Interferon Gamma Release Assays (IGRAs) for the Diagnosis of Latent Tuberculosis Infection

Dr. Michael Gardam
Medical Director, Tuberculosis Clinic
University Health Network

Hosted by Paul Webber
paul@webbertraining.com

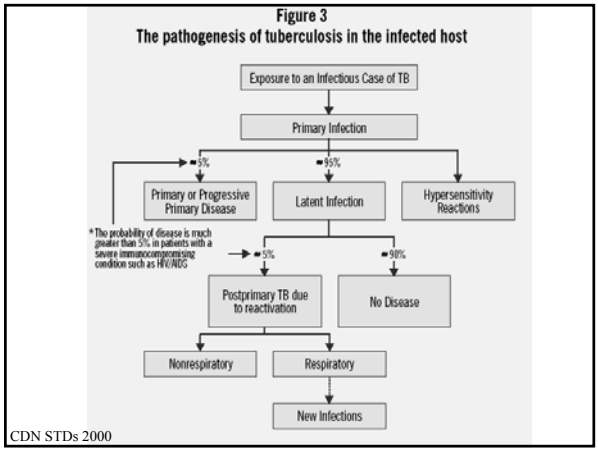
www.webbertraining.com

Conflict of interest statement

I have performed, and am currently performing, studies using the T-Spot. *TB* IGRA

Outline

- Background
  - Latent tuberculosis infection
  - Sensitivity and specificity
  - Tuberculin skin test
- Interferon-gamma assays
  - How they work
  - Understanding the literature
  - Limitations
- Clinical considerations



Test Sensitivity

$$\frac{\text{\# subjects with the disease who test positive}}{\text{Total \# of subjects with the disease}}$$

How to calculate sensitivity

- Test a population that you *know* has the disease you are looking for
- i.e. test people with culture-proven active tuberculosis
- Example: the sensitivity of the TST for active TB is about 70%

Specificity

subjects that **do not** have the disease who test **negative**

Total # of subjects who **do not** have the disease

How to calculate specificity

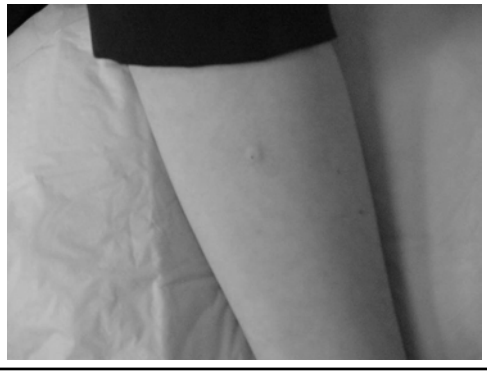
- Test a population that you know does not have the disease you are looking for
- i.e., test people who have no epidemiologic risk factors for tuberculosis infection or disease
- Example: test health, young CDN born individuals with no TB risk factors

Comparing tests

- Compare the new test to the "gold standard" (i.e., the test that allows you to *know* whether a subject has or does not have the disease).
- **There is no gold standard for latent tuberculosis infection**

Confused yet?

Tuberculin skin testing



Purified Protein Derivative aka "PPD", "Tuberculin"

- Different formulations used
- Contains approximately 200 antigens
- Many antigens are shared between different mycobacteria
  - *M tuberculosis* complex
  - *M bovis*
  - **BCG strain of *M bovis***
  - Environmental mycobacteria

Tuberculin skin testing

- Interobserver variability (2.5 mm)
- Intra-observer variability (1.3-1.9 mm)
- Biologic variability (<3mm)

- False negatives in active disease (30%)
- Requires 2 visits

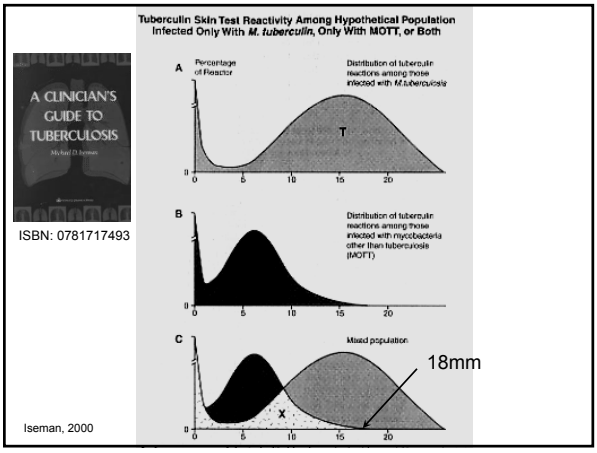
Sensitivity and specificity

- Sensitivity cited as 97% for latent TB infection in healthy individuals
  - How do we know?
  - Decreased in immunocompromised
- Sensitivity closer to 70% for active disease
- Specificity influenced by BCG vaccination, other mycobacterial exposure

Age at BCG vaccination and positive TSTs

- Infancy: no difference in positivity between vaccinated and unvaccinated after 5 years
- Primary school: 15-25% remain positive after 10 years

- Induration >18mm unlikely to be BCG



Summary

- The TST performs well in healthy, non-BCG vaccinated individuals
  - Decreased specificity if BCG vaccinated, especially later in life
  - Decreased sensitivity in immunocompromised populations
    - Low CD4 count
    - End stage renal disease
    - Chronic corticosteroid use
    - Hematologic malignancies
    - Transplant recipients

INFγ-based tests

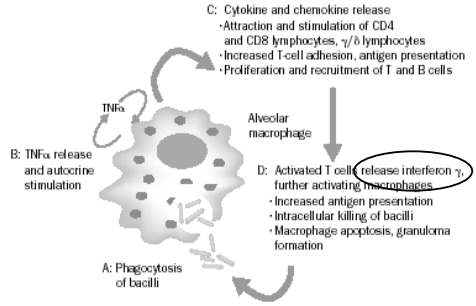


Figure 1. Schematic representation of the central role of TNFα in the cellular immune response to M tuberculosis infection.

Gardam et.al. Lancet ID

Interferon-gamma release assays (IGRAs)

- QuantiFERON Gold
  - ELISA
  - Licensed in Canada, USA
- T-Spot.*TB*
  - ELISPOT
  - Licensed in Canada, USA license pending

What is an ELISA?

- Enzyme-linked immunosorbant assay
- An enzyme is linked to an antibody direct against the molecule of interest (interferon gamma)
- Excess antibody is washed off and a substrate is added
- The enzyme acts on the substrate and causes a colour change

What is an ELISPOT?

- Enzyme-linked immunospot
- Similar to ELISA except individual positive cells are counted rather than measuring colour change
- In general, ELISPOTs are believed to be more sensitive than ELISAs because:
  - Individual cells are counted
  - Secreted cytokines are measured adjacent to the cell rather than diluted in the supernatant

Antigens used in latest IGRAs

- Two highly-specific proteins for *M. tuberculosis*
- Encoded on the Region of Difference-1 (RD-1) segment of the MTB genome
  - Early secreted antigenic target (ESAT-6)
  - Culture filtrate protein 10 (CFP-10)

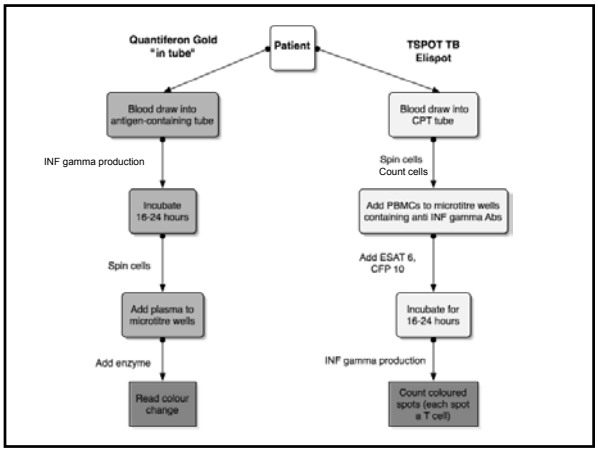
Specific antigens for TB?

- ESAT-6 and CFP-10 are also found in:
  - *M. leprae*
  - Wild type *M. bovis* (not the BCG strains)
  - *M. marinum*
  - *M. kansasii*
  - *M. szulgai*
  - *M. flavescens*

Reasonably common in Canada

Older IGRAs

- Most of the studies more than 3 years old looked at earlier versions of the tests
  - Different antigens
  - Incomplete antigens
- These studies are far less relevant to understanding the current IGRAs



Summary

- Both assays use RD-1 antigens
- The T-SPOT.TB requires white cell separation and cell counting
  - i.e., counting of gamma interferon-producing cells as spots at the bottom of a well
- The QuantiFERON measures gamma interferon production in the supernatant
- Theoretically, the T-SPOT.*TB* should be more sensitive

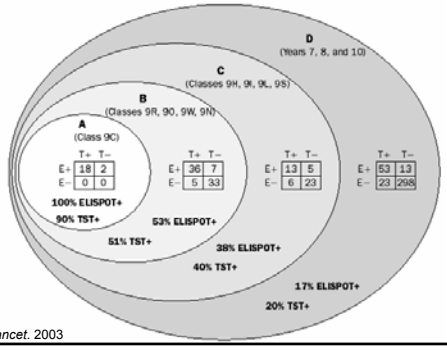
So how do you evaluate a test when there is no gold standard for latent tuberculosis?

Hint: You can't just compare it to the TST

Clinical assessment of IGRAs

- Measure the correlation between the TST and IGRA with:
  - Active TB
  - Outbreak setting (stratify according to exposure)
  - High risk setting (e.g. healthcare worker)
  - BCG vaccination
- Add in additional variables such as immunocompromised status
- **Unable to measure true sensitivity and specificity**

T-SPOT.*TB* in an outbreak setting



Ewer et. al. *Lancet*. 2003

HIV positive

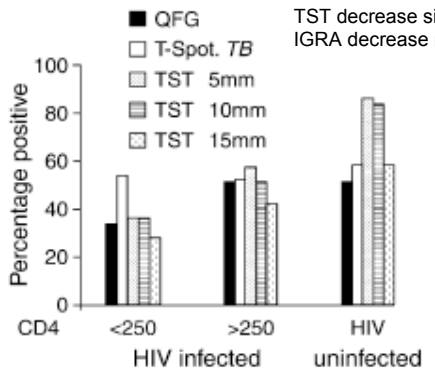
TABLE 1. BASELINE CHARACTERISTICS OF YOUNG ADULTS ATTENDING VOLUNTARY COUNSELING AND TESTING FOR HIV-1 INFECTION IN KHAYELITSHA TOWNSHIP, SOUTH AFRICA

	HIV Infected ($n = 74$)	HIV Uninfected ($n = 86$)	p Value
Sex, % (n)			
Female	73 (54)	60 (52)	0.10
Age, yr			
Mean (SD)	30 (6.37)	30 (9.08)	0.68
TB contact in last 6 mo, % (n)			
Yes	12 (9/73)	21 (18/85)	0.14
Deltoid/BCG scarring seen, % (n)			
Yes	51 (36/71)	71 (56/79)	0.01
CD4+ cell count			
Median (IQR)	392 (263–520)		
< 200/mm³, % (n)	16 (10)		
200–349/mm³, % (n)	27 (17)		
350–499/mm³, % (n)	30 (19)		
> 500/mm³, % (n)	28 (18)		

Definition of abbreviations: BCG = bacillus Calmette-Guérin; IQR = interquartile range; TB = tuberculosis.

A total of 160 adults were studied. *Note:* Numbers in parentheses indicate absolute number in group, and for factors where some information was missing, denominators are given.

Rangaka, Am. J. Respir. Crit. Care Med. 2007



TST decrease sig.
IGRA decrease not sig.

TABLE 4. AGREEMENT BETWEEN TUBERCULIN SKIN TEST AT VARIOUS CUTOFFS, T-SPOT.*TB* TEST, AND QuantiFERON-TB GOLD TEST

	Combined Groups			HIV Infected			HIV Uninfected		
	Agreement (%)	κ	p Value	Agreement (%)	κ	p Value	Agreement (%)	κ	p Value
QFT-G vs. T-SPOT.*TB*	68	0.37	< 0.001	67	0.34	0.002	70	0.40	< 0.001
QFT-G vs. TST 5 mm	65	0.31	< 0.001	79	0.58	< 0.001	53	0.07	0.189
QFT-G vs. TST 10 mm	65	0.33	< 0.001	76	0.52	< 0.001	55	0.12	0.078
QFT-G vs. TST 15 mm	65	0.31	< 0.001	66	0.31	0.006	65	0.30	0.003
T-SPOT.*TB* vs. TST 5 mm	72	0.40	< 0.001	80	0.60	< 0.001	65	0.17	0.035
T-SPOT.*TB* vs. TST 10 mm	72	0.40	< 0.001	80	0.60	< 0.001	65	0.18	0.034
T-SPOT.*TB* vs. TST 15 mm	68	0.37	< 0.001	74	0.49	< 0.001	64	0.24	0.014

T-Spot.*TB* against QFT-G

A

	QFT-G+	QFT-G-
T-Spot.*TB*+	28	17
T-Spot.*TB*-	8	21

McNemar p=0.07

TST positivity > 10mm

B

	T-spot.*TB*+	T-Spot.*TB*-
TST+	40	21
TST-	5	8

(Exact McNemar's p=0.003)

C

	QFT-G+	QFT-G-
TST+	32	29
TST-	4	9

(Exact McNemar's p<0.0001)

TST positivity > 15mm

D

	T-Spot.*TB*+	T-Spot.*TB*-
TST+	30	12
TST-	15	17

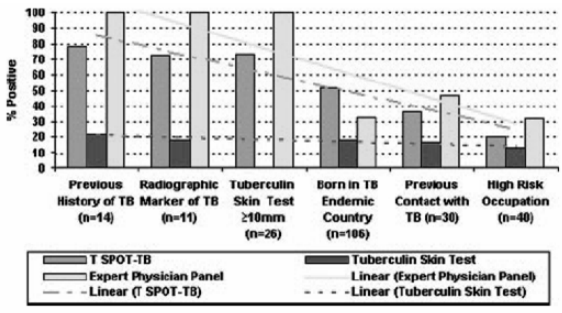
(McNemar's p=0.56)

E

	QFT-G+	QFT-G-
TST+	26	16
TST-	10	22

(McNemar's p=0.24)

Hemodialysis patients



Passalent et. al., CJASN 2006

Table 1. Logistic regression analysis: Factors associated with a positive T-SPOT.*TB* test, TST, and expert physician panel diagnosis of current or past tuberculosis infection[a]

Variable	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
T-SPOT.*TB*[b]				
age (yr)	1.01 (0.99 to 1.03)	0.21	1.01 (0.99 to 1.03)	0.52
history of TB	7.69 (2.07 to 28.6)	0.002	7.24 (1.70 to 30.8)	0.007
radiographic markers of TB[c]	5.33 (1.37 to 20.8)	0.02	5.48 (1.20 to 25.1)	0.03
born in TB-endemic country[d]	5.08 (2.66 to 9.70)	<0.0001	5.45 (2.72 to 10.9)	<0.0001
previous contact with TB	1.06 (0.48 to 2.38)	0.88	—	—
high-risk occupation[e]	0.39 (0.17 to 0.89)	0.03	—	—
history of BCG vaccination	1.90 (1.05 to 3.42)	0.03	—	—
TST[b]				
age (yr)	0.99 (0.97 to 1.02)	0.75	0.99 (0.97 to 1.02)	0.59
history of TB	1.97 (0.51 to 7.56)	0.33	2.73 (0.65 to 11.5)	0.17
radiographic markers of TB[c]	1.56 (0.32 to 7.63)	0.59	1.21 (0.24 to 6.21)	0.82
born in TB-endemic country[d]	2.81 (1.12 to 7.01)	0.03	—	—
previous contact with TB	1.45 (0.50 to 4.19)	0.50	—	—
high-risk occupation[e]	0.97 (0.34 to 2.74)	0.95	—	—
history of BCG vaccination	2.59 (1.12 to 5.99)	0.03	2.90 (1.22 to 6.92)	0.02
Expert panel[f]				
age (yr)	1.00 (0.98 to 1.02)	0.78	1.00 (0.98 to 1.02)	0.98
born in TB-endemic country[d]	2.16 (1.13 to 4.16)	0.02	2.64 (1.31 to 5.29)	0.007
previous contact with TB	3.01 (1.35 to 6.70)	0.007	3.81 (1.63 to 8.92)	0.002
high-risk occupation[e]	1.48 (0.70 to 3.14)	0.31	—	—
history of BCG vaccination	1.73 (0.92 to 3.28)	0.09	—	—

Passalent et. al., CJASN 2006

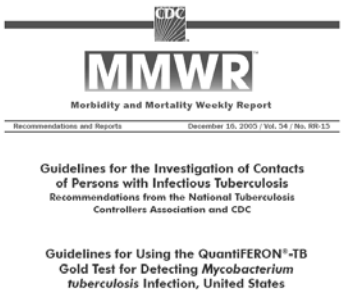
What does a positive IGRA mean in terms of active TB risk?

- + TST carries a 5-15% lifetime risk of active disease
- Most of the risk is in the first 2 years after infection
- There are **no** natural history studies with IGRAs

Discordant results

- TST +, IGRA –
  - Secondary to BCG or false negative IGRA?
- TST -, IGRA +
  - Poor sensitivity of the TST or false positive IGRA?
- **You cannot tell with certainty**

Clinical uses of IGRAs


MMWR
Morbidity and Mortality Weekly Report
Recommendations and Reports December 16, 2005 / Vol. 54 / No. RR-15

Guidelines for the Investigation of Contacts of Persons with Infectious Tuberculosis
Recommendations from the National Tuberculosis Controllers Association and CDC

Guidelines for Using the QuantiFERON®-TB Gold Test for Detecting *Mycobacterium tuberculosis* Infection, United States

Serial testing

- Small studies
  - IGRA response increased, decreased, or stayed the same……
- One study of HCWs in India
  - QuantiFERON test results showed reversion, conversion, or did not change…just like the TST
- **Continue to use TST**

Pai M, et. al, *Am J Respir Crit Care Med* 2006

Immigrant screening

- In general, screening of immigrants with TST is not recommended, unless they have underlying disease
- IGRAs will not significantly increase program efficiency
- **If going to screen, use TST**

Contact tracing

- QuantiFERON and T-SPOT.*TB* similar to TST in non-BCG vaccinated, better in BCG vaccinated
- But, considerable discordance found with both tests

Brock I, et. al. *Am J Respir Crit Care Med.* 2004
Kang et. al. *JAMA.* 2005
Ewer et. al. *Lancet.* 2003
Hill et. al. *Clin Infect Dis.* 2004
Richeldi et. al. *Am J Respir Crit Care Med.* 2004
Shams et. al. *Am J Respir Crit Care Med* 2005
Zellweger et. al. *Int J Tuberc Lung Dis.* 2005

Contact tracing recommendations

- Not enough evidence to recommend replacing the TST with IGRA
- IGRA should not be used alone
- If IGRA and TST both used, and patient high risk, treat if either is positive
- If TST + and patient considered low risk, may do an IGRA to confirm

Immunocompromised

- In general, IGRAs appear to be more sensitive than the TST
  - But, IGRAs have a higher percentage of "indeterminate" results
- T-SPOT.*TB* likely more sensitive than the QuantiFERON in immunocompromised

Lee et. al. *Eur Respir J.* 2006
Ferrara G et. al. *Lancet.* 2006

Immunocompromised recommendations

- Jury still out
- Some say evidence is enough to recommend them, at least in conjunction with TST
- Others say they is not enough evidence to recommend their use

Summary

- At present, the role for IGRAs is relatively limited
  - Natural history of + IGRA is unknown
  - Discordant results are common
  - Discordant results not well defined
  - If used with TST, treat if either is positive in high risk
    - Willing to accept discordance in low risk
  - A new study is out every week

Free-Access Teleclass Recordings & Handouts

More than 100 recordings

Handouts included

Free-access

World's most notable experts

Searchable

www.webbertraining.com/recordingslibraryc4.php