Laboratory Methods of Tuberculosis Testing

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CAMLT Sep 21st, 2019
Objectives

• US & California statistics of *Mycobacterium Tuberculosis*
• Interferon Gamma Releasing Assay testing:
  – Indications
  – methodologies for the laboratory professional
  – interpretation
• Discuss current diagnostic modalities for MTB infection and medical treatment options
• Review screening recommendations with current CDPH guidelines and the May 2019 MMWR guidelines
Magnitude of Tuberculosis Prevalence

*M. Tuberculosis* major cause of morbidity and mortality worldwide

- 2017 ~10 million people around the world became infected with TB
- 1 million are children and 230K died of Active TB
- Leading killer of HIV-positive people
- 100 million Health Care Personnel (HCP) tested/yr. in U.S. but U.S. only 0.00005% of global burden
- Multidrug-resistant TB (MDR-TB): 558K new cases
California Specific Statistics

- California reported 23% of the nation’s TB cases, and the greatest number of TB cases of any state
- For over 2 decades, is 1:4 states that account for about half of the TB cases in the US
- California is double the next highest state of Texas with 2x the cases annually 2091 vs. 1129 in 2018
- California-2018: Active TB Infection (ATBI) 2,091: 2017: 2,059
- >2 Million Californians have Latent TB Infection (LTBI) or 6% of pol’n
  - 80% TB from REACTIVATION LTBI

(CDPH Fact Sheet, 2018)
In 2017, 75% of California’s local health departments identified at least one patient with TB disease.
2/3rd are Foreign-Born (1st Asian)

Steady Decline in incidents since 1993

Current Decline is insufficient to Eliminate in the 21st Century

TB-Controllers: Focus on increased Risk i.e. LTBI Treatment
Conversion to Active TB

- TB infection
  No risk factors
  TB disease
  10% over a lifetime

- TB infection and diabetes
  TB disease
  30% over a lifetime

- TB infection and HIV
  TB disease
  7-10% per year
Testing Methodologies for TB
Testing Methodologies for TB
Per California Code of Regulations

Test for tuberculosis infection: Any test, including the tuberculin skin test and Blood Assays for *M. Tuberculosis* (BAMT) such as interferon gamma release assays (IGRAs) which:

- Has been approved by the Food and Drug Administration for the purposes of detecting tuberculosis infection
- Recommended by the CDC for testing for TB infection in the environment in which it is used
- Administered, performed, analyzed and evaluated in accordance with those approvals and guidelines – *MMWR 2005 Guidelines (12/30/05)*
  - TB Skin Test (TST)
  - Laboratory Test IGRA: Quantiferon Plus or T-Spot

- IGRA preferred (ATC-IDS-CDC, 12/8/16):
  - In persons that have low probability of read return
  - Those vaccinated with BCG vaccines
  - As a 2-step TST replacement
  - Children ≥ 2 years
TB Skin Testing (TST)

• Advantages
  – Price ($13 at Public Health)
  – Availability
  – Have been used worldwide for a century
  – Preferred method testing in children < 5yrs

• Disadvantages
  – Requires 2 visits, 4 for 2-step testing
  – 48-72 hours before result available
  – Subject to reader bias
  – Real costs $63-73 per person screened
  – Not as specific as IGRAs
  – Confounded by BCG, Non-TB Mycobacteria (M. avium, other mycobacterium complex) & Co-morbidity

≥5 mm Immunocompromised
≥10 mm for Everyone else

Sensitivity 89-95%
Specificity: 85-86%
*Lower in BCG-vaccinated cohorts
Shared antigenic determinates between M. *tuberculosis* Complex, BCG and known NTM species
# TST Limitations

## Reasons for False Positive TST

<table>
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<tr>
<th>Non-Tuberculosis Mycobacterium</th>
<th>i.e. M. avium</th>
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<td>PPD</td>
<td>Reading errors</td>
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<tr>
<td>BCG</td>
<td>Varied sub-strains</td>
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<td></td>
<td>Multiple vaccinations</td>
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<tr>
<td>Biologic Factors</td>
<td>Allergic reaction to PPD</td>
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## Reasons for False Negative TST

- **Technical Factors**
  - Too little antigen, too deep, leakage
- **PPD**
  - Improper storage or dilution
  - Reading errors
- **Biologic Factors**
  - Co-morbidity: Diabetes, HIV, MMR
  - Poor nutrition
  - Drugs; steroids, methotrexate
  - Chronic renal failure, Malignancy
  - Age (newborn, elderly booster)
  - Immunosuppressive drugs: RA, Lupus
History of the IGRA

- In the 1980’s the need for a better test for TB infection in cattle was addressed in Australia
- Injecting tuberculin from *M. bovis* into the caudal fold (base of tail) of a cow
- Using the tuberculin skin test in cattle had very similar problems to the TST in humans.
- Checking for induration was messy and dangerous.
- Many cows wouldn’t return for their reading
- IGRA’s ~2001.
How do IGRAs Work?

IGRAs measure a person’s immune reactivity to *M. tuberculosis*. WBCs from most persons that have been infected with *M. tuberculosis* will release interferon-gamma (IFN-g) when mixed with antigens (substances that can produce an immune response) derived from *M. tuberculosis*. To conduct the tests, fresh blood samples are mixed with antigens and controls.

<table>
<thead>
<tr>
<th>Table 1: Differences in Currently Available IGRAs</th>
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<td><strong>Initial Process</strong></td>
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<td><strong>M. Tuberculosis Antigen</strong></td>
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<td><strong>Possible Results</strong></td>
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**Immunological Basis for QuantiFERON Plus®**

- IFN-γ is a cytokine produced by T cells in response to viral and bacterial infections.
- In a healthy individual, there is little to no IFN-γ within the blood.
- In the presence of the TB specific antigens, T cells of infected persons are stimulated to produce IFN-γ.
- In the Quantiferon Plus (QFT+) test:
  - Whole blood is exposed to 2 Mycobacterial proteins (ESAT-6 & CFP-10).
  - Both CD4 & CD8 T-cells of infected persons are activated & secrete IFN-γ.
  - Measurement of IFN-γ using an ELISA assay is the basis for the QFT test. Measures cell-mediated immune reactivity to *M. tuberculosis* proteins.
Who should be tested w IGRAs?

- Persons at increased MTB infection risk and for who testing is recommended:
  - **Foreign-born** from areas with a high incidence of active TB
  - **Visitors** to areas with a high prevalence of active TB
  - **Residents and employees of congregate settings** whose clients are at increased risk for active tuberculosis
  - **Healthcare Personnel** who serve clients at increased risk for active TB
    - HCP only 3.4% of 8,654 cases in Ca. Almost all non-US born LTBI
  - **Populations** defined locally with increased risk factors of MTB infection
IGRA Advantages

- Requires a single visit
- Objective test results that are standardized and can be available in 24 hrs
- Specificity > 97% with high NPV = minimal doubt
- Sensitivity > 94% with high PPV = minimal doubt
- No impact by BCG, most NTMs or boosting → Fewer X-Rays
- Standardized objective laboratory-based testing: Set interpretation Criteria
- Better test for special populations: foreign-born, elderly, immunocompromised
- Proven reduction of global cost to a health system while offering improvements to IC: + or indeterminate TST requires a X-Ray ~$180
IGRA Disadvantages

- Cost higher direct costs of lab test in labor and test price
- “Lost in the Mail”
- Availability of Test
- Lab Errors: Collection, transport, processing 8-30 hours
- Indeterminate result (same as employee not return for reading)
- False +, False – [possible cross reaction with M. kansasii, M. szulgai and M. marinum]
- Productivity issues i.e. ↑ Lab work and ↓ Emp Health time
The Importance of SPECificity

- Specificity is the ability of a test to produce a negative result, when the subject is in fact free of the disease.

**Why does it matter?**

- Specificity combined with prevalence influences the positive and negative predictive value of a test (who is likely to progress to active disease).
QuantiFERON Plus®

- Results reported as:
  - Positive – The patient is **likely** to be infected
  - Negative – The patient is **unlikely** to be infected
  - Indeterminate –
    - Positive Control Fails
    - Negative Control Fails

Indeterminate results are valid and meaningful
- they provide information on QC and the Patient’s immune status.
Real-World Application

David Marder, MD, MPH
Medical Director of University Health Service
University of Illinois Medical Center Chicago

5000 HCWs screened annually (over 20,000 tests since 2006).

- In 2006, TST positivity rate reduced from 18% to 3% with QFT.
- A subset of the TST positives from 2005 received QFT in 2006. 365 of the 505 cohorts were QFT negative (72%).
- In 2006, Occupational Health had 2000 less clinic visits from the previous year.
- TB Program cost savings after adopting QFT (first year) = $80,000.
Positive and Negative Results

• 32 Year old Male with Hx of BCG. TST + 15mm
  ✓ QFT
    ✓ Nil (negative control) 0.05 IU
    ✓ TB1 3.5 IU
    ✓ TB2 3.4 IU
    ✓ Mitogen (positive control) >10 IU

Conclusion: Likely MTB infected with good controls

• Same Pt scenario
  ✓ QFT
    ✓ Nil 0.05 IU
    ✓ TB1 0.28 IU
    ✓ TB2 0.22 IU
    ✓ Mitogen >10 IU

Conclusion: Cross reaction to BCG. Not MTB infected and Negative
QFT + Indeterminate

- 32 Year old Male with Hx of BCG. TST + 15mm
  ✓ QFT
    ✓ Nil 3.65 IU
    ✓ TB1 (Antigens ESAT 6 & CFP10) 4.58 IU
    ✓ TB2 3.11 IU
    ✓ Mitogen >10 IU

Conclusion: QFT response to MTB Antigens but Indeterminate because Nil very high. Perhaps Pt. Immune system reacting to stimulus?

- Same scenario
  ✓ QFT
    ✓ Nil 0.09 IU
    ✓ TB1 0.23 IU
    ✓ TB2 0.21 IU
    ✓ Mitogen 0.45 IU

Conclusion: QFT response to MTB Ags negative but indeterminate due to low mitogen. Perhaps Pt. Immune system NOT reacting to stimulus?
QFT Mixed Results & Retesting

- 32 Year old Male with Hx of BCG. TST + 15mm

✓ QFT
  ✓ Nil 0.65 IU
  ✓ TB1 (Antigens ESAT 6 & CFP10) 0.55 IU
  ✓ TB2 0.11 IU
  ✓ Mitogen >10 IU

Conclusion: QFT+ Results Positive. Essentially 2 tests but 1 is positive & other is negative. Risk Stratification is the new norm. If the patient is at risk, consider this a positive result and treat. If the patient is low risk, consider retesting based on ”retesting zone” and high reversion rate.
When to Retest

- The SPECificity is 97% so expected retesting is ~3% of cohort
- Deciding when to retest? IF there are NO risks of infection
- QFT+ and QFT have same specificity and sensitivity
- Advise risk assessment to determine pre-testing probability of a positive result
- Example: 2018 at Mercy Medical Center, Redding HCP
  - 422 QFT testings
  - 27 positives = 6% positive rate
  - 3 reconfirmed positive-referred to provider
  - 7 reversion to negative
  - 7 referred to provider for treatment or lost to follow-up
  - If expected positive result or risk factor +, don’t retest, X-ray & treat
  - Wobblers should be offered treatment. Definitely have reaction to TB
  - If risk factor + but highly unlikely, i.e. me, ask ID Physician or retest
Positive QFT results...Now what?!

Positive Result for Quantiferon Gold Test (QFT) for TB

History: A Quantiferon + test is a form of blood test that detects Mycobacterium Tuberculosis (TB) in a person. Your patient has been tested using this method because of exposure to TB, screened as a new hire or because they have routinely tested positive by the TB skin test due to BCG or sensitivity to the TB Skin Test or PPD. Your patient tested POSITIVE using the QuantiFERON test and had a chest X-ray to confirm that this positive was NOT due to active disease.

The interpretation of this test indicates that your patient has a diagnosis of latent mycobacterium TB Infection, the non-contagious form of TB. The recommendation is treatment with the following medications per our Infectious Disease physician and current 2019 MMWR Guidelines. This is due to the fact that their latent status can change to active TB over their lifetime and these conversions represent the TB burden in the U.S.

Even after treatment, the person will likely continue to test positive on the Quantiferon test so no further testing is necessary.

If you have questions or your patient cannot tolerate the medication, please contact me, Dr. Miles or consider consulting with a local Infectious Disease physician. Thank you.

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Mercy Medical Center, Redding. Employee Health Department  530-225-6194
Treatment of QFT Positive

- QFT positive results rare in our area
- Repeat positive QFT with another QFT ASAP or 2 weeks later
- Those with confirmed quantitative #s are treated with current CDPH guidelines:
  - Priftin (Rifapentine)/Isoniazid and B6 50 mg q weekly x 12 weeks DOT
  - Or traditional INH 300 mg daily + Vit B6 50 mg daily x 9 months
  - Research on 1HP and other antibiotics –mycin-based

- Safety Profile of each drug must be considered
MMWR May, 2019

- Finds HCW are a low-risk/low-conversion population despite central dogma
- Finds majority of TB cases both ATBI and LTBI are from existing population that converts to active status (~80% unknown)
- Focus is on identification of high-risk population and treatment
- Adds Risk Assessment to Symptom Questionnaire
- Overall, remove annual screening or testing for HCW: OVER 96% neg
- https://www.cdc.gov/mmwr/volumes/68/wr/mm6819a3.htm?s_cid=mm6819a3_x#B1_down
Take Home of IGRA Testing

• Greater Specificity leads to better healthcare by clinicians

  ➢ 50 to 75% less positives by QFT compared to TST
  ➢ Significant reduction in clinic time
  ➢ Ability to screen past-positive, BCG-vaccinated people
  ➢ 100% compliance: Time SAVED!

• Greater Specificity offers better healthcare to patients

  ➢ Fewer patient x-rays
  ➢ Fewer patients directed for unnecessary treatment
  ➢ Less uncertainty for foreign-born unable to TST due to BCG

CDPH (8/16/19) AFL 19-28 Updated Centers for Disease Control Tuberculosis Screening Recommendations for Health Care Personnel (HCP) and Nationwide Shortage of Tuberculin Skin Test Antigens. Retrieved from https://www.cdph.ca.gov/Programs/CHCQ/LCP/Pages/AFL-19-28.aspx


Tuberculosis Screening, Testing, and Treatment of U.S. Health Care Personnel: Recommendations from the National Tuberculosis Controllers Association and CDC, 2019 | MMWR. Retrieved from https://www.cdc.gov/mmwr/volumes/68/wr/mm6819a3.htm?s_cid=mm6819a3_x#B1_down

Negative Predictive Value of QuantiFERON®

- 55% of QFT-negative were TST-positive
- No progression to active TB at 3.5 years
- QFT demonstrates 100% NPV* in this study

*Diel R, Am J Respir Crit Care Med. (Aug, 2010)*
Positive Predictive Value of QuantiFERON®

198 QFT-positive

142 QFT-positive TST-positive
- Not treated
  - 17 developed active TB

5 QFT-positive TST-negative
- Not treated
  - 2 developed active TB

51 QFT-positive (49 TST-positive)
- Chemoprophylaxis RIF and/or INH
  - No active TB

QFT-positive contacts

- All 51 QFT-positive contacts who received treatment - NO active TB
- Ignoring those who agreed to treatment, QFT had 147 positive compared to 555 TST.
- All 19 untreated contacts who progressed to active TB were QFT-positive.

_Diel et al AJRCCM (Aug 2010)_