

Using Interferon Gamma Release Assays for Diagnosis of TB Infection

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Learning Objectives

1. Describe available Interferon Gamma Release Assay tests for TB infection and how they work.
2. Understand interpretation and implications of test results.
3. Explain common community provider misconceptions and misuses of IGRA tests and strategies for intervention.

Testing for *M. tuberculosis* Infection

- Two testing methods available for the detection of *M. tuberculosis* infection in the United States:
 - Mantoux tuberculin skin test (TST)
 - Interferon-gamma release assays (IGRA)
- These tests *do not* exclude LTBI or TB disease
- Decisions about medical and public health management should include other information, and not rely only on TST or IGRA results

Limitations of the TST

- Subjective interpretation
- Difficult to maintain proficiency
- Requires 2 visits
- Affected by prior BCG vaccination
- Limited use by primary care providers
- Despite > 100 years of use, there is no standard way to record and retrieve results

LTBI Diagnostic Tests Developed in the 20th Century

- There were NO new diagnostic tests for LTBI throughout the entire 20th century!

Interferon-Gamma Release Assays (IGRA)

Interferon Gamma Release Assays

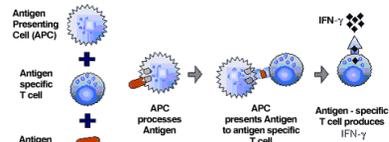
- Three IGRAs approved by the U.S. FDA and are commercially available in the U.S.:
 - QuantiFERON®-TB Gold test (QFT-G);
 - QuantiFERON®-TB Gold In-Tube test (QFT-GIT);
 - T-SPOT®.TB test (T-Spot)



Note: Latest guidelines: CDC guidelines for QFT-GIT and T-SPOT published June 2010 www.cdc.gov/mmwr Vol. 59, No. RR-5
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Whole Blood Gamma Interferon Assay

- IGRAs use purified antigens from MTB to stimulate peripheral-blood lymphocytes to produce gamma interferon



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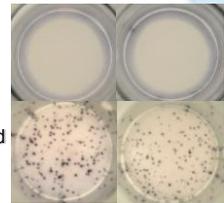
QuantiFERON-TB (Cellestis)

- Originally developed in Australia to test cattle for *M. bovis* infection
- Measures IFN- γ in stimulated whole blood relative to a nil and mitogen control
 - IFN- γ in the supernatant of the cell suspension
- QFT: PPD
- QFT-Gold: ESAT-6 and CFP-10
- QFT-Gold in tube (QFT-GIT): ESAT-6, CFP-10 and TB 7.7

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T-SPOT.TB (Oxford Immunotec)

- Developed in England
- Uses a modified ELISpot platform
- Measures IFN- γ production from effector T-cells after separated PBMCs are stimulated with ESAT-6 and CFP-10
 - Quantifies # cells producing IFN- γ
- Approved in Europe 2004 and in the U.S. 2008



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Table1: Differences in Currently Available IGRAs

	QFT-Gold	QFT-Gold In Tube (QFT-GIT)	T-Spot
Format	Process whole blood within 12h	Process whole blood within 16h	Process peripheral blood mononuclear cells (PBMCs)
M. Tuberculosis antigen	Separate mixtures of synthetic peptides representing ESAT-6 & CFP-10	Single mixture of synthetic peptides representing ESAT-6 & CFP-10, and TB 7.7	Separate mixtures of synthetic peptides representing ESAT-6 & CFP-10
Measurement	IFN- γ concentration	IFN- γ concentration	# of IFN- γ producing cells (spots)
Possible Results	Positive, negative, indeterminate	Positive, negative, indeterminate	Positive, negative, indeterminate, borderline

Note: CDC guidelines for QFT-GIT and T-SPOT published June 2010 www.cdc.gov/mmwr Vol. 59, No. RR-5
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IGRA: General Points

- IGRAs are highly specific (~95%)
 - Both commercially available tests (QFT and T-SPOT TB) are substantially more specific than the PPD since they contain antigens not found in BCG
 - Also distinguish most non-tuberculous mycobacteria (except *M. Kansasii*, *M. marinum*, *M. szulgai*, *M. flavescens*)
- IGRAs have moderate to high sensitivity vs. PPD
 - QFT being as sensitive as PPD (70-80%) in immunocompetent
 - T-SPOT TB is more sensitive (~90%) than QFT and PPD in the immunocompromised

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QFT vs. T-SPOT

QFT-GIT

- Positive (> 0.35 IU/mL)
- Negative (< 0.35 IU/mL)
- Indeterminate
 - Low mitogen
 - High nil
- Failed
 - Inadequate blood volume
 - Broken tube
 - Delayed incubation

T-SPOT.TB

- Positive (> 8 spots)
- Negative (< 4 spots)
- Borderline (5-7 spots)
- Invalid
 - Low mitogen
 - High nil
- Failed
 - Inadequate blood volume
 - Broken tube
 - Delayed incubation

Indeterminate QFT

- Retrospective review; public chest clinics in NYC using QuantiFERON
- 28,864 tested □ 522 (2%) indeterminate
 - 264 low mitogen (assoc. with age < 10, females, Asian, U.S. born)
 - 258 high nil (assoc. with foreign-born and Hispanic)
- Repeat test with valid result (pos/neg) in 68%
- For indeterminate IGRA result, repeat test

Q: IGRAs are reliable tests to tell practitioners who among those with latent TB infection will progress to active disease.

1. True
2. False

IGRA: Advantages

- Requires a single patient visit to conduct the test
- Controlled laboratory test
- Results can be available within 24 hours
- No booster responses with subsequent tests
- More specific than TST
 - Prior BCG does not cause a false + IGRA test result
 - No cross reactivity with most NTM
 - In healthy individuals with no known TB exposure
 - IGRA: 90-100% vs. TST: 70-95%
- Use of IGRA may increase acceptance of LTBI treatment

IGRA: Disadvantages

- Blood samples must be processed within 8-16 hours after collection while white blood cells are still viable
- Errors in collecting or transporting blood specimens or running and interpreting the assay can decrease the accuracy of IGRAs
- Limited data on the use of IGRAs to predict who will progress to TB disease in the future
- Limited data on the use of IGRAs for:
 - Children younger than 5 years of age
 - Persons recently exposed to *M. tuberculosis*
 - Immunocompromised persons
 - Serial testing
- Tests may be expensive

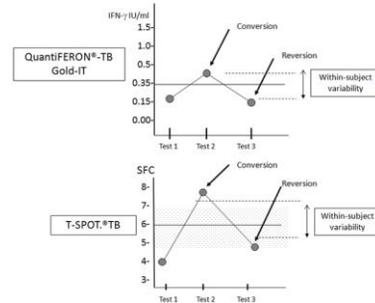
Remaining Questions about IGRA (1)

- How long does it take for an IGRA to become positive after exposure?
- Do IGRA results change after treatment for LTBI?
 - If so, can IGRAs detect re-infection after LTBI treatment?
 - Do IGRAs have a role monitoring response to LTBI treatment?
- Can sensitivity and specificity of IGRAs be improved by modifying the interpretation criteria?
- Will treatment of IGRA+ individuals reduce the incidence of TB disease?
- How do IGRAs perform in special populations, e.g., children, individuals with HIV infection, individuals on immunosuppressive therapy?
 - Which subgroups most likely to benefit from use of IGRAs?

Remaining Questions about IGRA (2)

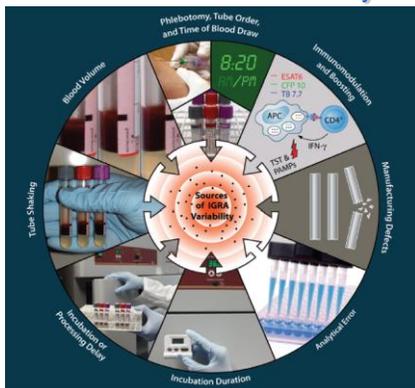
- Are individuals with higher IFN- γ responses associated with a greater risk for TB disease?
- How common are IGRA conversions and reversions?
 - How should they be defined?
- How should we interpret TST and IGRA discordance?
 - What proportion of the discordance is due to variations around TST and IGRA cutoff points?
 - When discordant cases are re-tested, what proportion become concordant?
- What is cost effectiveness of switching from TST to IGRA?
- What is the impact on clinic/staff workload and patient compliance with testing and follow-up?
- How does each IGRA compare with TST in predicting progression from LTBI to TB disease?

Figure 2. Schematic of the concept of "conversion and reversion" and "within-subject variability".



van Zyl-Smit RN, Zwerling A, Dheda K, Pai M (2009) Within-Subject Variability of Interferon- γ Assay Results for Tuberculosis and Boosting Effect of Tuberculin Skin Testing: A Systematic Review. PLoS ONE 4(12): e48517. doi:10.1371/journal.pone.0008517
<http://www.plosone.org/doi/10.1371/journal.pone.0008517>

Sources of IGRA variability



Pai M, CMR 2014

William Osler

"Medicine is a science of uncertainty and an art of probability."

"It is much more important to know what sort of a patient has a disease than what sort of a disease a patient has."

Specific CDC Recommendations

- IGRAs preferred
 - Person not likely to return for test reading (homeless, drug users, etc.)
 - Persons who received BCG for vaccine or cancer treatment
- TST preferred: Children <5 years (*IGRA can be used*)
- TST or IGRA
 - Contacts; periodic screening of health care workers

Note: CDC guidelines for QFT-GIT and T-SPOT published June 2010
www.cdc.gov/mmwr. Vol. 59, No. RR-5

Specific CDC Recommendations

- Use of both tests not recommended, except in specific situations. Consider TST **AND** IGRA:
 - If initial test is negative
 - Increased risk of infection, progression to TB, poor outcome (e.g., HIV) and initial test was negative
 - Clinical suspicion of TB and confirmation of infection desired
 - If initial test is positive:
 - Additional confirmation of LTBI will help with compliance
 - Healthy low risk persons with positive test
 - single pos. test not reliable evidence of infection in low risk person

Note: CDC guidelines for QFT-GIT and T-SPOT published June 2010
www.cdc.gov/mmwr. Vol. 59, No. RR-5

BCG: True or False

- BCG vaccine prevents infection with *M. tuberculosis*.
- A. True
B. False

LTBI in Pregnant Women: True or False?

- IGRA testing should be deferred until after the first trimester.
- Pregnant women with a positive IGRA should not have a CXR until after delivery.
- If a pregnant woman is at high risk of progression from LTBI to active TB, LTBI therapy can be considered.

LTBI Testing Summary: Comparison of IGRA and TST

IGRA:

- In vitro test
- Specific antigens
- No boosting
- 1 patient visit
- Minimal inter-reader variability
- Results in 1 day
- Requires blood test
- Not affected by BCG, most atypical mycobacteria

TST

- In vivo test
- Single antigen
- Boosting phenomenon
- 2 patient visits
- Inter-reader variability
- Results in 2-3 days
- No phlebotomy
- Cross-reacts with BCG, atypical mycobacteria

Cases

Case 1a

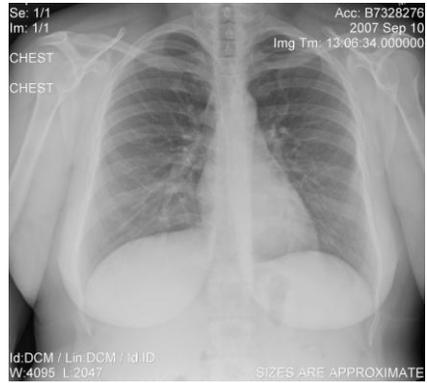
- The first patient is a US born 45 year old woman who had a PPD for work. She has no other medical problems and she is asymptomatic. Here is her x-ray.



Case 1a

Case 1b

- The second is a 50 year old woman from Florida is also asymptomatic and had a PPD as a work requirement but she denies any exposures to anyone with TB. She too has a 10 mm PPD but had a documented negative PPD 7 years ago. Here is her x-ray.



Case 1b

Case 1a and b

a. US born 45 year old F, 10mm PPD for work, no other medical problems, asymptomatic. x-ray normal.

b. 50 year old F from Florida but born in Colombia, also asymptomatic, PPD done as a work requirement 10mm, was negative 7 y ago, denies any exposures to anyone with TB.

Which of the following statements is true about these 2 women?

- A. Both are likely latently infected with TB given the PPD reaction size
- B. Both should be evaluated to begin treatment for LTBI
- C. An IGRA would be helpful to determine if these truly represent LTBI
- D. An IGRA would not be helpful since you have already diagnosed them with LTBI
- E. All of the above are false

Case 2

A patient with a vague history of a positive PPD (undocumented) comes in to be evaluated after being identified as a contact to an active case of TB. He is refusing a PPD but is willing to have blood drawn for an IGRA. He has a history of prednisone use for COPD. A Quantiferon is drawn and the result is read as "Indeterminate". Possible reasons for the indeterminate result include:

- A. His concurrent treatment with prednisone
- B. May have other undiagnosed cause of immune suppression
- C. He may be in the "window" since his last exposure
- D. Collection or processing error
- E. All of the above

Case 2b

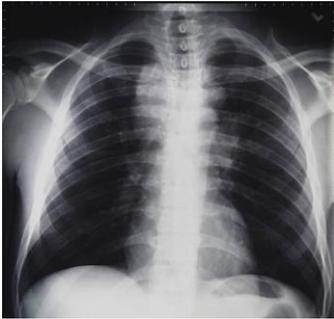
For this patient with "Indeterminate" QFN GIT test result, what should you do next?

- A. Evaluate for active TB
- B. Treat for LTBI without retesting since immunosuppressed
- C. Order a TST since immunosuppressed
- D. Re-test with QFN-GIT
- E. Re-test with a T-spot test

Case 3

- A 37 year old man with a history of HIV infection is referred to the health department for evaluation prior to beginning LTBI therapy. He was a contact to an active case and had a positive IGRA. He reports no symptoms, and has the following x-ray.

Case 3



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Case 3

37 year old HIV (+) man, known contact to an active TB case, positive IGRA, no symptoms, with shown x-ray. The next step would be:

- A. Start LTBI therapy with INH 900mg daily if there are no contraindications
- B. Start LTBI therapy with INH and rifampin by DOT because he is HIV (+) and therefore a high priority candidate to complete therapy
- C. Obtain sputum specimens for AFB
- D. Withhold therapy for LTBI at this time because given his immune suppression, his positive IGRA was likely a false positive
- E. None of the above would be appropriate next steps

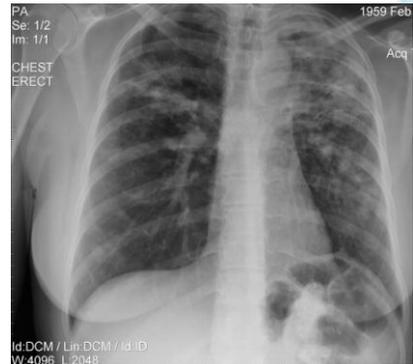
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Case 4

- A patient with a history of homelessness is referred to the health department for evaluation of a positive PPD that was done during a contact investigation at the homeless shelter. He is a poor historian but you have information that he had a negative IGRA during the contact investigation. For some reason a chest x-ray was obtained.

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Case 4



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Case 4

Homeless patient with (+)PPD, (-) IGRA during CI at homeless shelter. Based on CXR shown, which of the following is true?

- A. TB ruled out by (-) IGRA, so he should begin tx for community acquired pneumonia with quinolone as per IDSA/ATS glns
- B. He should begin LTBI treatment soon because of his recent close contact to an active case and associated increased risk for progressing to TB disease rapidly
- C. After appropriate evaluation, consider LTBI tx with 3HP by DOT because he's homeless and high risk for non-adherence
- D. Evaluated for active TB with sputum for AFB and start TB tx
- E. Refer to pulmonary for bronchoscopy to diagnose abnormalities on CXR

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CONSULTS FROM THE SNTC

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Consult #1

A physician calls you regarding a patient from Guatemala with a positive PPD documented at private provider's office. He had a negative PPD and CXR 2 years ago but recently returned home for a few months.

What should you do now?

- A. Because it is a documented conversion, start LTBI treatment after counseling him of the risks and benefits.
- B. Repeat the PPD to confirm the results since it was done by a private provider.
- C. Order an IGRA to confirm the positive TST test.
- D. Evaluate him for active TB before starting LTBI therapy.



Consult #2

Patient from Germany with 17mm PPD, clear CXR. He is a health care worker but has no known exposure to TB. He has no signs and symptoms of active disease and has a normal chest x-ray. He is refusing LTBI therapy.

- How should this patient be followed?
 - A. Perform serial symptom screening and CXR annually
 - B. Repeat IGRA annually to see if test converts/reverts
 - C. Conduct annual screening using a symptom/risk assessment
 - D. He is low risk and does not need any further screening.



Consult #3

Provider calls you regarding a 17 month old with 22 mm PPD induration. By history, another 12 mo. old with 15 mm PPD induration lives in the same home. Both are immigrants from high burden countries; probable BCG vaccination.

What further evaluation is recommended?

- A. Routine follow up at next available appointment to determine need for LTBI therapy.
- B. Overbook patient to quickly evaluate for signs and symptoms of active TB and consideration of LTBI treatment.
- C. Evaluate all household and close contacts.
- D. Both A and C
- E. Both B and C



Consult #4

Manager of Infection Control/Immunizations for an HMO that provides health care contacts you regarding a police officer who was born in Korea, adopted at 2 and moved to US but does not know if he had BCG. He had a negative PPD in Feb 2012, and a positive PPD in March 2014. QFN-GIT was negative. He has no clinical or radiologic evidence of TB disease.

- Does he need treatment for LTBI?
 - A. Yes
 - B. No
 - C. It depends....



Consult #5

PCP calls you about a patient had an indeterminate QFN-GIT. Patient being evaluated to initiate Humira therapy for osteoarthritis. She is from the islands, however, has not had contact with an active case of TB nor does she have any other risk factors for LTBI or TB disease.

What should PCP do next?

- A. Re-test with QFT-Gold
- B. Order the T-spot next
- C. Order the TST
- D. Treat for LTBI regardless of indeterminate test results.



Consult #5 (cont.)

Repeat testing confirms positive result. Evaluation reveals no symptoms of TB disease and normal CXR.

What should be done next?

- A. Complete a full course of LTBI therapy prior to initiating TNF-blocker therapy (Humira)
- B. Concurrently start Humira and LTBI therapy
- C. Start LTBI therapy, and after at least one month start Humira.



Thank You!
1-800-4TB-INFO

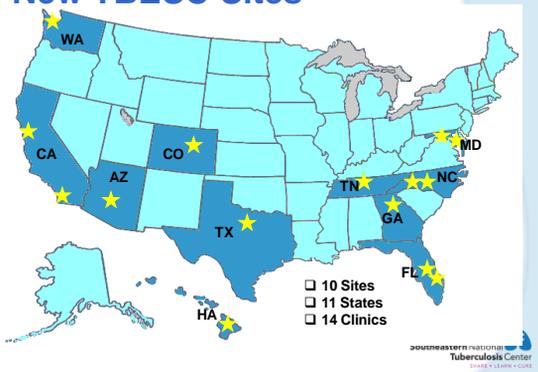


“To have striven, to have made the effort, to have been true to certain ideals - this alone is worth the struggle.”

William Osler



New TBESC Sites



TBESC Main Study (TO 1)

- Main objective
 - To evaluate and compare the performance of TST and IGRAs in:
 - Diagnosing LTBI
 - Predicting progression from LTBI to TB disease
- Study design
 - Multicenter (14 clinics), prospective, longitudinal cohort
 - Each person will get TST, QFT, T-SPOT
 - Persons positive by any test will get f/u for 2 yrs
- Study duration: 10 years
- Enrollment: over 40,000 high risk persons



TBESC Primary Objectives

1. Evaluate the agreement between the TST and 2 Interferon Gamma Release Assays (QFT-GIT and T-SPOT)
2. Compare the ability of each test to predict progression to TB disease

Secondary Objectives

1. Determine if sensitivity and specificity of IGRAs can be improved by changing the cut-off values



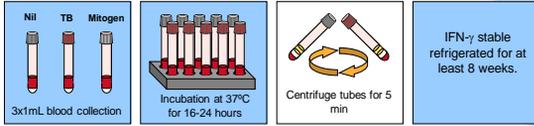
Baye's Theorem

The accuracy of a test is dependent upon the prevalence of the disease in the population



QuantiFERON®-Gold In Tube

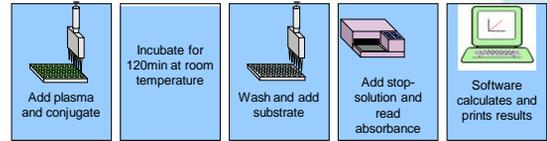
Stage One – Blood collection and harvesting



- ✓ Option 1: Shipment of the blood collection tubes within 16 hours to a laboratory prior to incubation
- ✓ Option 2: Shipment of the blood collection within 3 days after incubation to the laboratory.
- ✓ Possibility to batch samples.

QuantiFERON®-Gold In Tube

Stage 2 – Interferon-γ ELISA



- ✓ Easy "Standard"-ELISA.
- ✓ User-friendly software supplied free-of-charge from Cellestis.
- ✓ No need for new equipment.

Step 1 – Preparation of cells



- Blood collected into Vacutainer CPT™ tube
- Tube centrifuged
- Lymphocyte band removed
- Cells washed & counted
- Cells added to 96-well plate
- Antigens added to wells
- Incubate overnight



Step 2 – Forming spots

- Plate washed
- Add detection reagent for 60 minutes
- Plate washed
- Add substrate; spots in 7 minutes
- Plate washed and dried

