

TB Infection Testing

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TB 101
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Outline of Talk

- Test for TB infection
 - Tuberculin Skin Test
 - IGRA
 - Quantiferon Gold
 - T-Spot
- Clinical scenarios- using and interpreting the tests
- Take home messages

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Tests for TB infection – how do they work?

- Presently all available tests do NOT check for the presence of the TB organism
- They query the immune system to see if it has been sensitized to antigens contained in TB mycobacteria
 - Thus, if the immune system is not optimally functional, the test may not be accurate

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Testing for Latent TB Infection

- Tuberculin Skin Test
- Interferon Gamma Release Assay



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Latent TB Infection Tests

TST (PPD, C-TB)

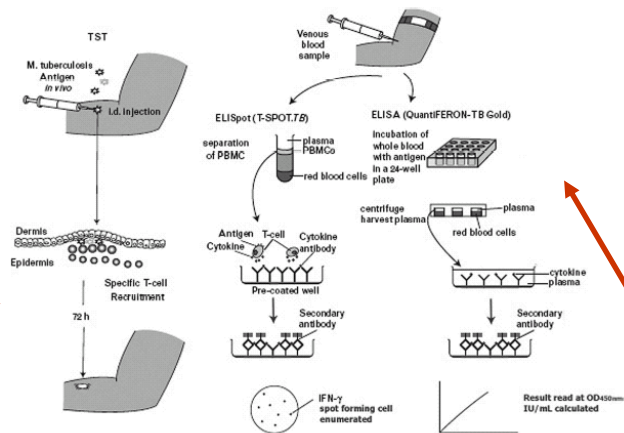
- 2 Patient Visits
- Requires expertise in planting and measuring
- Inexpensive
- False positives/False negatives?

IGRA (Quantiferon Gold, T-Spot)

- 1 Patient Visit*
- Requires expertise in phlebotomy and laboratory
- Expensive
- False Positives/ False Negatives?

Neither test distinguishes between infection and disease – in disease both tests may be negative or indeterminate

TST and IGRA



Mycobacterial Slurry

TB specific antigens: ESAT-6, CFP-10, TB7.7

Tuberculin Skin Testing

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TB infection testing interpretation, PPD, TST, Mantoux test

- Intradermal injection of tuberculin material
- Stimulates a delayed-type hypersensitivity response mediated by T lymphocytes
- In patients with prior mycobacterial exposure, causes induration at the injection site within 48 to 72 hours



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PPD Testing: a 2-step process

- Mantoux skin testing
 - Size measurement of induration is recorded
- Epidemiologic risk assessment
 - risk of having been exposed
 - known contact to active disease
 - risk of developing disease if infected
 - comorbid disease

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Reading the TST

- Measure reaction in 48 to 72 hours
- Measure induration, not erythema
- Record reaction in millimeters, not “negative” or “positive”
- Ensure trained health care professional measures and interprets the TST



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Interpretation of PPD

- Interpretation depends on the **risk for TB infection** and the **risk for progression to active TB disease**

≥ 5mm	HIV + (any CD4 count)	Close contact of active contagious case	Abnormal chest radiograph with fibrotic changes consistent with old TB	Immunosuppressed patients: -TNF-alpha inhibitors -chemotherapy -organ transplantation -glucocorticoid treatment (equivalent of ≥15 mg/day prednisone for ≥1 month)
≥ 10mm	Certain conditions that increase the risk of reactivation -HD -Silicosis -Certain malignancies -DM -Malnutrition -Jejunal bypass -IVDU	Residents and employees in high-risk settings -Prisons -Jails -Healthcare facilities - Mycobacteriology labs -Homeless shelters	Children < 4 yrs age	Foreign born from countries with high incidence TB, in particular those immigrated in past 5 years
≥15mm	Healthy individuals age 4 years and older with low likelihood of true TB infection			

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TB infection testing interpretation
booster phenomenon vs. conversion

- **Booster response:**
 - Positive PPD performed 1-4 weeks after an initial negative PPD in the absence of TB exposure
 - Sometimes done in a person who is planned for serial testing, ie.. health care workers
 - May be a sign of a remote TB infection
 - Helps to avoid misclassifying someone as a new conversion
- **Conversion:**
 - An increase in induration of ≥10 mm since the previous test in the setting of ongoing risk of exposure to TB
- **Remember: serial PPDs will not lead to a false + PPD!**

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TB infection testing interpretation PPD false positive

- Non-TB mycobacteria
 - Remember TB is not the only mycobacteria- in fact there are 100's of others out there!
- BCG vaccine, made from *Mycobacterium bovis*
 - More likely if received a booster BCG after infancy
 - Effect on the PPD wanes with time (12mm PPD in a 8 yr old vs. 12mm PPD in a 60 yr old)
 - Can look for the scar on the upper lateral arm
- Misreading
 - Should read horizontally across the forearm
 - Read induration, not erythema! Close your eyes!

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TB infection testing interpretation, PPD false negative

- Immunosuppression
 - HIV (esp low CD4), steroids, malignancy, TNF alpha
 - Malnourished
 - CRI
- Any active infection including active TB!
- Recent infection
 - Close contact of an active case repeat PPD in 8 wks to look for conversion
- Recent live vaccine, MMR
- Improper storage, improper administration, improper reading

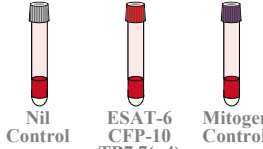
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IGRA

Interferon Gamma Release Assays (Quantiferon Gold, T-Spot)

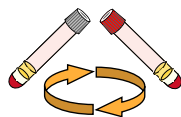
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Stage One – Blood Incubation and Harvesting



Nil Control ESAT-6 CFP-10 TB7.7(p4) Mitogen Control

1. Collect 1mL of blood (X3).
 Incubate at 37°C for 16-24 hrs.

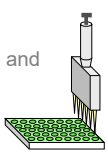
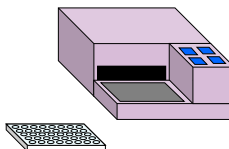


2. Centrifuge tubes for 5 minutes.




Stage Two – Human IFN-γ ELISA

3. Add plasma and conjugate to ELISA plate. Incubate for 120 minutes at Room Temperature.

4. Wash and add substrate. Read absorbance after 30 min.



5. Software calculates results and prints report.

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Species specificity of ESAT-6 and CFP-10

Tuberculosis complex	Antigens		Environmental strains	Antigens	
	ESAT	CFP		ESAT	CFP
<i>M tuberculosis</i>	+	+	<i>M abcessus</i>	-	-
<i>M africanum</i>	+	+	<i>M avium</i>	-	-
<i>M bovis</i>	+	+	<i>M branderi</i>	-	-
BCG substrain			<i>M celatum</i>	-	-
gothenburg	-	-	<i>M chelonae</i>	-	-
moreau	-	-	<i>M fortuitum</i>	-	-
tice	-	-	<i>M gordonii</i>	-	-
tokyo	-	-	<i>M intracellulare</i>	-	-
danish	-	-	<i>M kansasii</i>	+	+
glaxo	-	-	<i>M malmoense</i>	-	-
montreal	-	-	<i>M marinum</i>	+	+
pasteur	-	-	<i>M oenavense</i>	-	-
			<i>M scrofulaceum</i>	-	-
			<i>M smegmatis</i>	-	-
			<i>M szulgai</i>	+	+
			<i>M terrae</i>	-	-
			<i>M vaccae</i>	-	-
			<i>M xenopi</i>	-	-

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TB infection testing interpretation Quantiferon Gold Plus

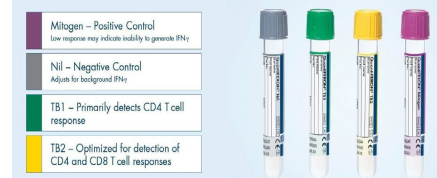
- Mitogen – Positive Control
Low response may indicate inability to generate IFN- γ
- Nil – Negative Control
Adjusts for background IFN- γ
- TB1 – Primarily detects CD4 T cell response
- TB2 – Optimized for detection of CD4 and CD8 T cell responses



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TB infection testing interpretation Quantiferon Gold Plus

- Results in IU/ml
- Positive result
 - + Mitogen, - Nil, + result in EITHER tube 1 or tube 2
 - tube 1 or tube 2 minus nil > **0.35 IU/ml**
- Negative result
 - + Mitogen, - Nil, - result in BOTH tube 1 and tube 2
- Indeterminate result
 - - Mitogen: immunosuppression
 - + Nil: high background noise



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Quantiferon Gold Plus

False Negative

- Window period after TB infection, up to 8-12 wks

False Positive

- *M bovis* (but NOT BCG)
- *M kansasii*, *M szulgai* and *M marinum*
- **ANALYTIC ISSUES**

Indeterminate result- means the control tubes reveal a problem
 TB Nil- the gamma interferon level in patient's bloodstream if high with no stimulation
 TB Mitogen- the white cells in the sample are unable to be stimulated by AG exposure

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Quantiferon Gold Plus -Analytic Issues

Laboratory testing

- Phlebotomy
- Handling of Specimen
- Transportation of specimen
- Incubation
- Batch issues



Issues in any of these
can lead to a false positive

The Lab should be reporting a quantitative number and a qualitative interpretation of the test.

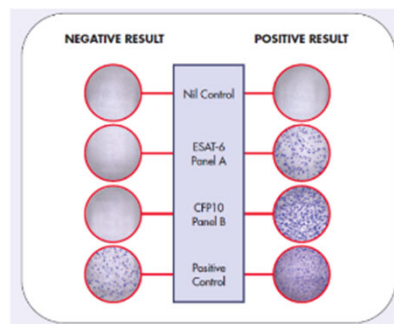
Low positive results (0.35 to 1.00) need to be interpreted in the context of the patient's risk profile (remember the 5-10-15 cutoffs for a TST reading).

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T-Spot (Elispot assay)

➤ T-spot:

- Mitogen, nil, ESAT-6, and CFP10 panels
- Positive:
 - # of spots in Panel A or Panel B – nil ≥ 8 + nil < 10
- Negative:
 - # spots in antigen panels – nil < 5 + mitogen ≥ 20
- Borderline: 5, 6, or 7 spots



T-Spot may be a better choice when the patient has low WBC counts

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Let's do a few cases

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LTBI?

- 18 year old b. RI - PPD 11mm
 - No medical problems
 - No travel
 - Lives in Providence
 - 2nd generation US born (Cambodian parents)
 - CXR – normal
 - Leaving for 6 month pre college tour of the world on bicycle
 - PE – 4cm matted nodes in supraclavicular fossa

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Authorizing Provider Edward L Zuroweste, MD		F: 717-705-2171
1-22-24		
QFT Gold Plus - 4 (Final result)		
QUANTIFERON(R)-TB GOLD PLUS, 4T, INCUBATED	Value NEGATIVE	Range NEGATIVE
Negative test result. M. tuberculosis complex infection unlikely.		
NIL	0.02	IU/mL
MITOGEN-NIL	9.96	IU/mL
TB1(CD4+)-NIL	0.00	IU/mL
TB2(CD4/8+)-NIL	0.00	IU/mL
<p>The Nil tube value reflects the background interferon gamma immune response of the patient's blood sample. This value has been subtracted from the patient's displayed TB and Mitogen results.</p> <p>Lower than expected results with the Mitogen tube prevent false-negative Quantiferon readings by detecting a patient with a potential immune suppressive condition and/or suboptimal pre-analytical specimen handling.</p> <p>The TB1 Antigen tube is coated with the M. tuberculosis-specific antigens designed to elicit responses from TB antigen primed CD4+ helper T-lymphocytes.</p> <p>The TB2 Antigen tube is coated with the M. tuberculosis-specific antigens designed to elicit responses from TB antigen primed CD4+ helper and CD8+ cytotoxic T-lymphocytes.</p>		
For additional information, please refer to		

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See Values: QUANTIFERON(R)-TB GOLD PLUS, 4T, INCUBATED (A)		
Authorizing Provider Edward L Zuroweste, MD		F: 717-705-2171
3-24-24		
QFT Gold Plus - 4 (Final result)		
QUANTIFERON(R)-TB GOLD PLUS, 4T, INCUBATED	Value POSITIVE (A)	Range NEGATIVE
In healthy persons who have a low likelihood of M. tuberculosis infection, a single positive QFT result should not be taken as reliable evidence of M. tuberculosis infection. Repeat testing, with either the initial test or a different test, may be considered on a case-by-case basis.		
NIL	0.31	IU/mL
MITOGEN-NIL	5.67	IU/mL
TB1(CD4+)-NIL	8.37	IU/mL
TB2(CD4/8+)-NIL	8.31	IU/mL
<p>The Nil tube value reflects the background interferon gamma immune response of the patient's blood sample. This value has been subtracted from the patient's displayed TB and Mitogen results.</p> <p>Lower than expected results with the Mitogen tube prevent false-negative Quantiferon readings by detecting a patient with a potential immune suppressive condition and/or suboptimal pre-analytical specimen handling.</p> <p>The TB1 Antigen tube is coated with the M. tuberculosis-specific antigens designed to elicit responses from TB antigen primed CD4+ helper T-lymphocytes.</p> <p>The TB2 Antigen tube is coated with the M. tuberculosis-specific antigens designed to elicit</p>		
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TB Infection Testing Interpretation, Quantiferon Gold Plus

QFT PLUS INTERPRETATION INDETERMINATE !

Comment:

An Indeterminate interpretation can be caused by a number of factors, including sample collection. Testing should be reordered and a new sample collected on patients with indeterminate results. Samples with a low mitogen response (<0.5) may be due to anergy or immune suppression. Samples with a high Nil response (>8.0) may be due to interferon gamma production independent of TB stimulation or heterophile antibodies. For more details, please see TB Testing Guidance on Staffnet.

QFT PLUS NIL VALUE	10.000	IU/mL
QFT PLUS TB AG1 MINUS NIL	0.000	IU/mL
QFT PLUS TB AG2 MINUS NIL	0.000	IU/mL
QFT PLUS MITOGEN MINUS NIL	0.000	IU/mL

QUANTIFERON TB GOLD

Collected: 11/19/15 1021

Resulting lab: LABCORP

Reference range: Negative

Value: **Indeterminate !**

Comment: Mitogen (positive control) gave low response. This may indicate anergy or immune suppression. Early draws and extended transit time may also result in low positive control and indeterminate results. The specimen received for QuantIFERON testing was incubated by the ordering institution. Specific procedures outlined in our Directory of Services and in the package insert for the QuantIFERON Gold (In Tube) test must be followed to enable for proper stimulation of cells for the production of interferon gamma.

QFT TB AG VALUE	0.04
QFT NIL VALUE	0.04
QFT MITOGEN VALUE	0.14
QFT TB AG MINUS NIL VALUE	0.00
QFT INTERPRETATION	

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TB infection testing interpretation, Quantiferon gold plus

	3 11/22/2019 0953	2 9/1/2021 1842	1 9/13/2021 1732
QUANTIFERON-TB			
QFT PLUS INTERPRET...	NEGATIVE *	POSITIVE * !	NEGATIVE *
QFT PLUS NIL VALUE	0.346 *	0.092 *	0.064 *
QFT PLUS TB AG1 MI...	-0.096 *	0.283 *	0.009 *
QFT PLUS TB AG2 MI...	0.039 *	0.346 *	0.013 *
QFT PLUS MITOGEN M...	9.654 *	9.908 *	9.936 *

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Do not just look at the Positive or Negative answer!

- TB Nil
 - Checks that the patient does not have a pre-existing condition that could cause a False + (≤ 8.0)
- TB Mitogen
 - Checks that the patient has a health immune system that can react ($0.5 >$ TB Nil tube)
- TB Ag1 and 2 Nil
 - Tube coated with TB AG ($0.35 >$ TB Nil tube)

Low positives- pause to THINK

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Important take home messages

- When interpreting results of PPD or IGRA, always interpret in context of the patient
- No gold standard to diagnose latent TB infection, pre-test probability matters!
- Ask yourself:
 - what risk factors does this patient have for being infected with TB?
 - what risk factors does this patient have for reactivation of a latent TB infection?
 - what are the consequences of TB reactivation?
 - what are the possible reasons for a false + or a false - PPD or IGRA?

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Important take home messages

- Neither PPD or IGRAs differentiates b/w latent or active TB infection
- Neither is used to follow response the treatment in a patient with active TB
- After TB infection (and treatment) both should remain positive going forward
 - Repeat testing is not indicated as it adds little addition information

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Intent to screen should be
coupled with intention to treat

(to completion of therapy)

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Appendix Table 1. Characteristics of the 3 Tests for Latent Tuberculosis Infection*

Variable	Tuberculin Skin Test	QuantIFERON-Gold and QuantIFERON-Gold In-Tube	Elispot T-SPOT.TB
Administration	In vivo (intradermal)	Ex vivo, ELISA-based	Ex vivo, Elispot-based
Antigens	PPDS or RT-23	ESAT-6, CFP-10, and T87.7	ESAT-6 and CFP-10
Standardized	Mostly	Yes	Yes
Units of measurement	Millimeters of induration	Units of IFN- γ	IFN- γ spot-forming cells
Definition of positive test results	5, 10, and 15 mm	Patient's IFN- γ ≥ 0.35 U/mL (after subtracting IFN- γ response in nil control)	≥ 6 spot-forming cells in the antigen wells, with 250 000 cells/well, and at least double-negative well
Indeterminate	If any (rarely tested)	Poor response to mitogen (<0.5 U/mL in positive control) or high background response (>8.0 U/mL in nil well)	Poor response to mitogen (<20 spot-forming cells in positive control well) or high background (>10 spot-forming cells in negative well)
Time to result	48–72 h	16–24 h (but longer if run in batches)	16–24 h (but longer if run in batches)
Cost per test, \$†			
Materials		19 (A3)	63 (A3)
Labor/other		22 (A1, A3)	22 (A1)
Total cost	12.73 (A1, A2)	41	85

* ELISA = enzyme-linked immunosorbent assay; IFN = interferon; IGRA = interferon- γ release assay; PPDS = purified protein derivative standard.
 † All costs are in Canadian dollars (\$1 Canadian = \$0.91 U.S.). The numbers in parentheses are references. For the IGRA tests, the materials cost is based on quotes from the manufacturers for shipment to Canadian centers in September 2006. The cost for IGRA labor, shipping, and handling is taken from published field experience with QuantIFERON testing, as reported in reference A1. Costs may vary widely in different countries.

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Questions?

Acknowledgement to Dr Linda Shipton of Massachusetts and Dr Ed Zuroweste of Pennsylvania for IGRA case studies

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