

## What is an IGRA? Are they available here? How do I use them?



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## Learning Objectives



Participants will be able to:

- Explain the immunologic basis for QuantiFERON-TB Gold, T-Spot TB and TST testing
- List three CDC recommendations for use of the tests available to detect TB infection
- Identify potential resources for IGRA testing near their local health department
- List three factors of populations served that might indicate preference for IGRA use vs. TST
- List the test requirements and constraints of each of the IGRA tests

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## What is an IGRA?



- Blood assay that measures an immune response that reflects prior contact with M.tb and a few other Mycobacteria
- There are two IGRAs currently produced:
  - QuantiFERON-TB Gold-in-Tube by Cellestis, Inc.
  - T-Spot.TB by Oxford Immunotec, Ltd.

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## How To Detect TB Infection

First, there was the TST

- Used for more than 100 years (since 1890)
- 0.1 ml of 5 TU PPD tuberculin injected intradermally
- Induration in millimeters read 48-72 hours after injection

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## TST Limitations

- Variability in administration and reading
- > 1 visit needed
- False negative responses
- Boosting of the immune response with repeated TSTs
- False positive responses

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## Basic Principle of the TST

- Old tuberculin - a sterile solution of a concentrated filtrate of *M. tuberculosis* in culture
- PPD – purified protein fraction precipitated from old tuberculin
- PPD contains many antigens; some are also found in BCG and NTM

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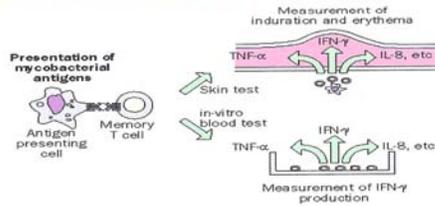
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## What Do These Tests Measure?

### IGRA: rationale



Anderson, et. al., Lancet 2000:356:1099

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## Basic Principles of IGRAs

- Peripheral blood lymphocytes from a person suspected of having tuberculosis infection are exposed to antigens (different from those in PPD/more specific) from *Mycobacterium tuberculosis*
- If person has been infected with *M. tuberculosis*, lymphocytes will respond by producing IFN-γ
- The tests measure the total IFN-γ produced (QFT-GIT) or number of cells that produce IFN-γ (T.Spot TB)

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## Antigens Specific to *M. tuberculosis*

- Located in the genetic region of difference, not found in BCG or most NTM
  - Exceptions! *M. kansasii*, *M. szulgai*, *M. marinum*, and *M. riyadhense*
- Codes for 9 proteins
  - Two are used that produce strong immunologic responses in persons infected with *M. tuberculosis*
    - 10-kDa culture filtrate protein (CFP-10)
    - 6-kDa early-secreted antigenic target (ESAT-6)
  - A third is used in QFT-GIT
    - TB7.7

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### QuantiFERON TB Gold In-Tube

- Whole blood is collected in three special collection tubes, filled precisely to 1 ml line, and agitated vigorously for 5 seconds
- Tubes must begin incubation within 16 hours of the blood draw
- The tubes are incubated for 16-24 hours
- Usually requires phlebotomy at a patient service center to meet time deadlines or spin and incubate on-site
- Results usually available within 1 week

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### QuantiFERON TB Gold In-Tube



The Tubes:  
Mitogen (positive)  
TB Antigen  
Nil (negative)

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### QuantiFERON TB Gold In-Tube



The black fill line

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## QuantIFERON TB Gold In-Tube

- ELISA is performed to detect IFN- $\gamma$  produced in
  - Nil (negative control)
  - Mitogen (positive control) and
  - TB Antigen tube
- The result is the difference between the concentration of IFN- $\gamma$  in plasma from blood stimulated with TB antigen minus the IFN- $\gamma$  concentration in plasma from blood incubated without antigen (Nil tube)
- The QFT-GIT can have a result of negative, positive, or indeterminate

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## QuantIFERON TB Gold In-Tube

- Available at commercial labs and some local medical facilities
  - Quest
  - Labcorp
  - Riverside in Newport News
  - Call your local resources to locate
- Cost – varies from \$160 negotiated rate to \$230 or more
- Covered by most insurances if testing indicated

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## QuantIFERON TB Gold In-Tube Sample Results

QuantIFERON TB Gold	Positive	Abnormal	Negative
QFT Positive Criteria			
To be considered positive a specimen should have a TB Ag minus Nil value greater than or equal to 0.35 IU/mL and in addition the TB Ag value must be greater than or equal to 25 % of the Nil value. There may be insufficient information in these values to differentiate between some negative and some indeterminate test values.			
QuantIFERON TB Ag Value	>10.00		IU/mL
QuantIFERON Nil Value	0.43		IU/mL
QuantIFERON Mitogen Value	>10.00		IU/mL
QFT TB Ag minus Nil Value	>10.00		IU/mL

**Interpretation:**  
 The QuantIFERON TB Gold (in Tube) assay is intended for use as an aid in the diagnosis of TB infection. Negative results suggest that there is no TB infection. In patients with high suspicion of exposure, a negative test should be repeated. A positive test indicates infection with *Mycobacterium tuberculosis*. Among individuals without tuberculosis infection, a positive test may be due to exposure to *M. kansasii*, *M. szulgai* or *M. marinum*. On the Internet, go to [cdc.gov/tb](http://cdc.gov/tb) for further details.

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### QuantiferON TB Gold In-Tube Results - 1

- **Positive:** Nil  $\leq 8.0$  and  
TB Response  $\geq 0.35$  IU/ml and  $\geq$  of 25% of Nil  
Mitogen response: Any
- **Negative:** Nil of  $\leq 8.0$  and  
TB Response  $< 0.35$  IU/ml or  $< 25\%$  of Nil  
Mitogen response:  $\geq 0.5$

Table 2. Interpretation Criteria for QFT-GIT, CDC Guidelines, pg. 16

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### QuantiferON TB Gold In-Tube Results - 2

- **Indeterminate:** Nil  $\leq 8.0$  and  
 $< 0.35$  IU/ml or  $< 25\%$  of Nil with  
Mitogen  $< 0.5$  Under-active immune system
- OR
- Nil  $> 8.0$  Over-active immune system  
Any TB Response  
Any Mitogen Response
- REDRAW RECOMMENDED

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### T.Spot TB

- Whole blood is collected in one or two green top tubes (lithium heparin), depending on presence immuno-compromising conditions



- Tubes must begin incubation within 32 hours of the blood draw

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## T.Spot TB

- Blood is shipped via FedEx or processed in-house if equipped
- Oxford Immunotec provides shipping materials/mailling label




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## T.Spot TB

- At the lab the peripheral blood mononuclear cells are separated, washed, counted and inoculated into 4 wells with TB antigen and IFN- $\gamma$  antibodies, and incubated for 16-20 hours
- Wells are for Nil control, ESAT-6, CFP-10, and positive control
- Several processes occur resulting in spots appearing where IFN- $\gamma$  was secreted by T-cells
- Spots are counted

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## T.Spot TB

- Available after signing a contract with Oxford Immunotec
- The TB Program is exploring a contract that would apply statewide
- Current price \$59, negotiated for one county, which includes supplies and mailing
- WebVision code is TSpotTB

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## T Spot Sample Result

T-SPOT.TB

Positive

A patient's test result is positive when the difference between the number of spots present in patient's sample and the negative (NTS) control is  $\geq 8$ . Note: Diagnosing or excluding tuberculous disease, and assessing the probability of TB, requires a combination of epidemiological, historical, medical and diagnostic findings that should be taken into account when interpreting T-SPOT.TB test results. Refer to the most recent CDC guidance (<http://www.cdc.gov/ncidod/d/tb>) for detailed recommendations about diagnosing TB infection (including disease) and selecting persons for testing.

Nil (Neg) Control Spot Count	1
Panel A Spot Count	>50
Panel B Spot Count	>50
Positive Control Spot Count	>20

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## T.Spot TB Results

- Positive - Nil  $\leq 10$  spots  
TB Antigen  $\geq 8$  spots  
Mitogen - Any Response
- Negative - Nil  $\leq 10$  spots  
TB Antigen  $\leq 4$  spots

Table 3 Interpretation Criteria for T.Spot TB, CDC Guidelines, pg. 16

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## T.Spot TB Results

- Borderline - Nil  $\leq 10$  spots  
TB Antigen 5, 6, or 7 spots  
Mitogen Any Response
  - Indeterminate - Nil  $\geq 10$  spots Overactive Immune System  
TB Antigen and Mitogen - Any Response
- OR
- Nil  $\leq 10$  spots  
TB Antigen  $< 5$  spots  
Mitogen  $< 20$  spots Underactive Immune System

REDRAW RECOMMENDED

Table 3 Interpretation Criteria for T.Spot TB, CDC Guidelines, pg. 16

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### Immune response being measured!

- There still is no way to detect M.tb directly
  
- “Because TST, QFT-GIT and T Spot each measure different aspects of the immune response and use different antigens and interpretation criteria, test results might not be interchangeable. Different tests can yield different results.” CDC IGRA Guidelines, pg. 5
  
- **The test results should be used in combination with clinical assessment**

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### Which Test is Better?

- Sensitivity and Specificity vary depending on:
  - Cut points used for TST and a variety of standards used for interpretation of IGRAs
  - No good standard for determining true TB infection and TB disease
  
- Very few head-to-head trials of the two approved IGRAs
  
- With current limited data, they are approximately equal, with a few exceptions

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### Which Test is Better?

- It depends on the population to be tested
  
- All are recommended to be used in high-risk populations only

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## Overview of the 2010 CDC Guidelines

Centers for Disease Control and Prevention

### Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection- United States, 2010

MMWR 2010: 59(No. RR-5);1-26

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## General Recommendations for Use of IGRAs - 1

- TSTs and IGRAs should be used as aids in diagnosing infection with M. tuberculosis.
- IGRAs should be performed and interpreted according to established protocols using FDA-approved test formats.
- BOTH standard qualitative test interpretation (positive or negative) AND the quantitative assay measurements should be reported, along with the criteria used for test interpretation.

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## General Recommendations for Use of IGRAs - 2

- Arrange for IGRA testing prior to blood collection.
- Each institution and TB control program needs to evaluate the availability, overall cost, and benefits of IGRA use for their own setting. Consider your population!
- As with TSTs, IGRAs generally should not be used for testing persons with a low risk for both infection and progression to TB disease.

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## Test Selection Principles

- Select the most suitable test or combination of tests for detection of M.tb on the basis of the reasons and context for testing.
- An IGRA can be used in place of (but not in addition to) a TST in all situations in which CDC recommends a TST, with few exceptions. In spite of the special exceptions, use of an alternative test is acceptable medical and public health practice.

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## Situation in Which an IGRA is Preferred but TST is Acceptable

- Testing persons with low rates of return for reading
  - the homeless
  - substance abusers
- Testing persons who have received BCG
  - as a vaccine
  - for treatment of bladder cancer therapy

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## Situations in which a TST is preferred but an IGRA is Acceptable

- TST is preferred for testing children less than 5 years of age.
- Some have advocated an IGRA in conjunction with a TST to increase diagnostic sensitivity in this group. The American Academy of Pediatrics states\* that IGRAs may be useful in determining whether a BCG immunized child with a reactive TST more likely has LTBI or has a false-positive TST caused by the BCG.

\*American Academy of Pediatrics, Red Book: 2009, pg. 687.

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### Situations in Which Either TST or IGRA May Be Used Without Preference

- To test recent contacts of persons known or suspected to have active TB disease.
  - IGRA with greater specificity than TSTs in this setting
  - Unlike TSTs they do not boost subsequent test results
  - Offer a single patient visit
  - Need to be repeated at 8-10 weeks after contact broken if first test is prior to that time, as with TSTs
- To conduct periodic screening of persons who might have occupational exposure to TB (HCWs)

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### Situations in Which Testing with Both an IGRA and a TST May BE Considered

- When the initial test (TST or IGRA) is negative and
  - When the risk for infection, progression and risk of poor outcome are increased (HIV or children aged < 5 years)
  - When clinical suspicion exists for active tuberculosis
- When more evidence of infection might encourage compliance with Tx for LTBI
- In healthy persons with low risk for both infection and progression to disease; to support conclusion of false positive test
- When initial IGRA result is indeterminate, borderline or invalid and a reason for testing persists

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### What to do with IGRA Results Medical Management

- Decisions about treatment include medical history, clinical picture, and epidemiologic information
- Persons with positive TST or IGRA should be evaluated for likelihood of M.tb infection, for risks for progression to active disease, and for symptoms of TB disease
- A diagnosis of LTBI requires that a diagnosis of active disease has been ruled out
- Neither a TST nor IGRA can distinguish LTBI from active TB disease

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### What to do with IGRA Results Medical Management – positive results

- In persons with symptoms or radiographic evidence of active TB who are at increased risk of progression to TB if infected, a positive result of either TST or IGRA should be taken as evidence of M. tuberculosis infection
- Negative results are NOT SUFFICIENT TO EXCLUDE INFECTION

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### What to do with IGRA Results Medical Management – negative results

- In healthy persons who have a low likelihood of both infection and progression to TB disease, a single positive TST or IGRA SHOULD NOT BE TAKEN AS RELIABLE EVIDENCE OF M.tuberculosis infection.  
A false positive is more likely.
  - Reassess likelihood of infection and progression to disease
  - Repeat testing with the same or different test on a case-by-case basis
  - Can also assume, without additional testing, that the initial test is a false positive (Why were they tested?)

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### What to do with IGRA Results Medical Management-discordant results (+ and -)

- Decisions about management include:
  - Assessment of the quality and magnitude of each test result
  - The probability of infection
  - The risk for disease if infected
  - The risk for poor outcome if disease occurs

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### What to do with IGRA Results Medical Management – discordant results

- Taking a positive result from either test is reasonable when:
  - Clinical suspicion exists for active tuberculosis
  - The risks for infection, progression, and a poor outcome are increased
    - HIV +
    - Children aged < 5 years

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### What to do with IGRA Results Medical Management – discordant results

- For healthy persons with low risk for infection and progression to disease DISCOUNTING A POSITIVE RESULT IS REASONABLE. This choice will:
  - increase specificity of the test
  - Decrease unnecessary treatment

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### What to do with IGRA Results Medical Management – discordant results

- For persons who have received BCG who are not at increased risk of poor outcome if infected:
  - TST <15 mm may be discounted as a false positive when an IGRA is clearly negative
- In other situations, evidence is lacking in which to base recommendations
  - Diagnostic decisions can be deferred if no risk of progression or poor outcome

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## What is next??

- More studies are needed!
- Sites using either IGRA are encouraged to publish results

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## Questions??

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