Role of the Laboratory in TB Diagnosis and Management

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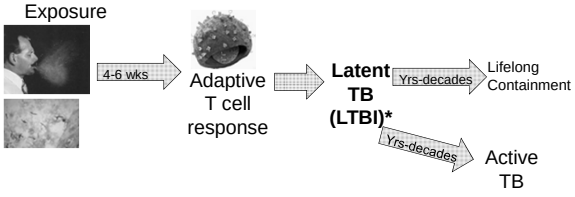
Objectives

- At the completion of this TB webinar, participants will:
  - Be familiar with the tests to diagnose latent tuberculosis and active tuberculosis
  - Recognize the tests available to detect Mycobacterium tuberculosis in clinical specimens
  - Understand the value of molecular tests to detect TB

History of TB Diagnostics

- Robert Koch announced in 1882 that he had found a microbe, *Mycobacterium tuberculosis*, that was the cause of "White Death", a disease responsible for one-seventh of all deaths in Europe in the late part of the 1800's.

Timeline of TB Infection



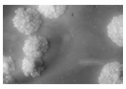
*Prevention efforts focus on detecting LTBI, most LTBI do not advance to active disease but those patients are at high risk particularly if they become immunocompromised.

TB Infection vs. **TB Disease**

TB in the body	TB in the body
Chest X-ray normal	Chest X-ray abnormal
Sputum not done	Sputum smear and culture positive
No symptoms	Symptoms: cough, fever, weight loss
Not infectious	Infectious
Not a case of TB	Case of TB

TB Algorithm

- Collect sputum specimens at 3 different times and 8 hours apart (at least one must be a first morning specimen) for AFB smear and mycobacterial culture.
- Perform MTD or NAAT test on the first smear positive sputum specimen



Diagnosis of TB

- Clinical picture
  - History and symptoms
- Chest XRay
- Antigen Test
  - Skin test (TST)
  - Blood Test (IGRA)
- AFB (Acid Fast Bacilli) microscopy of sputum
- NAAT Testing
- Culture (up to 6 weeks)
  - Solid medium
  - Liquid (MGIT)
- Nucleic Acid Amplification Testing (NAAT)
  - Molecular probes
  - PCR
- Sensitivity Testing
- Genotyping

Requirements to get a high quality specimen to the laboratory

- Collect specimens before therapy started
- Even after a few days of therapy, AFB may be killed or numbers decreased to longer be detectable
- Specimens must be handled properly to guarantee successful cultures
- Promptly transport specimens

Specimen Type: Varies with symptoms

- Pulmonary
  - Sputum (spontaneous, induced)
  - Bronchoalveolar Lavage
- Gastric Lavage (children)
- Tissue and Body fluids (CSF, pleural, blood)
- Wounds, skin lesions (exudates)

Specimen Collection and Processing: special considerations

- Biohazard
  - Aerosol transmission
- Prevent contamination of specimen
  - Slow growth rate of TB
- Evaluate at least 3 specimens per patient

Sputum collection considerations

- Instruct patients that nasopharyngeal discharge and saliva are not sputum
- Sputum = thick, yellowish (sometimes blood-tinged) exudative material brought up from the lungs after a deep, productive cough
- First rinse mouth with mouthwash to decrease bacterial contamination
- Collect specimen into appropriate container

Sputum cont…

- About 10 ml of sputum is sufficient
- If patient cannot provide an adequate specimen then sputum induction is acceptable
  - Warm, aerosolized hypertonic salt solution
  - Be certain to label the specimen as “induced sputum”

Specimen Transport

- From the time of collection until the specimen is processed, the other bacteria present will over grow (contaminate) the slower growing *Mycobacteria* sp.
  - Speed is important
    - Courier
    - Ship cold when possible
    - Shipping cold slows bacterial growth

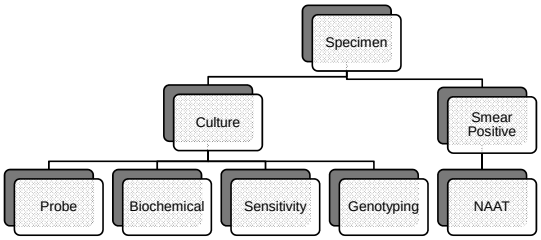
Specimen Processing

- If collected from a non-sterile site (sputum), then digest and decontaminate before culture
  - Kill off other microbes
  - Liquefy mucin
  - Remove organic debris
  - Homogenize tissue
- N-acetyl-L-cysteine (NALC)-NaOH method
- Concentrate specimen

Summary of Standard Diagnostic Techniques

- Direct from specimen
  - AFB Smear – cold kinyoun and fluorescent
  - Culture in broth and on solid media
  - Direct detection by NAAT
- From growth of organism
  - Probe (accuprobe)
  - Biochemicals
  - 16S ribosomal RNA
  - Sensitivity Testing

TB Specimen Processing

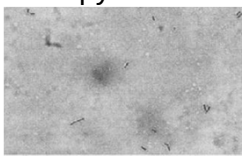


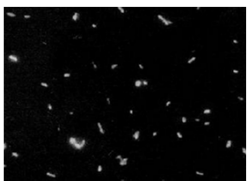
Laboratory Tests: Non-specific

- AFB smear
  - Semi-quantitative as a measure of patient infectiousness
- Culture
  - Liquid and solid media (up to 6 weeks)
  - Automated commercial systems widely used
  - Semi-quantitative

Diagnosis of TB: AFB Smear Microscopy

- Make a “smear” on a slide
- Stain for acid-fast bacteria
  - Cold Kinyoun
  - Ziehl Neelsen
  - Fluorochrome (Auramine-Rhodamine)





Diagnosis of TB: AFB Smear Microscopy

- Strengths
  - Easy, fast, cheap (ZN)
- Weakness
  - 50-60% of TB patients are smear negative
    - Need at least 10,000 CFU/ml sputum for positive result
  - Cannot differentiate *Mycobacteria* species

Importance of acid-fast bacilli smear microscopy as a primary diagnostic tool

- Initial diagnosis
- Monitoring treatment
- Determination of time to release from isolation

How sensitive is the smear?

- Peterson et. al. JCM 1999 vol. 37:3564-68.

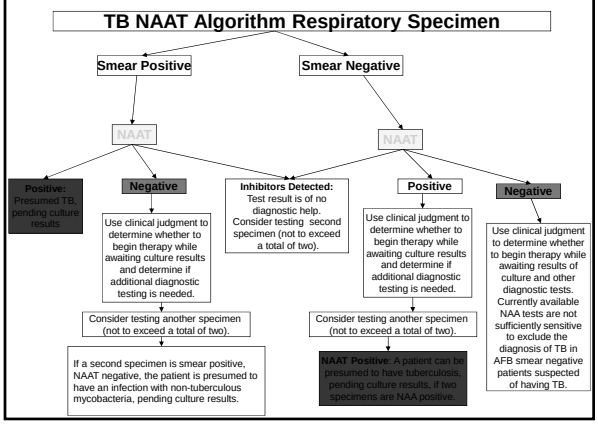
Number of specimens	Direct AFB smear sensitivity	Concentrated AFB smear sensitivity	Comment
353 culture positive for Mycobacteria	34%	58%	Direct smear cannot be relied on
208 cultures positive for *M. tuberculosis*	42%	74%	Concentrated smear most reliable
Analysis of 3 specimens per patient	81%	91%	Concentrated smear is still the most reliable

Direct detection of TB in the specimen

- MTD test – Genprobe – transcription mediated amplification
- In house developed Nucleic Acid Amplification test (NAAT)
- GeneXpert Cepheid NAAT

Interpretation of NAAT Result

Smear	NAAT	Interpretation
+	+	**Presumed Positive TB**, No Additional Testing
+	--	If first sputum specimen: smear positive and NAAT-negative, repeat on one additional specimens, if negative then presume negative for TB.
--	+	Additional specimens (limit 2). **Presumptive positive for TB** if the subsequent specimen positive
--	--	**Presumptive negative for TB**. Two specimens recommended.



Diagnosis of TB: Culture

- Solid Media Culutre
  - Agar Middlebrook 7H10/7H11
  - Egg based Lowenstein-Jensen
- Liquid – Broth Culture
  - 7H9
  - Commercial broth and monitoring systems
    - Becton Dickinson MGIT
    - ThermoScientific, TREK Diagnostic Systems,Versa TREK Myco
- Use a solid and a liquid media

Laboratory Tests: Specific

- Biochemical tests
  - Require sub-culture
  - Ex. Niacin, Nitrate, Tween 80 Hydrolysis, 68 Catalase
- High performance liquid chromatography (HPLC) of cell wall mycolic acids
- Molecular probes
  - Culture confirmation
  - Direct from growth in broth or on slant
- DNA sequence analysis
  - 16S rRNA gene

Molecular Probes for Mycobacteria identification

- **MYCOBACTERIUM TUBERCULOSIS Complex Culture Identification Test** – For identification of *M. tuberculosis, M. bovis, M. bovis* (BCG), *M. africanum, M. canetti, M. microti* etc. isolated from culture.
- **MYCOBACTERIUM AVIUM Culture Identification Test** - For the identification of *Mycobacterium avium* isolated from culture.
- **MYCOBACTERIUM INTRACELLULARE Culture Identification Test** - For the identification of *Mycobacterium intracellulare* isolated from culture.
- **MYCOBACTERIUM AVIUM Complex Culture Identification Test** - For the identification of Mycobacterium avium complex (*M. avium, M. intracellulare*, and other members) isolated from culture.
- **MYCOBACTERIUM GORDONAE Culture Identification Test** - For the identification of *Mycobacterium gordonae* isolated from culture.
- **MYCOBACTERIUM KANSASII Culture Identification Test** - For the identification of *Mycobacterium kansasii* isolated from culture.

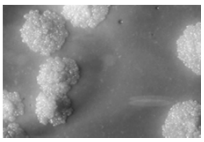
Molecular Probe Performance Characteristics

Organism	Sensitivity	Specificity
M avium	99.3%	100%
M intracellulare	100%	100%
M avium complex	99.9%	100%
M gordonae	98.8%	99.7%
M kansasii	92.8%	100%
M tuberculosis complex	99.2%	99.0%

Biochemical tests for *M. tuberculosis* complex

- 8 species make up the complex
- *Mycobacterium tuberculosis*
- *Mycobacterium africanum*
- *Mycobacterium bovis*
- *Mycobacterium bovis (BCG)*
- *Mycobacterium microti*
- *Mycobacterium canettii*
- *Mycobacterium pinnipedii*
- *Mycobacterium mungi*

- Differentiate by biochemical testing
  - Niacin
  - Nitrate
  - Others



Antimicrobial susceptibility testing

- Required for all MTB complex patients
  - Absolute concentration
  - Resistance ratio
  - Proportion
- Recommended for some NTM species

Drug susceptibility testing of *M. tuberculosis*

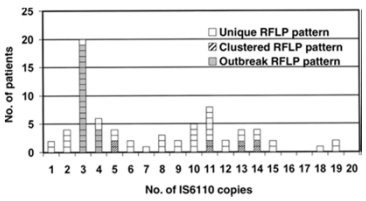
- Culture based DST remains the gold std
  - Reliable for INH & Rif, inconsistent for Ethambutol resistance
- Genotypic methods
  - Sequencing
  - Line probe hybridization assays (commercial)
  - Molecular beacons (GeneXpert)
  - Loop mediated isothermal amplification

Genotyping

- MMWR Controlling TB in the US Nov. 2005
  http://www.cdc.gov/tb/publications/reportsarticles/iom/TaskForcePlan/strategies_accelerate.htm
- Refers to procedures to identify *M. tuberculosis* isolates that are identical in specific parts of the genome
- Along with epi investigation, genotype used to confirm transmission

CDC program for genotyping *M. tuberculosis* isolates

- DNA Fingerprinting – Restriction Fragment Length Polymorphism (RFLP)



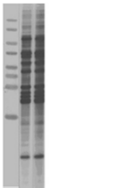


TABLE 3. Essential laboratory tests for tuberculosis control

Test	Maximum turnaround time
Microscopy for acid-fast bacilli	≤24 hours from specimen collection or, if test is performed offsite, ≤24 hours from receipt in laboratory; if latter, time from specimen collection to laboratory receipt should be ≤24 hours
Nucleic acid amplification assay	≤48 hours from date of specimen collection
Mycobacterial growth detection by culture	≤14 days from date of specimen collection
Identification of cultured mycobacteria	≤21 days from date of specimen collection
Drug susceptibility testing	≤30 days from date of specimen collection
Drug susceptibility testing of second-line drugs	≤4 weeks from date of request

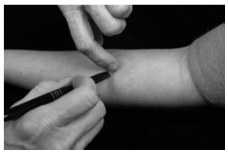
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5412a1.htm

What are the expected TAT?

Test	Standard	SHL
AFB smear	<24 h	7 h
NAAT	<48 h	<24 h
Growth in culture	<14 d	NA
ID of culture	<21 d	14 d
Sensitivity Testing	<30 d	21 d

Tuberculin Skin Test (TST)

- In routine use since 1910
- TST is the most used test for *M. tuberculosis* infection in U.S.
- Delayed type hypersensitivity reaction to PPD, a polyvalent mycobacterial antigen mixture



TST Pro's Con's

Advantages

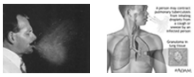
- Inexpensive
- Good performance
- No special equipment
- Long history of experience

Limitations

- Reader variability
- "Boost" response
- Low specificity
  - Cross reaction with BCG and NTM
- Low sensitivity
- Need for 2 visits

Interferon Gamma Release Assays:

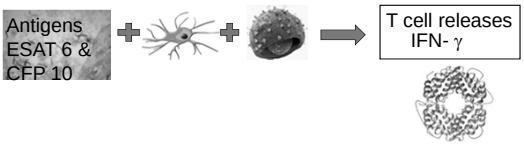
- Principle:
  - Persons exposed to *M. tuberculosis* develop T-cells (lymphocytes that recognize and respond to TB-specific antigens



Dendritic cell processes antigen and presents antigen to T Cell

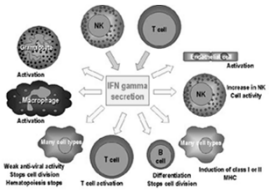
IGRA (continued)

- When stimulated with TB-specific antigens, these primed T-cells release the cytokine, interferon-gamma (IFN- γ)



IGRA (continued)

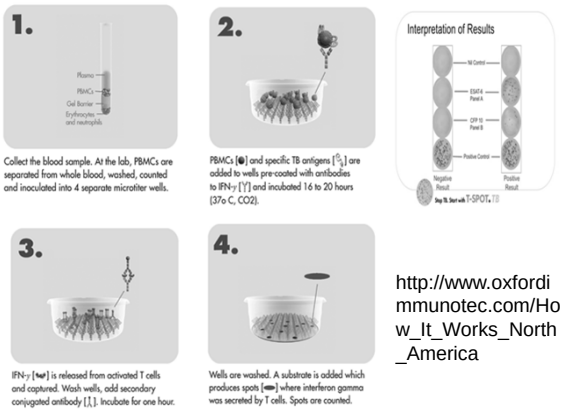
- The released IFN- γ can then be detected and serves as an indirect indicator of exposure to TB.



T-SPOT

FDA approved August 2008

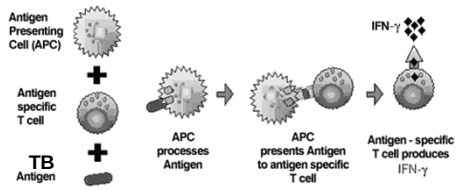
http://www.oxfordimmunotec.com/na/healthcare/howitworks.html



http://www.oxfordimmunotec.com/How_It_Works_North_America

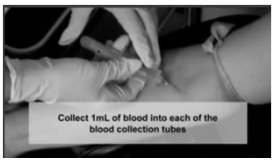
QuantiFERON TB- Gold In-Tube

- Blood test that measures and compares amount of interferon-gamma (IFN-γ) released by blood cells in response to antigens
- FDA approved in May 2005 –Cellestis, Carnegie, Australia

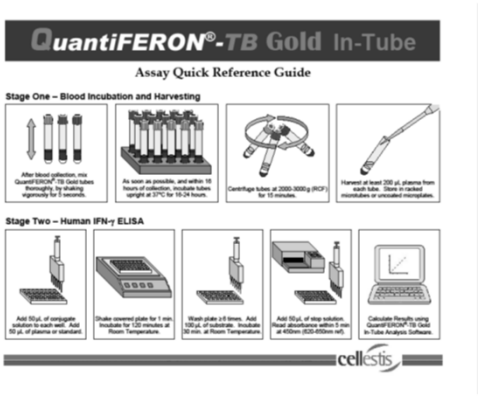


QFT Procedure – Clinic and Lab

- Procedures in Clinic
  - Blood Collection
  - Shaking of Tubes
  - Blood Incubation
  - Plasma Separation

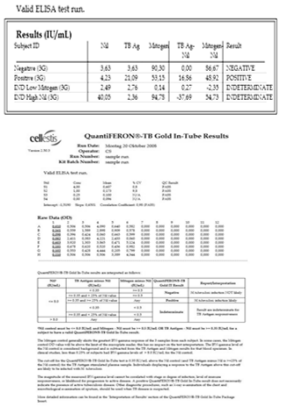


- Procedures in Lab
  - ELISA
  - Data Analysis



Data Analysis and Results

- Results are reported as
  - Positive
  - Negative
  - Indeterminate
- Indeterminate
  - Low mitogen
    - CMI response
  - High Nil
    - live vaccines
    - secondary infection


Valid ELISA test run.

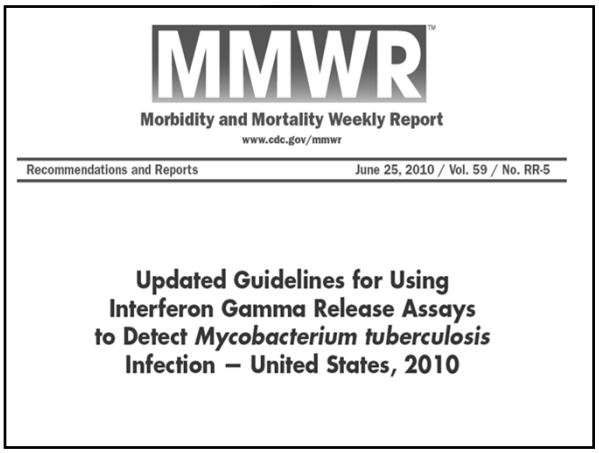
Results (IU/mL)

Subject ID	Nil	TB Ag	Mitogen	TB Ag - Nil	Mitogen - Nil	Result
Negative (SG)	3.63	3.63	90.30	0.00	56.67	NEGATIVE
Positive (SG)	4.23	21.09	53.13	16.86	48.90	POSITIVE
IUD Low Mitogen (SG)	2.49	2.76	0.14	0.27	-2.35	INDETERMINATE
IUD High Nil (SG)	40.05	2.36	94.78	-37.69	54.73	INDETERMINATE

QuantiFERON®-TB Gold In-Tube Results

Technology Comparison

	T-SPOT	QFTB In Tube	TST
Antigens	ESAT-6 & CFP10	ESAT-6 , CFP10, TB7.7	PPD
Boosting effect with repeat tests	No	No	Yes
TAT	16-20 h	16-24 h	48-72 h
Readout units	IFN-Gamma spot forming cells	International units of IFN-Gamma	Millimeters of induration
Technology	ELISpot	ELISA	NA
Readout system	Count of spots	Measurement of optical density values using an automated reader	Palpable induration
Subjective Reading	Yes	No	Yes



CDC advises that IGRA's can be used in all circumstances in which the TST is used, including…

- Contact investigations
- Evaluation of recent immigrants who have had BCG vaccination
- TB screening of health care workers and other individuals in high risk settings
- IGRA is in place of (not an addition to) TST

General Benefits of IGRA over TST

- Requires only one patient visit
- Assesses responsiveness to *M. tuberculosis* antigens
- Does not boost previous responses
- Interpretation less subjective than for TST

Limitations of IGRAs

- Cross-reactivity is possible with some atypical Mycobacteria infections:
  - *M. kansasii, M.szulgai*, and *M. marinum*
- Testing logistics:
  - Specimen transport time
  - Requirement for specialized testing equipment
- Additional data needed in certain patient populations
  - Children, Immunocompromised, Pregnancy

BCG Vaccinated Patients

- IGRA benefit the BCG vaccinated patient
- Many false positive TST due to vaccination status
- Treatment is costly, carries risk of significant side effects
- Treatment is not always needed since most do not have LTBI

Performance of IGRAs and the TST:

An up-to-date TB Test Meta-Analysis

R Diel, R Loddenkemper and A Nienhaus *Evidence based comparison of commercial interferon-gamma release assays for detecting active tuberculosis – a meta-analysis.* Chest, 2009, Published on Dec 18, 2009 in electronic format;

Chest April 2010 137:952-968; doi:10.1378/chest.09-2350

Contact Investigations

- For persons with recent TB exposure, negative IGRA results should be confirmed with a repeat test 8-10 weeks after exposure (end of window period) per CDC. This is the same as for a negative TST.
- Yoshiyama, et al. Timing of Quantiferon TB-G test for the contact examination of tuberculosis. *Kekkaku. 2007 Aug;82(8):655-8.*
  - "3 months interval from the diagnosis of the index case will be enough for the final decision of the infection of contacts."
    - N=25, 8 positive QFTB-G

For high risk contacts…

- When "window prophylaxis" has been started for high-risk contacts exposed to an infectious TB patient, a negative IGRA result at the end of the window period should be interpreted in light of all other clinical and epi data
  - A full course of LTBI TX should be considered even with a negative result when the rate of TB transmission to other contacts is high or when a false-negative is suspected because of immune status.

Use of IGRA Baseline and Serial Testing

- Baseline testing with IGRA
  - Establish baseline with single negative IGRA
  - HCWs with positive IGRA result should be referred for diagnostic evaluation
- Serial testing for infection control
  - A conversion is a change from negative to positive

Cost Barrier?

- **Cost-effective alternative to TST**
  - **Reduction in false positive test results**
  - **No second visit needed to complete testing**
  - **Two-step testing not needed**
  - **Reduction in rates of CXR (due to higher specificity for *M. tuberculosis)***

Are IGRAs cost effective?

- DePerio et al: Arch Intern Med. 2009
  - Use of IGRA "leads to superior clinical outcomes and lower costs than the TST and should be considered in screening non-BCG-vaccinated and BCG vaccinated new HCWs for LTBI."
- Marra et al: Int J Tuberc Lung Dis. 2008
  - "Selected use of QFT-G appears to be cost effective if used in targeted fashion."

IGRA Summary

- IGRAs are more specific than TST and are not confounded by previous BCG vaccination
  - Less unnecessary preventive treatment
- IGRA are more sensitive than TST

TB antibody tests

- Tests that detect IgG antibody to TB
- Highly variable results for sensitivity and specificity
- Do not have a roll in the diagnosis of TB
- Not FDA approved
- Recently confused with IGRA in the news.

Take Home Message

- Culture of TB remains the gold standard
- AFB smears are the most cost effective
- NAAT are sensitive and rapid but cannot differentiate between dead and viable TB
- IGRA do not differentiate between active and latent TB
- There are new tests on the horizon

Let's not forget…

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