 66%, smear positive cases (85% - 96%) is better than in smear negative cases (55% - 62%)\(^2\)
- Sensitivity of 94% and specificity of 98% for rifampin resistance (also detected by Xpert MTB/RIF platform); positive results require confirmation with another method in low prevalent areas of multi-drug resistant TB
- With WHO recommendations, 4.8 million test cartridges were procured in 2014 by 116 low- and middle-income countries at concessional prices, up from 550,000 in 2011

CDC Recommendations for NAATs

- Guidelines available since 1996, last updated in 2009
- NAA testing can be performed on at least one respiratory specimen to rule out pulmonary TB
- Culture remains the gold standard

Table 2: Interpretation of NAATs

<table>
<thead>
<tr>
<th>Smear</th>
<th>NAAT</th>
<th>TB</th>
<th>Next Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Start Anti-TB Treatment</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>?</td>
<td>Use clinical judgement to start Anti-TB treatment; May repeat NAAT or wait for cultures</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>?</td>
<td>Consider presence of inhibitors (likely 3%-7% specimens); Repeat NAAT, repeat smear; If inhibitor present, NAAT is not useful; If no inhibitor, 2(^{nd}) NAAT neg and AFB +, consider diagnosis of nontuberculous mycobacteria</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>?</td>
<td>Use clinical judgement; Sensitivity is 50%-80% in smear (-) cases</td>
</tr>
</tbody>
</table>

The New Diagnostic Modalities in Pipeline

There are many\(^{10}\) but I will leave you with this special one to read on your own. See an editorial in the Wall Street Journal\(^{14}\) and the actual Journal article in Nature Chemistry in 2012.\(^{15}\) Dr. Jeffrey Cirillo and his team at the Texas A&M Health Science Center have developed a compound that binds specifically to TB enzymes and a portable reader device that would detect a positive sample in 10 minutes. The reader device costs around $500, and processing each sample would cost less than $2. They hope to have a TB drug sensitivity platform as well.
References


Contact Us
Send us your updates, TB vignettes, and thoughts.
Join our listserv.
Email: gmirani@tulane.edu

Wetmore Clinic
3308 Tulane Ave.
6th Floor
New Orleans, LA 70119

Clinical: 504.826.2063
TB Hotline (Ms. M. Vincent): 504.638.7053

Helpful links:
dhh.louisiana.gov/index.cfm/page/1005
www.medschool.lsuhsc.edu/tb
www.cdc.gov/tb/
www.who.int/tb/en/