Interferon Gamma Release Assays for the Laboratory Detection of *Mycobacterium tuberculosis* Infection

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Objectives

Describe the FDA approved interferon gamma release assays (IGRAs) for the laboratory detection of *Mycobacterium tuberculosis* infection.

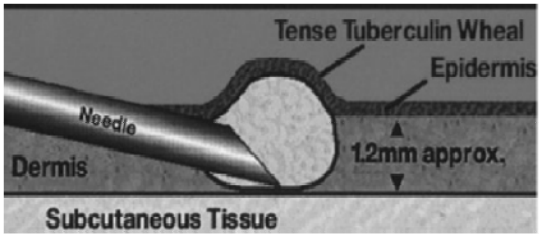
Describe the CDC recommendations for the use of IGRAs

Describe potential problems with IGRAs and future areas for research.

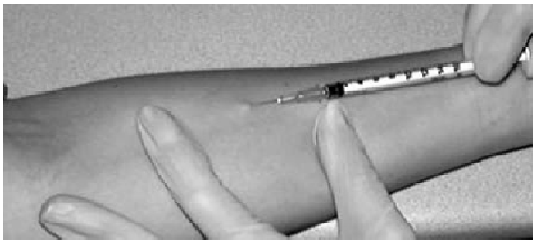
Historical Perspective of LTBI

- 1890 Robert Koch produced "tuberculin"
  - Broth culture filtrate that he thought might cure TB
  - Observed a local reaction at site of inoculation in a TB patient, but no such reaction in non-TB patients
  - Foundation for use of tuberculin for TB diagnosis
- 1908 Charles Mantoux described intradermal injection with a controlled dose of Koch's tuberculin
- 1934 PPD (purified protein derivative) of "old tuberculin" was developed using a precipitate of filtrates from heat treated cultures of M.tb.
  - Neither pure nor specific for M. tb
- 2001 First FDA approved IGRA

Mantoux Test

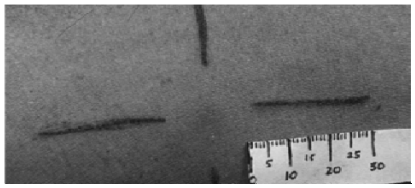


TST



TST Interpretation

- Based on
  - Measurement of the induration (mm)
  - Risk of being infected with TB and/or progression to disease if infected



Cut points for interpretation

>=5 mm positive in	>=10 mm in	>=15 mm in
HIV-positive pts	Recent immigrants	Persons with no known risk factors for TB
Recent contacts of TB case patients	Injection drug users	Persons at low risk for TB who are tested at start of employment
Organ transplants and other immunosuppressed	Residents of high risk congregate settings; prisons, LTCF, etc.	
	Mycobacteriology lab personnel	
	Children <4 yrs	

TST Short-comings

False-Positive Reactions

- Infections with NTMs
- BCG Vaccination
- Incorrect administration
- Incorrect interpretation
- Incorrect antigen used

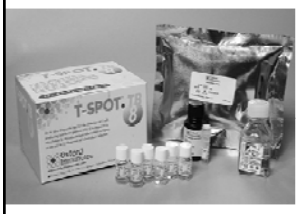
False-Negative Reactions

- Cutaneous anergy
- Recent TB infection (within 8-10 weeks)
- Very old TB infection
- Very young age (<6mo)
- Recent live-virus vaccination
- Recent viral illness
- Overwhelming TB disease
- Incorrect administration
- Incorrect interpretation

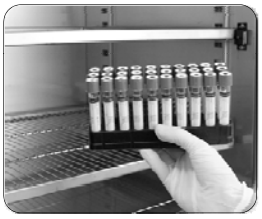
Next Generation

- 2001 QuantiFERON-TB (QFT)
  - The first blood based test for detection of LTBI
  - First generation test using PPD and an M.avian antigen
- 2005 QFT-TB Gold
- 2007 QFT-GIT
- 2008 T-Spot.*TB*

Interferon Gamma Release Assays (IGRAs)



QuantiFERON®



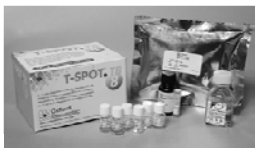
Interferon Gamma Release Assays

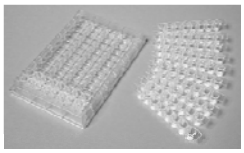
- Blood tests for detecting M. tuberculosis infection
  - Measure cell mediated immunity to M. tb
  - Sensitized white blood cells release IFN-gamma in response to contact with TB antigens
    - ESAT-6
    - CFP 10
    - TB7.7 (QFT-GIT)
- Do not differentiate latent infection from active disease

No Cross-reactivity to BCG and Most NTMs

Tuberculosis Complex	Antigens		Environmental Strains	Antigens	
	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. gordonae*	+	+
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. xenopii	-	-

T-SPOT.*TB* Test Kit



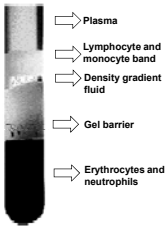


- 96-well format
  - Twelve, 8-well strips
  - 4 wells used per patient; 24 patients per kit
  - Positive and Negative control for each patient test
- Utilizes standard blood collection tubes

Oxford Immunotec

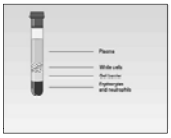
Performing the T-SPOT.*TB* Test

- Peripheral blood mononuclear cells (PBMCs) are separated from whole blood and washed
  - Removes any source of background interference
- Washed PBMCs are counted to ensure a *standardized number of cells* are added to the assay
- Blood must be processed within 8 hours of collection
  - Xtend® reagent increases time to 32 hours

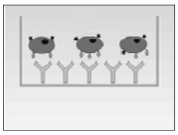


Oxford Immunotec

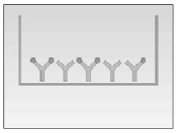
The Science behind T-SPOT™



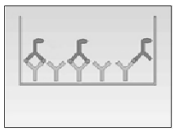
Collect white cells using BD CPT tube or Ficoll extraction.



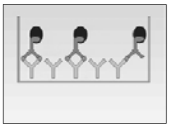
Add white cells and TB antigens to wells. Effector T-cells release interferon gamma.



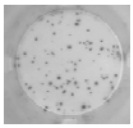
Interferon gamma captured by antibodies.



Incubate, wash and add conjugated second antibody to interferon gamma.

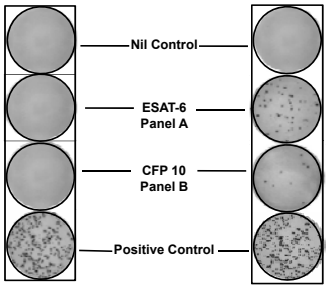


Add substrate and count T-SPOTs



Oxford Immunotec

Interpretation of Results



Oxford Immunotec

TABLE 3. Interpretation criteria for the T-SPOT.TB Test (T-Spot)

Interpretation	Nil*	TB Response†	Mitogen§
Positive¶	≤10 spots	≥8 spots	Any
Borderline**	≤10 spots	5, 6, or 7 spots	Any
Negative††	≤10 spots	≤4 spots	
Indeterminate**	>10 spots	Any	Any
	≤10 spots	<5 spots	<20 spots

Source: Based on Oxford Immunotec Limited. T-Spot.TB [Package insert]. Available at http://www.oxfordimmunotec.com/USpageInsert.

* The number of spots resulting from incubation of PBMCs in culture media without antigens.

† The greater number of spots resulting from stimulation of peripheral blood mononuclear cells (PBMCs) with two separate cocktails of peptides representing early secretory antigenic target-6 (ESAT-6) or culture filtrate protein-10 (CFP-10) minus Nil.

§ The number of spots resulting from stimulation of PBMCs with mitogen without adjustment for the number of spots resulting from incubation of PBMCs without antigens.

¶ Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.

** Interpretation indicating an uncertain likelihood of *M.tuberculosis* infection.

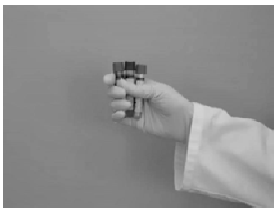
†† Interpretation indicating that *M. tuberculosis* infection is not likely.

MMWR June 25, 2010 59:RR-5

QuantiFERON® -TB Gold In-Tube

Stage 1 – Blood Stimulation and Harvesting

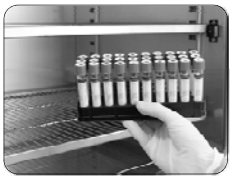
- **QFT Collection tubes**
- **After blood collection, QuantiFERON® blood tubes (nil, TB Ag, PHA) mix thoroughly by shaking.**



Courtesy Cellestis

Stage 1 – Blood Stimulation and Harvesting

- As soon as possible, and within 16 hours of collection, incubate tubes upright at 37°C for 16-24 hours.
- After incubation can hold blood tubes up to 3 days at 2-27C
  - If separate plasma, up to 28 days at 2-8C



Courtesy Cellestis

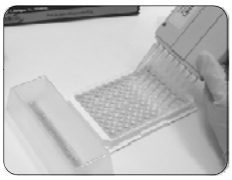
Stage 1 – Blood Stimulation and Harvesting

- Centrifuge tubes at 2000 – 3000 g (RCF) for 15 minutes to separate plasma.

Courtesy Cellestis

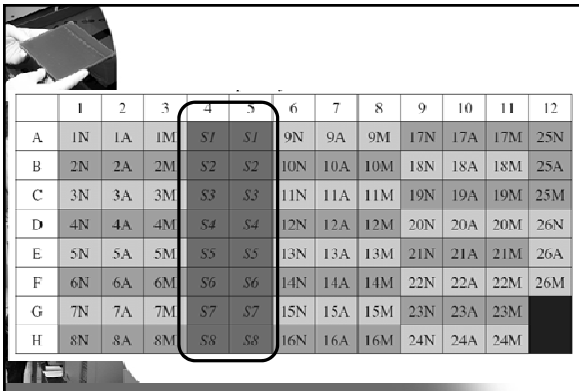
Stage 2 – Human IFN- γ ELISA

- Add conjugate to each well, then add plasma or standards.
- Shake plate and incubate for 120 minutes at room temperature.



* Can be automated

Courtesy Cellestis



	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	1A	1M	S1	S1	9N	9A	9M	17N	17A	17M	25N
B	2N	2A	2M	S2	S2	10N	10A	10M	18N	18A	18M	25A
C	3N	3A	3M	S3	S3	11N	11A	11M	19N	19A	19M	25M
D	4N	4A	4M	S4	S4	12N	12A	12M	20N	20A	20M	26N
E	5N	5A	5M	S5	S5	13N	13A	13M	21N	21A	21M	26A
F	6N	6A	6M	S6	S6	14N	14A	14M	22N	22A	22M	26M
G	7N	7A	7M	S7	S7	15N	15A	15M	23N	23A	23M	
H	8N	8A	8M	S8	S8	16N	16A	16M	24N	24A	24M	

Stage 2 – Human IFN- γ ELISA

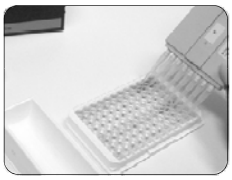
- Wash plate 6 times. Add substrate.
- Incubate for 30 minutes at room temperature.

* Can be automated

Courtesy Cellestis

Stage 2 – Human IFN- γ ELISA

- Add stop solution.
- Read absorbance within 5 min at 450nm (620-650nm ref).



* Can be automated

Courtesy Cellestis

Stage 2 – Human IFN- γ ELISA

- Calculate results using QuantiFERON® Analysis Software.
- >0.35 IU gamma interferon considered positive

Courtesy Cellestis

TABLE 1. Interpretation criteria for the QuantiFERON-TB Gold Test (QFT-G)

Interpretation	Nil*	TB Response†	Mitogen Response§
Positive¶	Any	≥0.35 IU/ml and ≥50% of Nil	Any
Negative**	≤0.7	<0.35 IU/ml	≥0.5
Indeterminate††	≤0.7	<0.35 IU/ml	<0.5
	>0.7	<50% of Nil	Any

Source: Based on Cellestis Limited. QuantiFERON-TB Gold [Package insert]. Available at http://www.cellestis.com/IRM/Company/ShowPage.aspx?CPID=1247.

* The interferon gamma (IFN-γ) concentration in plasma from blood incubated with saline.

† The higher IFN-γ concentration in plasma from blood stimulated with a cocktail of peptides representing early secretory antigenic target-6 (ESAT-6) or a cocktail of peptides representing culture filtrate protein 10 (CFP-10) minus Nil.

§ The IFN-γ concentration in plasma from blood stimulated with mitogen minus Nil.

¶ Interpretation indicating that *Mycobacterium tuberculosis* infection is likely.

** Interpretation indicating that *M. tuberculosis* infection is not likely.

†† Interpretation indicating an uncertain likelihood of *M. tuberculosis* infection.

Performance Characteristics

No good Gold Standard for LTBI

Test	Sensitivity*	Specificity*
Quanti-FERON Gold in-Tube	70-84%	96% (BCG Vac) 99% (non-BCG)
T-SPOT.TB	88-90%	86-93%
TST	70-77%	59% (BCG Vac included) 97% (BCG Vac excluded)

*Compared to active TB

CDC Guidelines for the use of IGRAs

- MMWR June 25, 2010 59:RR-5
- TSTs and IGRAs should be used as aids in diagnosing infection with M. tb
  - Surveillance purposes
  - To identify persons likely to benefit from treatment
- As with TST, IGRAs should not be used for testing person who have a low risk for both infection and progression to active TB if infected
- An IGRA may be used in place of (but not in addition to) a TST in all situations in which CDC recommends TST--- with preferences and special considerations

CDC Guidelines (Cont.)

- IGRAs FDA-Approved as in vitro diagnostic aids for detection of Mtb infection
  - Including active disease and LTBI
  - Used in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations

CDC Guidelines (Cont.)

- QFT-GIT, T-Spot and TST results may not be interchangeable
  - Measure different aspects of the immune response
  - Use different antigens
  - Use different interpretation criteria
- Therefore, different tests can yield different results!

CDC Guidelines (Cont.)

- **Situations in which IGRA preferred**
  - For persons who have received BCG
  - IGRA preferred for persons/groups that have low rates of returning to have TST read.
- **Situations in which TST preferred**
  - For children <5 years
    - IGRA in conjunction with TST advocated by some experts
    - Recommendations: Amer. Acad Ped, 2009:680-701.

CDC Guidelines (Cont.)

- Situations in which testing with both TST and IGRA may be considered
  - When risk for infection, progression, and poor outcome is increased
  - When clinical suspicion exists for active TB and confirmation is desired.
    - Positive second test increases detection sensitivity, but multiple negative results cannot exclude TB infection
  - When initial IGRA is indeterminate, borderline or invalid, repeat IGRA or perform TST

CDC Reporting Recommendations

- Qualitative test interpretation
  - Positive
  - Negative
  - Borderline (T-Spot.*TB*)
  - Indeterminate
- Quantitative assay measurements
  - IU/ml
  - Number of spots

Serial Testing of HCWs

Cleveland Clinic Study

Fong, K. et al. Chest 2012; 142: 55-62

- HCWs who underwent preemployment QFT-GIT testing 2007-2010
  - 7,374 IGRAs performed
    - 486 (6.6%) positive at baseline
    - 305 (4.1%) indeterminate
    - 6583 (89.3%) negative
  - 2.8% (52/1,857) identified as converters
    - 71% with values ≤1 IU/ml
    - None with active TB or part of outbreak investigation
  - Previous annual conversion rate of 0.09% using TST

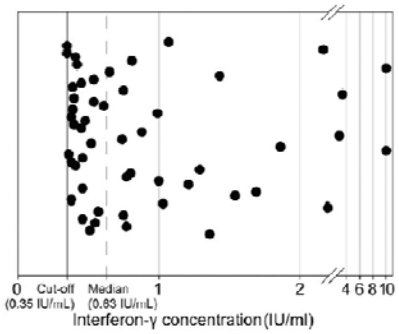


FIGURE 2. Quantitative interferon-γ release assay results in T-cell assay converters (n = 52).

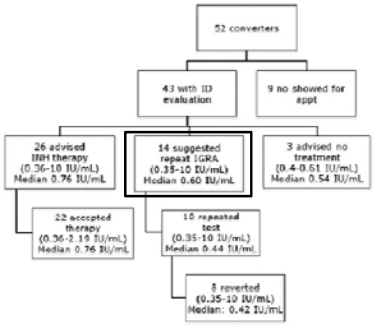


FIGURE 3. Management of converters (n = 52). Quantitative IGRA values available from August 2006. INH = isoniazid. See Figure 1 legend for expansion of other abbreviations.

Cleveland Protocol

- If new converter
  - Repeat IGRA, chest x-ray, ID evaluation
  - If no identifiable risk factors, consider treatment if >1 IU/ml
    - 15/52 would have been considered converters (0.8% vs 2.8%)
    - May reduce unnecessary treatment

Study Conclusions

- Conversions/reversions tend to occur around the cut-off
  - Did see reversions in HCWs with values >3 IU/ml
- Studies needed to establish new cutoffs for conversions in serial testing of HCWs

Factors influencing IGRA Results

Effects of Incubation Delays on QFT-GIT Results

- Doberne, D. et al. J. Clin Microbiol. 49: 3061-3064
  - Compared results when blood incubated immediately after collection to results after 6- and 12-hour delays
  - 128 HCWs from Stanford Hosp and Clin

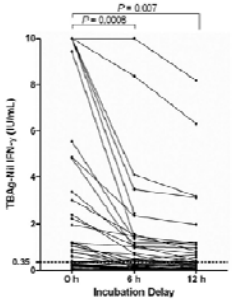
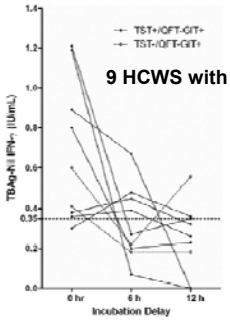


FIG. 1. QFT-GIT TB Ag-nil values for 128 volunteers tested with immediate and delayed incubations. The cutoff for positive results (TB Ag-nil value of >0.35 IU/ml) is marked with a dashed line. TB Ag-nil values of <0 IU/ml are shown as 0 IU/ml. Two 12-hour-delay results were excluded due to overincubation. The Student *t* test was used to compare differences in means.



9 HCWS with Discordant Results

FIG. 2. QFT-GIT TB Ag-nil values for 9 volunteers with discordant results between immediate and delayed incubations. The cutoff for positive results (TB Ag-nil value of ≥0.35 IU/ml) is marked with a dashed line. TB Ag-nil values of <0 IU/ml are shown as 0 IU/ml. Prior TST and QFT-GIT results are shown.

Positive-to-Negative Reversions

- 19% (5/26) with 6-hr delay
- 22% (5/23) with 12-hr delay
- Underscore importance of preanalytical practices

Effect of Delayed Incubation on QGT-GIT

Whitworth, WC ,et al. Presented at 8th National Conference on Laboratory Aspects of Tuberculosis, Atlanta, June 2012

- <1hr delay to incubation
  - 25% (37/148) positive
- 11-12 hr delay to incubation
  - 20.9% (31/148) positive
    - p=.03

Effect of Incubation Duration on QFT-GIT

Whitworth, WC ,et al. Presented at 8th National Conference on Laboratory Aspects of Tuberculosis, Atlanta, June 2012

- 23-24 hr incubation
  - 25.8% (39/151) positive
- 16-17 hr incubation
  - 23.2% (35/151) positive
    - p= 0.08

Effect of Incubation Temperature on QFT-IT

- Incubation at 37C
  - 20.6% (21/102) positive
- Incubation at 35C
  - 20.6% (21/102) positive
- No impact on result interpretation, but signficant differences in the qualitative IGRA results

Whitworth, WC ,et al. Presented at 8th National Conference on Laboratory Aspects of Tuberculosis, Atlanta, June 2012

Advantages of IGRAs

- Require a single patient visit
- Not subject to reader bias
- Use defined TB antigens
- Not affected by prior BCG vaccination
- Controlled laboratory based test
- Objective result
- No possibility of adverse reactions in hypersensitive individuals
- Do not boost responses upon subsequent testing

Disadvantages of IGRAs

- Blood must be processed within 8-32 hours after collection
- Possibility of errors in collection or transport of samples
- Lab variability and errors in performance or interpretation of the test
- Limited data on use in certain populations
  - Immunocompromised
  - Patients on immunosuppressive drugs
  - Patients with hematological disorders, diabetes, malignancies
- Limited data on the use of IGRAs to determine risk for developing TB disease

Logistical and Economic Laboratory Issues

- Processing of blood within required time frame
- Test verification more difficult
- Need sufficient test volume to make economically feasible
- Transfer of cost from employee health or TB Program to the laboratory

Outstanding Issues

- Reproducibility in the "real world"
  - Impact of time from blood draw to incubation
  - Impact of incubation time
  - Impact of time of day blood drawn
  - Impact of how collection tubes handled
  - Impact of technical variations on the test performance
- Serial testing of HCWs
  - The "wobble" phenomenon
  - Do we need a gray zone and/or different cutoff values?
- Which test is the best predictor of progression to active TB?

Thank You!