

# Blood-based Diagnostic Tests for TB Infection

## World TB Day 2007

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# Tuberculosis

- 1/3 of world's population is currently infected with *M. tb*
- Need to identify those with latent TB infection (LTBI) who are at highest risk for progressing to active TB & ensure they complete effective treatment
- Need an accurate test that is easy to use and is acceptable to both patients and providers

# Tuberculin Skin Test (TST)



# TST

- Associated with exposure to *M. tb*
- Conversion associated with increased risk of active disease
- On a cohort level TST can predict who will get TB, but on an individual level it is not as clear
- Rates of active TB in MRC BCG trial
  - TST negative and no BCG  $\approx$  TST positive

# TST

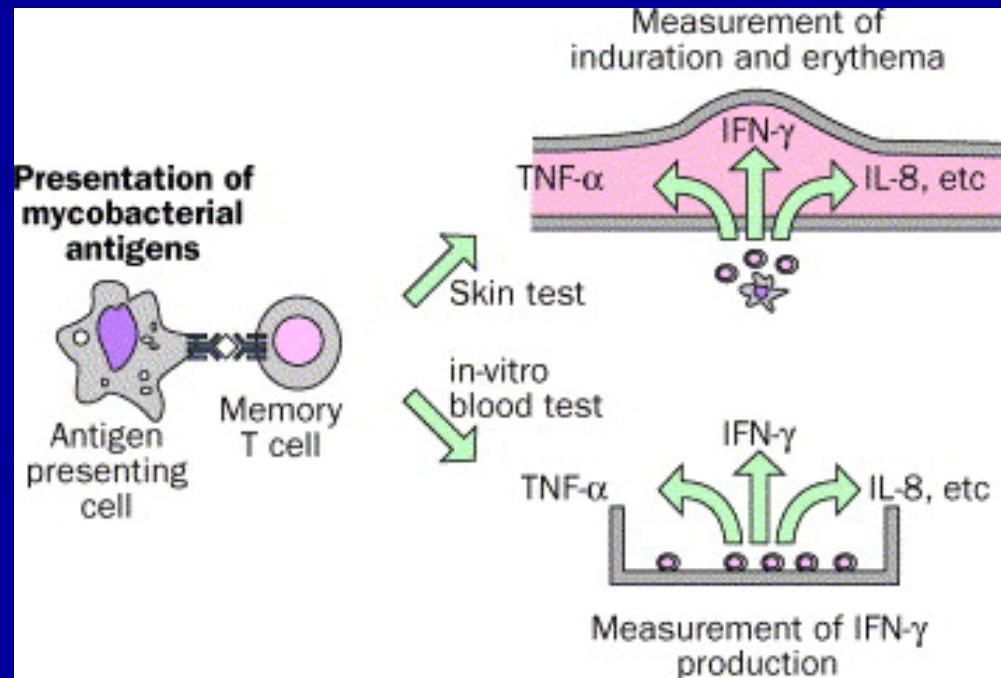
- Dependent on skills of tester and reader
- Inter-reader variability
- Patient needs to return for the reading
- Size of the TST does not reflect
  - Time from infection
  - Active vs. latent disease
  - Risk of progression
  - Response to treatment

# TST and BCG Vaccination

- Strong evidence that BCG vaccination in infancy has little effect on TST results later in life (Farhat et al., *Int J Tuberc Lung Dis* 2006;10:1192-204)
- However, many patients and providers believe that it does
- Public perception often is no match for scientific evidence

# Interferon- $\gamma$ (IFN- $\gamma$ )

- Component of cell mediated immune (CMI) response
- Antigen specific secretion
- Associated with protective adaptive immunity
- Stable & measurable
- Not produced by macrophages



Andersen et al., *Lancet* 2000;356:1099-104.

# QuantiFERON<sup>®</sup>-TB Gold (QFT-G)

- Whole blood IFN- $\gamma$  release assay (IGRA) (Cellestis Ltd, Australia)
- Stimulate cells with TB specific peptides (ESAT-6 & CFP-10) measure  $\Delta$  IFN- $\gamma$  concentration with ELISA
- Utilizes a positive control (mitogen) and a nil (saline) to measure background IFN- $\gamma$
- FDA approved as an aid for diagnosing LTBI & TB disease but does not differentiate between them

# Species specificity of ESAT-6 and CFP-10

Tuberculosis complex	ESAT-6 CFP-10		Environmental strains	ESAT-6 CFP-10	
	ESAT-6	CFP-10		ESAT-6	CFP-10
M tuberculosis	+	+	M abcessus	-	-
M africanum	+	+	M avium	-	-
M bovis	+	+	M branderi	-	-
BCG substrain			M celatum	-	-
gothenburg	-	-	M chelonae	-	-
moreau	-	-	M fortuitum	-	-
tice	-	-	M gordonii	-	-
tokyo	-	-	M intracellulare	-	-
danish	-	-	M kansasii	+	+
glaxo	-	-	M malmoense	-	-
montreal	-	-	M marinum	+	+
pasteur	-	-	M oenavense	-	-
			M scrofulaceum	-	-
			M smegmatis	-	-
			M szulgai	+	+
			M terrae	-	-
			M vaccae	-	-
			M xenopi	-	-

## QFT-G

- *in vitro*
- TB specific antigens
- No boosting → do not need 2 step testing
- Not affected by BCG/most NTMs
- Have results after 1<sup>st</sup> visit
- Minimal inter-reader variability

## TST

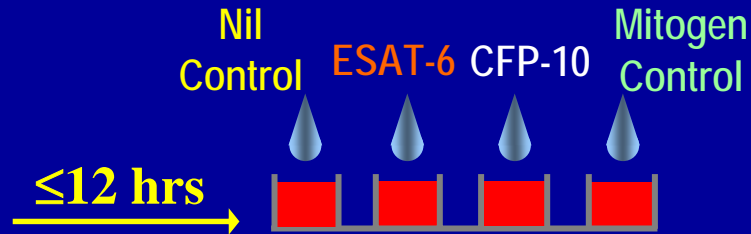
- *in vivo*
- Less specific PPD
- Boosting
- False+ due to BCG/NTMs
- Patient must return to have a result
- Inter-reader variability (placement & reading subject to errors & bias)

# QFT-G Steps

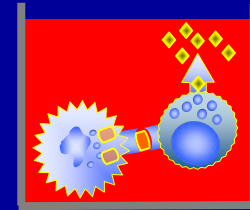
## Stage 1 Whole Blood Culture



Draw 5-10 mL blood into Heparin tube

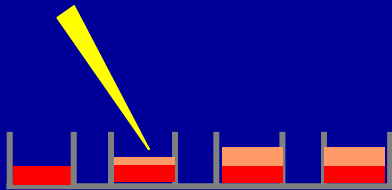


Make 1 mL aliquots & add antigen

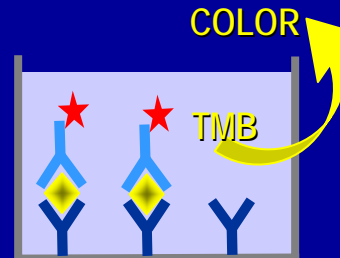


Incubate 16-24 hrs → sensitized T-cells release IFN- $\gamma$

## Stage 2 IFN- $\gamma$ ELISA



Harvest plasma from above settled cells



Measure IFN- $\gamma$  in 'Sandwich' ELISA



Computerized interpretation

# QFT-G Results

- IFN- $\gamma$  calculated in ESAT-6, CFP-10, and mitogen-stimulated plasmas by subtracting out the IFN- $\gamma$  amount detected in the nil

ESAT-6–Nil &/or CFP-10–Nil	Nil	Mitogen–Nil	Result
$\geq 0.35$ IU/mL & $>50\%$ $\uparrow$ nil	Any	Any	Positive
$< 0.35$ IU/mL	$\leq 0.7$	$\geq 0.5$	Negative
$< 0.35$ IU/mL	Any	$< 0.5$	Indeterminate
$\leq 50\%$ above nil	$> 0.7$	Any	Indeterminate

# QFT-G In Tube (QFT-GIT)

- Next version of QFT-G
- Commercially available in Europe
- Submitted for FDA review
- Collect blood directly into tubes containing 3 antigens and controls
- Eliminates need to submit to lab in  $\leq 12$  hrs
- Several studies have found increased positivity compared to the QFT-G

# T-SPOT.TB<sup>®</sup>

- IGRA that uses peripheral blood mononuclear cells
- Measures  $\Delta$  # of cells releasing IFN- $\gamma$  using enzyme-linked immunospot (ELISPOT)
- Technically more difficult than QFT-G
- May be more sensitive than QFT-G especially in immunosuppressed
- Submitted for FDA review

# IGRA Sensitivity

- Meta-analysis (Menzies, *Ann Intern Med* 2007;146:340-54)
- Pooled sensitivity (95% CI) in patients with active TB
  - QFT-G: 80% (73%-87%)
    - Adult: 76%, Pediatric: 66%
  - T-SPOT.TB/Elispot: 87% (78%-95%)
- LTBI
  - Unknown sensitivity
  - Immune differences: LTBI  $\neq$  TB
    - Cell-mediated immunity usually depressed in active TB → expect reduced performance of tests (TST, IGRA) that measure it

# IGRA Specificity

- Pooled specificity (95% CI) in populations at very low risk for LTBI (Menzies, *Ann Intern Med* 2007;146:340-54)
- QFT-G: 96% (94%-99%)
- T-SPOT.TB/Elispot: 92% (88%-95%)
- Some people may actually be infected

# Serial Testing

- Few published studies on serial testing
- In 2 studies reversions occurred in 24-28% of subjects in the absence of treatment
  - Associated with being TST negative and having an IGRA response close to cut-point
- IGRA response
  - Decreased with treatment in patients with active TB but not in patients with LTBI

# QFT & TST Agreement

		QFT	
		+	-
TST	+	22%	24%
	-	5%	49%

Combined results from 12 studies (Menzies, *Ann Intern Med* 2007;146:340-54)

# IGRA & TST Agreement

- Poor agreement may be a good thing
- Agreement varies widely
  - $\uparrow$  with  $\uparrow$  risk of infection
- Positive TST & Negative QFT-G discordance
  - Associated with BCG, NTM, TB Prevalence
- Negative TST & Positive QFT-G discordance
  - Less frequent & random?

# Natural History of TB Infection

- Much of how we define the natural history of TB is based on the TST
- Recent vs. Remote Infection
  - Short incubation (i.e. overnight) assays such as QFT and T-SPOT.*TB* sample effector T-cells
  - Longer incubation (i.e. 2-5 days) assays such as TST sample memory T-cells
- Transient infection
  - Evidence for transient infections where bacterial replication is controlled
- IGRA such as QFT and T-SPOT.*TB* are likely detecting recent, non-transient infections only

# Natural History of TB

- A rise in IFN- $\gamma$  levels may predict transition from LTBI to active disease
- A decrease in IFN- $\gamma$  levels may indicate an effective response to treatment
- Conversions and Reversions
  - Dynamic and prone to conversions and reversions
  - Reproducibility fairly good in those with negative results
  - More variation in those with positive results
  - Conversions higher than for TST
    - $\uparrow$  IFN- $\gamma$  from 0.31 to 0.36 IU/mL  $\Rightarrow$  conversion, but is this clinically meaningful?

# QFT-G vs. TST Korea

<b>Population</b>	<b>n</b>	<b>QFT-G+</b>	<b>TST<sub>10mm+</sub></b>
<b>Low risk + BCG</b>	<b>99</b>	<b>4%</b>	<b>51%</b>
<b>Casual Contacts</b>	<b>72</b>	<b>10%</b>	<b>60%</b>
<b>Close Contacts</b>	<b>48</b>	<b>44%</b>	<b>71%</b>
<b>TB Patients</b>	<b>54</b>	<b>81%</b>	<b>78%</b>

**Kang, JAMA 2005;293:2756-61**

# QFT-G vs. TST

## United States

Population	QFT-G+ (n)	TST+ (n)
Low risk & No BCG	0.2% (573)	0.9% (548)
Increased LTBI risk	1.4% (284)	12% (287)
Contacts	20.3% (580)	30.7% (580)
Untreated TB	79% (63)	79% (63)

CDC unpublished

# QFT vs. TST in HIV+

- Concern about performance, especially indeterminate rates
- QFT-GIT in 590 HIV(+) persons in Denmark (Brock, *Respir Res* 2006;7:56)
  - 3% indeterminate rate – associated with CD4 count
  - Positive QFT-GIT results associated with TB risk factors
- QFT-G, T-SPOT.TB, and TST in 74 HIV(+) and 86 HIV(–) persons in South Africa (Rangaka, *Am J Respir Crit Care Med* 2007;175:514-20)
  - QFT-G indeterminate rates: 5% in HIV(+), 2% in HIV(–)
  - IGRA % + not significantly lower for HIV(+) vs. HIV(–)
  - % + higher for T-SPOT.TB
  - IGRA and TST agreement fair in HIV(+), poor in HIV(–)

# QFT vs. TST in Children

- QFT-GIT and TST in 105 children who had suspected TB or history of contact with TB case recruited from a hospital in rural India (Dogra, *J Infect* 2007;54:267-76)
  - No indeterminate results
  - Agreement high between the 2 tests
- QFT-GIT and TST in 207 children in Nigeria (Nakaoka, *Emerg Infect Dis* 2006;12:1383-8)
  - % QFT-GIT+ much higher in contacts to smear(+) case (74%) than in contacts with smear(-) case and controls (both 10%)
  - TST less like to be positive in contacts to smear(+) case (49%), however, used  $\geq 10$  mm

# QFT-G Guidelines

- CDC released interim guidelines in Dec 2005  
*MMWR Recomm Rep* 2005;54:49-55  
<http://www.cdc.gov/mmwr/PDF/rr/rr5415.pdf>
- Can use QFT-G in all settings in which the TST is currently used
  - contact investigations
  - evaluation of recent immigrants
  - sequential-testing surveillance programs
- Use QFT-G in place of, not in addition to, TST

# QFT-G Guidelines

- QFT-G results “...should be considered in conjunction with other epidemiologic, historic, physical, and diagnostic findings.”
- A negative QFT-G result should not be used alone to exclude *M. tb* infection in symptomatic persons
- HCWs and others undergoing serial testing do not need initial 2-step testing

# Changes to NY Health Codes

- NYC health code changed to allow use of an approved blood test or the TST
  - See revised *TB and the Law*
- We are working with New York State to allow the use of QFT-G for HCWs
- NYC will also update its regulations to allow the use of QFT-G for HCWs

# QFT-G at the NYC DOHMH

- On October 2, 2006, QFT-G was implemented as the standard test for TB infection at two of the largest volume TB clinics (Ft. Greene and Corona)
- QFT-G testing was not available on Saturdays until December 9, 2006 (TST was used)
- All persons presenting at these clinics for a TB test were informed that the standard test for TB infection is a blood-based test called “Quantiferon” and given a QFT-G fact sheet

# Methods

- Did not perform QFT-G test and performed TST if
  - Patient was less than 1 year of age due to technical difficulty of phlebotomy in this age group
  - Patient expressed a religious prohibition against getting blood drawn
  - Phlebotomy was contraindicated due to medical condition
  - Patient was a health care worker (HCW) because New York State has not approved QFT-G for meeting HCW testing requirements
- Did not perform QFT-G test (or TST) if patient had previously or was currently taking treatment for LTBI or active TB

# Results

- Data analyzed from patients receiving a TB test October 2, 2006 through January 15, 2007
- 2197 TB tests performed
  - 295 (13%) TST implants
    - 75 because it was a Saturday visit before QFT-G was available on Saturdays
  - 1902 (87%) QFT-G tests

# QFT-G Results

<b>Negative</b>	1661 (87%)
<b>Positive</b>	159 (8%)
<b>Indeterminate</b>	29 (2%)
<b>Not tested*</b>	53 (3%)

\*Not enough blood, received by the laboratory >12 hours after being drawn, or another logistical problem (49 occurred in the first 2 months of testing)

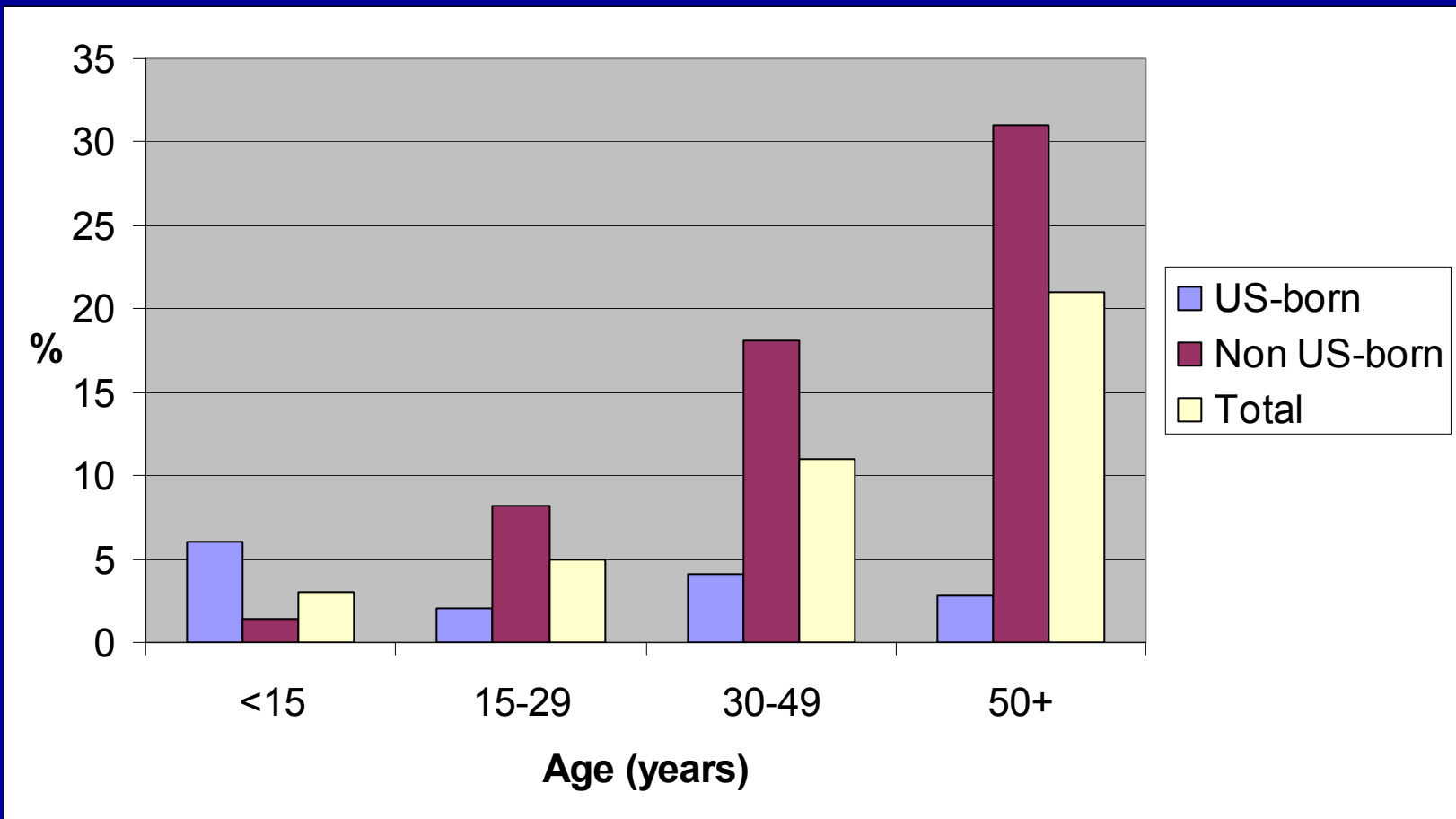
# TB Test Results by Country of Birth

	QFT-G*		TST in 2005**	
	US born	Non-US born	US born	Non-US born
Positive	25 (3%)	134 (14%)	236 (7%)	1673 (39%)
Negative	813 (97%)	806 (86%)	3247 (93%)	2596 (61%)

\*p<0.0001 for QFT-G+ for US born vs. non-US born

\*\*Results from patients who had a TST at the same clinics in 2005

# % QFT-G Positive by Age and Country of Birth



Tests for trend, US-born:  $p=0.59$ , Non-US born  $p<0.0001$ , Total  $p<0.0001$

# QFT-G Use at NYC DOHMH

- Lower rate of TB infection found with QFT-G compared to TST may be due to the increased specificity (or decreased sensitivity) of QFT-G
- On January 16, 2007, expanded QFT-G to 4 other clinics (Chelsea, Bedford, Bushwick, Brownsville)
- Plan to expand to remaining 4 clinics during the course of 2007
- A post-marketing evaluation comparing QFT-G to TST is planned in a similar population

# Conclusions on IGRA

- Sensitivity for active TB appears to be as good as the TST
- Specificity is much improved over TST
- More closely associated with exposure to *M. tb* than TST
- The commercially available assays are likely only detecting recent, non-transient infections

# Unanswered Questions

- Sensitivity for LTBI?
  - Probably impossible to determine since no gold standard for detecting LTBI
- Risk of TB if QFT-G(+)?
  - May be higher than TST if QFT-G is more accurate and is picking up just recent infection
- Risk of TB if TST(+) but QFT-G(-)?
  - May be minimal if the TST is a false-positive or if the infection is remote
- Risk associated with recent conversion?
  - For QFT-G also likely to be higher than prevalent +

# Unanswered Questions

- Length of time between TB infection & QFT-G conversion?
  - May be impossible to determine due to lack of gold standard
- Cost-effectiveness?
  - Findings so far have been mixed and will likely be population specific
- Acceptable indeterminate rate?
  - Currently a rate at or below ~2% is considered acceptable
  - Can a low mitogen provide useful information?

# Research Needs

- Reproducibility including laboratory variability, influence of field conditions, biological variability, conversions
- Comparisons of IGRA and TST in HIV+ and other immunocompromised, IV drug users, children, elderly
- Discordance of IGRA and TST and how that is affected by cutpoints, NTM, time to conversion
- Feasibility, utility, and cost-effectiveness in different populations
- Risk of progression to active disease especially in those who are TST positive and IGRA negative

# QFT-G Availability in NYC

- Available at 6 DOHMH chest centers
  - Queens: Corona
  - Brooklyn: Ft. Greene, Bedford, Bushwick, Brownsville
  - Manhattan: Chelsea
  - Plan to expand to remaining 4 chest centers over course of 2007
- Providers interested in providing QFT-G directly to their patients can contact
  - Cindy Jacke at Quest Diagnostics, 1-800-222-0446, ext. 5162
  - Cellestis at 1-800-519-4627

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