Targeted Tuberculin Testing and Treatment of Latent Tuberculosis Infection (LTBI)

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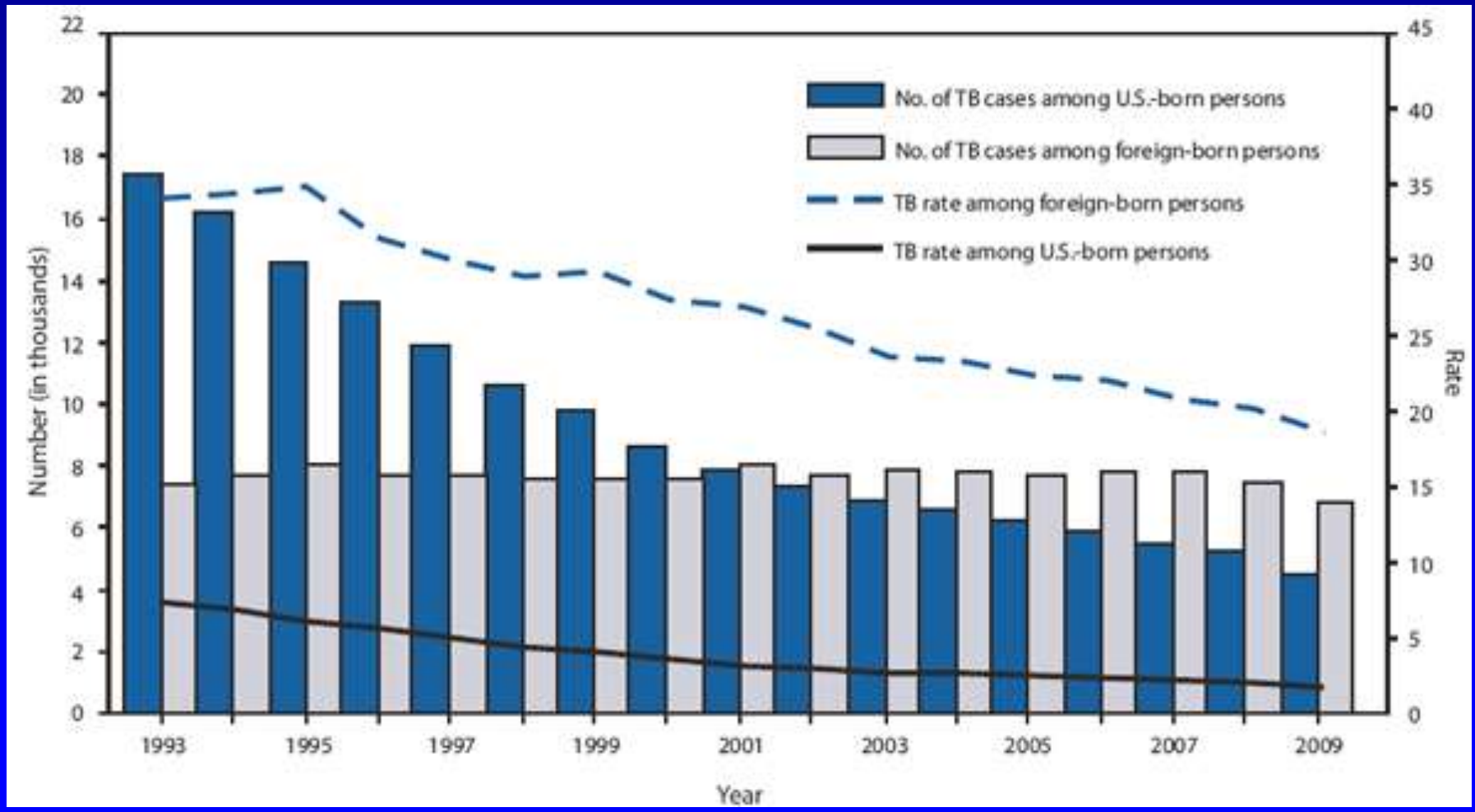
Tuberculosis Estimates

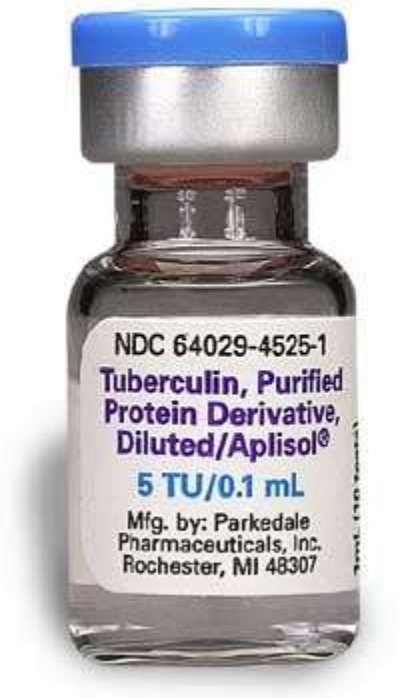
	USA	World
Infection	15,000,000	2,000,000,000
Disease	14,000	9,000,000
Death	1,000	2,000,000

Number and rate of tuberculosis (TB) cases among U.S.-born and foreign-born persons, by year reported --- United States, 1993--2009



121 years old

71 years old


NDC 64029-4525-1
Tuberculin, Purified
Protein Derivative,
Diluted/Aplisol®
5 TU/0.1 mL
Mfg. by: Parkedale
Pharmaceuticals, Inc.
Rochester, MI 48307

103 years old

•Indications for Screening and Treatment

< 5 mm	≥ 5 mm	≥ 10 mm	≥ 15 mm
•Recent contacts who are immuno-suppressed (HIV+, prednisone, chemotherapy) should receive a full course of therapy •Children < 5 yo who are recent contacts (treat 8-12 weeks and retest). Consider treatment for children<15 yo, and for severe exposures.	•Immunosuppressed (e.g., HIV+, prednisone ≥15 mg/day for ≥ 1 month, organ transplants) •Recent contacts (not immunosuppressed) •Fibrotic changes	•Recent immigrants (5 years) •Injection drug users •Residents of prisons, health facilities (long or short term), homeless shelters •Employees of prisons, health facilities (long or short term), homeless shelters, AIDS residential facilities, mycobacteriology labs •Clinical conditions (silicosis, diabetes, cancer, ESRD, malig, gastrectomy, JI bypass, wt loss) •Children < 4 yo •Children exposed to adults in high-risk categories	No risk factors: screened upon entry into a high-exposure setting

A recent conversion is defined as an increase of ≥ 10 mm within a 2-year period

Medical Risk Factors

- Silicosis
- Postgastrectomy
- Jejunoileal bypass
- Loss of $\geq$ 10% of ideal body weight
- Chonic renal failure
- Diabetes Mellitus
- Immunosuppressive therapy
- Malignancy

BCG and Subsequent Skin Test Reactivity

Author	Time between BCG and skin test	Strata	PPD≥10 mm	Comments
Menzies ARRD 1992	10-25 y	< 1 yo 2-5 yo ≥ 6 yo	8% 18% 25%	Infant rate not significantly different from controls
Sepulveda ARRD 1990	5-19 y	No scars 1 scar 2 scars 3 scars	12% 34% 63% 74%	Retrospective. Study entrants were supposedly vaccinated at 0, 6, and 14 years old.
Comstock ARRD 1971	8-15 y	Control BCG	2% 16%	Navy recruits vaccinated after age 5.

- Can you use a skin test to help diagnose active tuberculosis?

Specific Anergy

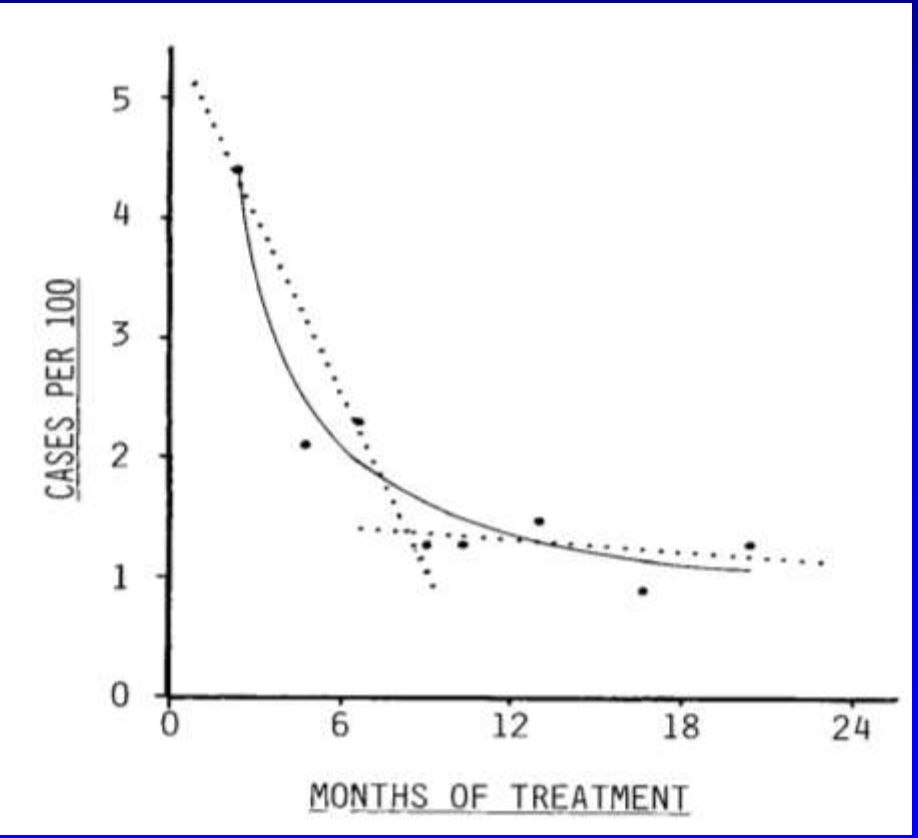
Author	Active TB	Anergy to 5 TU	Specific anergy to 250 TU with positive controls
Nash	200	25%	8%
Holden	115	19%	
McMurray	117	20%	9%

Recommended Drug Regimens for LTBI

Drug	Interval and Duration	HIV -	HIV +
Isoniazid	Daily for 9 months Twice weekly for 9 months	A (II) B (II)	A (II) B (II)
Isoniazid	Daily for 6 months Twice weekly for 6 months	B (I) B (II)	C (I) C (I)
Rifampin	Daily for 4 months	B (II)	B (III)
Isoniazid + Rifapentine	Once a week for 12 weeks on DOT		

A=Preferred, B=Acceptable, C=If A and B cannot be given
I = RCT, II= Non RCT, III=Expert opinion
Rifabutin can be substituted for rifampin

Bethel Isoniazid Studies 1957-59



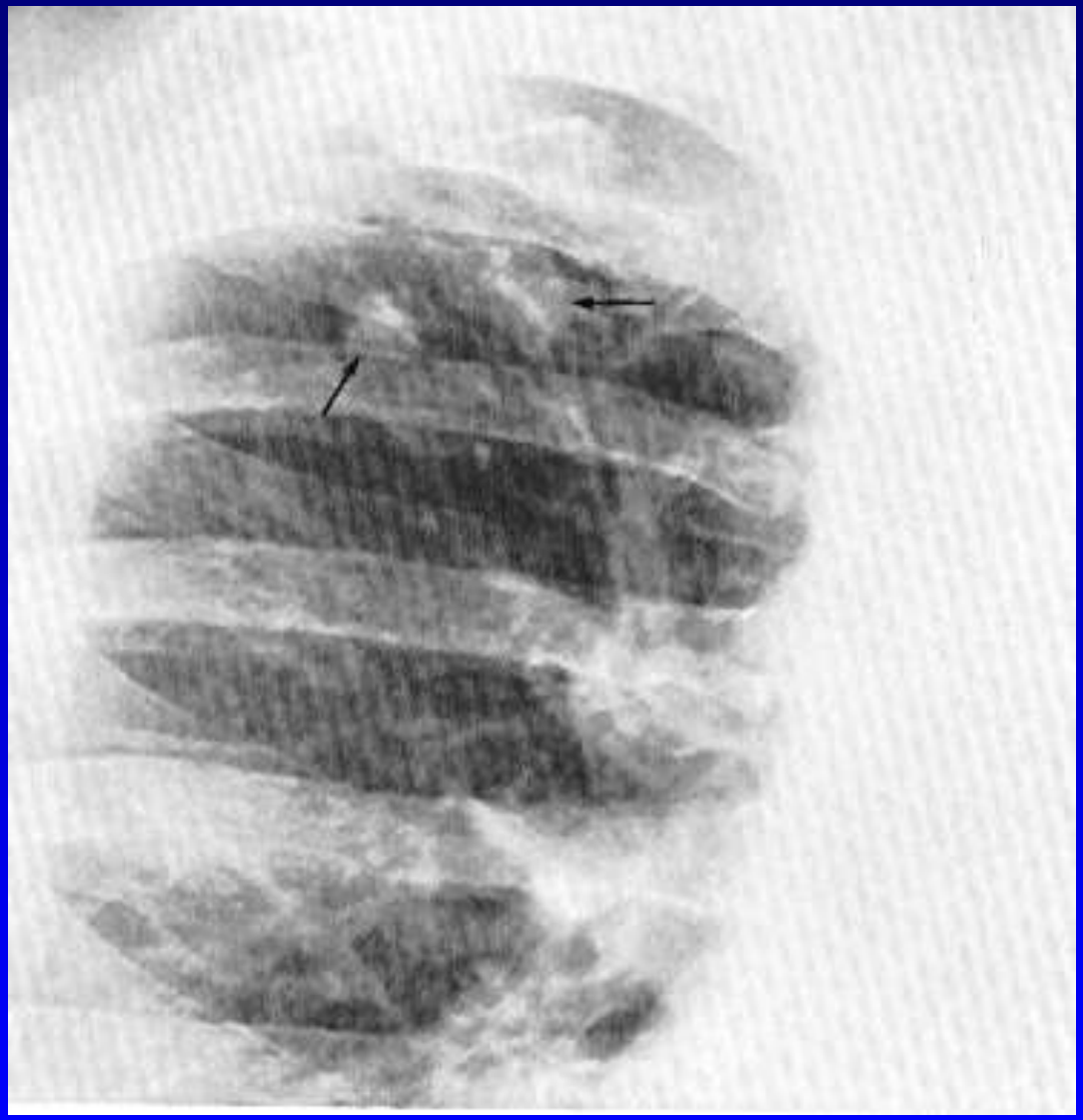
Comstock, GW. How much isoniazid is needed. Int J Tuberc Lung Dis 1999; 3:847-50.

TBTC Study 26

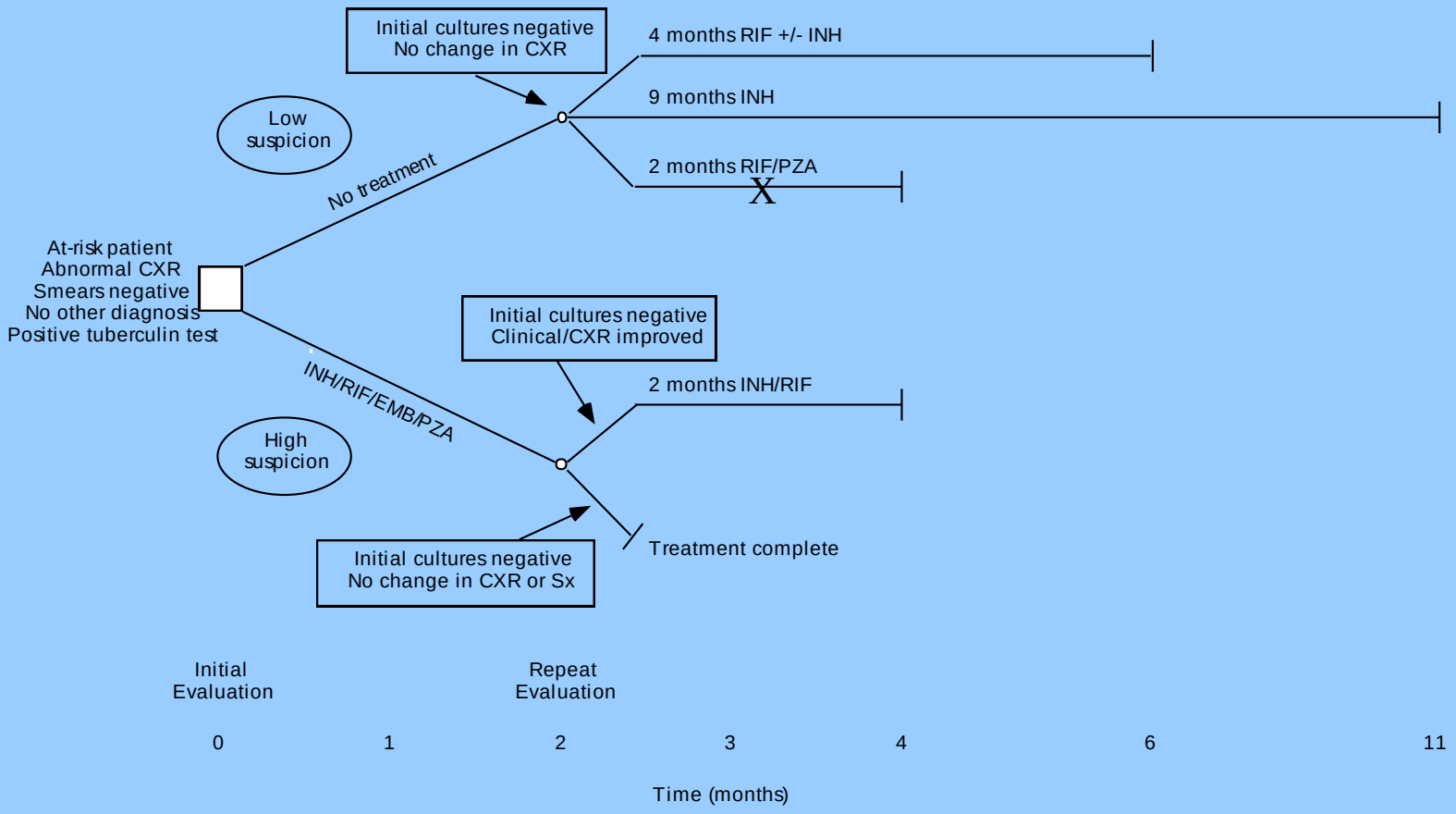
- Daily INH for 9 months versus once weekly INH (900)/RPT (weight based) for 12 weeks DOT (outcomes at 33 months)
- 72% household contacts, 24% new convertors, 2% fibrosis, 2% HIV
- INH:15/3794 (69% compliance): 0.40% TB
- I/P: 7/4052 (81% compliance): 0.17% TB and better tolerated
- Not recommended for children<2, pregnancy, antiretrovirals, exposure to known resistance to INH or RIF.

NEJM 2011; 365:2155-66
MMWR 2011; 60 (No. 48):1650-3.

- Can you treat latent tuberculosis in 2 months?



Treatment Of Active Culture-Negative Pulmonary Tuberculosis And Inactive Tuberculosis



AJRCCM 2003;167:603

Tuberculosis Associated with Infliximab

- Pulmonary 22 (31%)
- Extrapulm (local) 23 (33%)
  - –LN (11), perit (4), pleural (2), mening (1), enteric (1), skel (2), gu (2)
- Extrapulm (dissem) 17 (24%)
- Not reported 8 (11%)

Keane. NEJM 2001;345:1098

Tuberculosis Associated with Infliximab

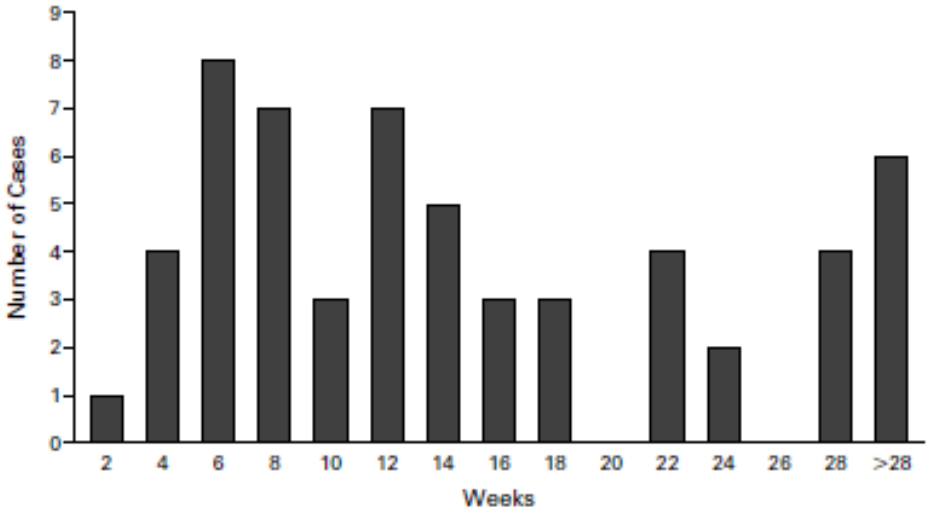


Figure 1. Time from the Initiation of Infliximab Therapy to the Diagnosis of Tuberculosis.
Data were available for 57 patients, most of whom had received monthly infusions of infliximab.

Keane. NEJM 2001;345:1098

Risk of TB is Higher with Infliximab and Adalimumab than with Etanercept

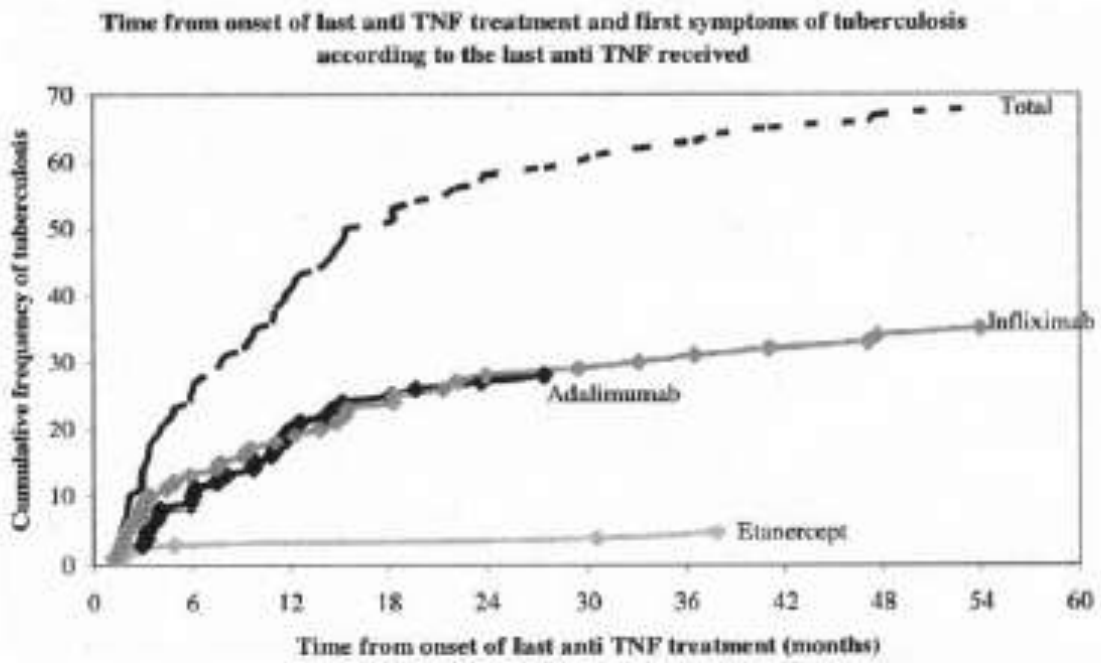


Figure 1. Cumulative incidence of tuberculosis as a function of the duration of anti–tumor necrosis factor (anti-TNF) treatment, in total and for individual anti-TNF agents.

Tubach.Arth Rheum 2009;60:1884

Tuberculosis and TNF-α Inhibitors

- Test with both TST and IGRA. Can do sequentially
- Treat TST ≥5 mm or positive IGRA
- Treat TST <5 and negative IGRA where circumstances suggest a high probability of LTBI –fibrotic lesions, recent exposure, endemic area, prisons, ivdu, etc.
- Start treatment for LTBI before starting anti-TNF-α therapy – use INH for 9 months, or consider RIF or INH/RIF for 4 months if you cannot wait that long.
- Active TB should be excluded in patients with an abnormal chest radiograph or a past history of TB not previously adequately treated.
- Patients with old TB or LTBI taking infliximab or adalimumab should be screened every month for the first three months by symptoms and with a CXR ± sputum
- Favor etanercept over other agents in patients with LTBI or old TB.
- Screen carefully for extrapulmonary disease. If TB develops in a patient on TNF-α inhibitors, stop therapy while treating for TB, at least until the TB is well under control. Watch for the development of a paradoxical reaction.

Completion of Therapy

Regimen	Minimum number of doses	Maximum duration of therapy
INH daily for 9 months	270	12 months
INH twice weekly for 9 months	76	12 months
INH daily for 6 months	180	9 months
INH twice weekly for 6 months	52	9 months
RIF/PZA daily for 2 months	60	3 months
RIF daily for 4 months	120	6 months

If interruption of therapy > 2 months, must r/o active TB
If course not completed, must renew entire regimen

Treatment of Drug-Resistant LTBI

INH Resistance

RIF for 4 months

INH/RIF Resistance

PZA/EMB for 6-12 months **12M for immunosupp**

PZA/FQ for 6-12 months **12M for immunosupp**

Follow for 2 years

Case 1

23 year old male receives a routine skin test before starting work at a health care facility. There is 10 mm of induration.

Case 2

28 year old Russian male who received BCG at birth now presents with a positive skin test (16 mm) and states he received BCG and should not be treated.

Case 3

78 year old white female with diet controlled diabetes has a history of a positive skin test (placed 3 years ago and not treated). She has no end organ damage.

Case 4

68 year old white male with COPD and a history of a positive skin test (never treated) who is not steroid dependent, but recently presented with an exacerbation of his disease and received 40 mg of prednisone tapered over 2 weeks

Case 5

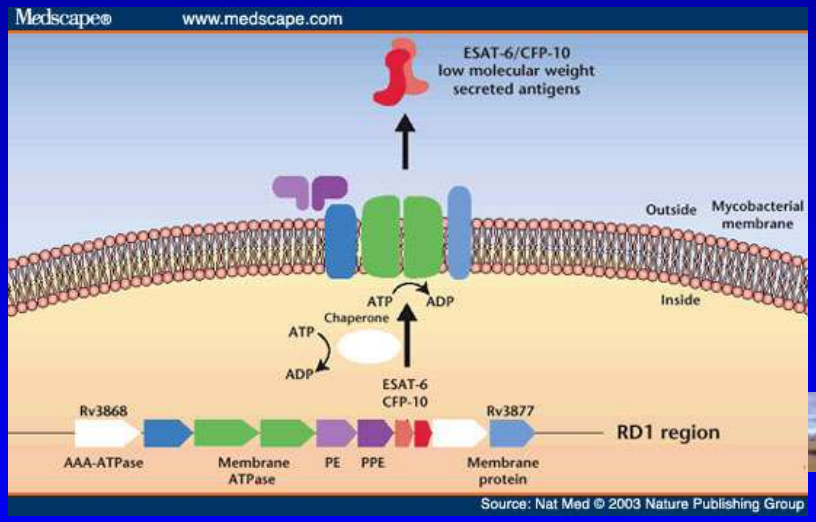
A 23 year old male has smear positive tuberculosis and knows that he was exposed to an individual with INH-resistant tuberculosis

103 years old

IGRA
Interferon-gamma release assay

- QuantiFERON-TB - 2001
- QuantiFERON-TB Gold - 2005
- QuantiFERON-TB Gold In-Tube - 2007
- ELISPOT
- T-SPOT.TB - 2008

Region of Difference 1 (RD1)



Region of Difference 1 (RD1)

- Specific region on the Mycobacterium tuberculosis genome that is not shared with BCG or MAI
  - ESAT-6 – early secreted antigen target 6
  - CFP-10 – culture filtrate protein 10
  - TB 7.7
- These antigens are also present in M. kansasii, M. leprae, M. marinum, and M. szulgai

QUANTIFERON-TB GOLD IT (*In-Tube Method*)

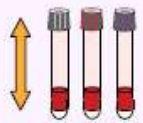
QuantiFERON®-TB Gold IT (*In-Tube Method*)

- An ***in vitro*** test approved by the FDA on October 12, 2007, as an alternative to TST for diagnosing TB infection
- Measures tuberculosis-specific antigen-induced secretion of interferon gamma (IFN-γ) by peripheral white blood cells
- A mixture of peptides representing ESAT-6, CFP-10, and TB 7.7 in one tube. Also there is a positive and a negative control tube.
- Blood must be incubated within 16 hours of collection, incubation ensues for 16-24 hours, plasma supernatant is drawn off and analyzed by ELISA for gamma interferon

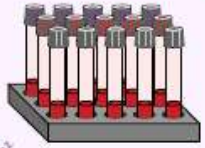
QuantiFERON®-TB Gold In-Tube

Assay Quick Reference Guide

Part 1. Blood Incubation and Harvesting



Step 1. After blood collection, mix QuantiFERON®-TB Gold tubes thoroughly, by shaking vigorously for 5 seconds.

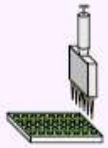


Step 2. As soon as possible, and within 16 hours of collection, incubate tubes upright at 37°C for 16-24 hours.

Step 3. Centrifuge tubes at 2000-3000 g (RCF) for 15 minutes.

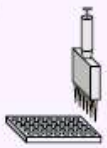
Step 4. Harvest at least 200 µL plasma from each tube. Store in racked microtubes or uncoated microplates.

Part 2. Human IFN-γ ELISA

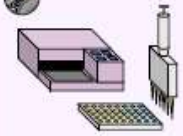


Step 5. Add 50 µL of working conjugate to each well. Add 50 µL of plasma or standard.

Step 6. Shake covered plate for 1 min. Incubate for 120 minutes at room temperature.



Step 7. Wash plate ≥ 6 times. Add 100 µL of substrate. Incubate 30 min. at room temperature.



Step 8. Add 50 µL of stop solution. Read absorbance within 5 min. at 450 nm (620-650 nm ref).

Step 9. Calculate results using QuantiFERON®-TB Gold In-Tube Analysis Software.

For comprehensive instructions, please refer to the QuantiFERON®-TB Gold In-Tube Package Insert

North America / South America
Email: customer.service@cellestis.com
Tel: +1 661 775 7480
Toll free: 800 519 4637 (USA only)
Fax: +1 661 775 7479

Europe / Middle East / Africa
Email: europe@cellestis.com
Tel: +49 6151 428 59 0
Fax: +49 6151 428 59 110

Asia / Oceania
Email: quantiferon@cellestis.com
Tel: +61 3 9571 3500
Fax: +61 3 9571 3544

Step not required with automation.

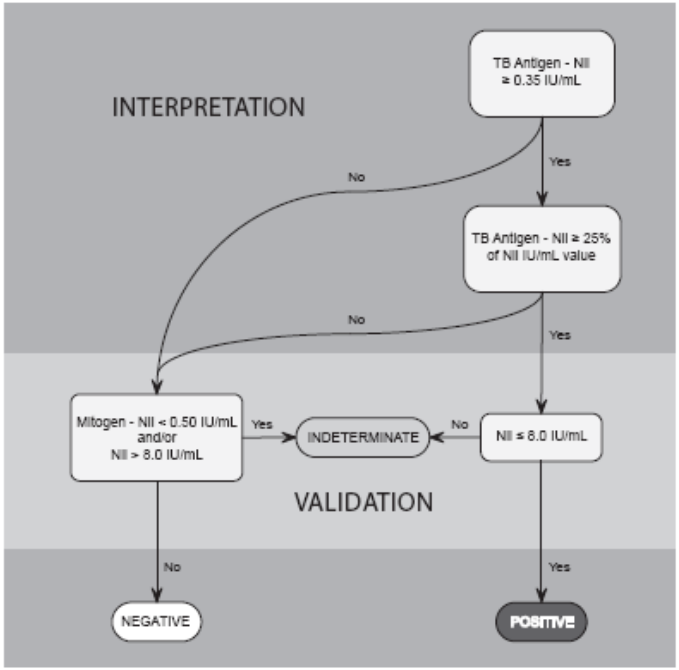
Step can be automated.

cellestis

LOS9860ITA Apr 2007

QuantiFERON®-TB Gold In-Tube

Results Interpretation Guide



For detailed results interpretation please refer to the QuantiFERON®-TB Gold In-Tube Package Insert.

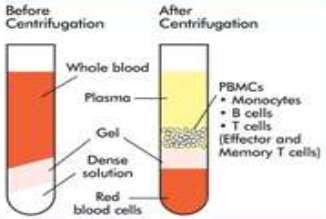
ELISPOT

- A T-cell based assay that measures the number of cells that release gamma interferon
- Potentially more sensitive because gamma interferon is released and is captured by antibodies directly under the cell in an ELISA (ELISPOT)

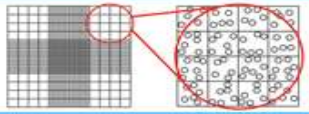
HOW TO USE T-SPOT.*TB*

Using **T-SPOT.*TB*** couldn't be simpler.
Follow these six easy steps:

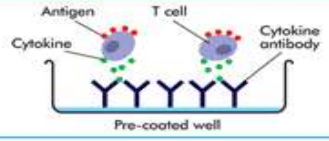
Step 1 Collect the blood sample in a Cell Preparation Tube and centrifuge to separate Peripheral Blood Mononuclear Cells (PBMCs)



Step 2 Wash and count the PBMCs using a microscope and counting chamber or simply run them on a hematology analyser



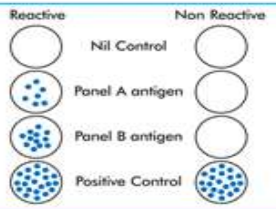
Step 3 Add PBMCs to wells with antigens and incubate overnight (37°C, CO_2)



Step 4 Wash and add secondary antibody

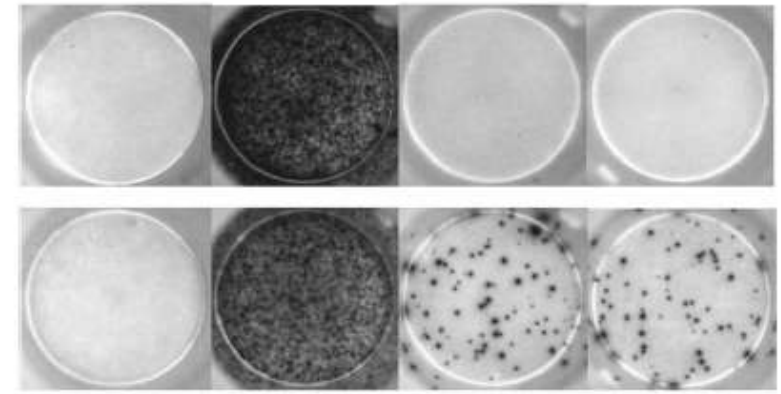
Step 5 Wash and add substrate

Step 6 Count spots
One spot = one T cell



Final suspension should be 250,000 PBMCs/ 100 uL

SPOTS



-ve

+ve

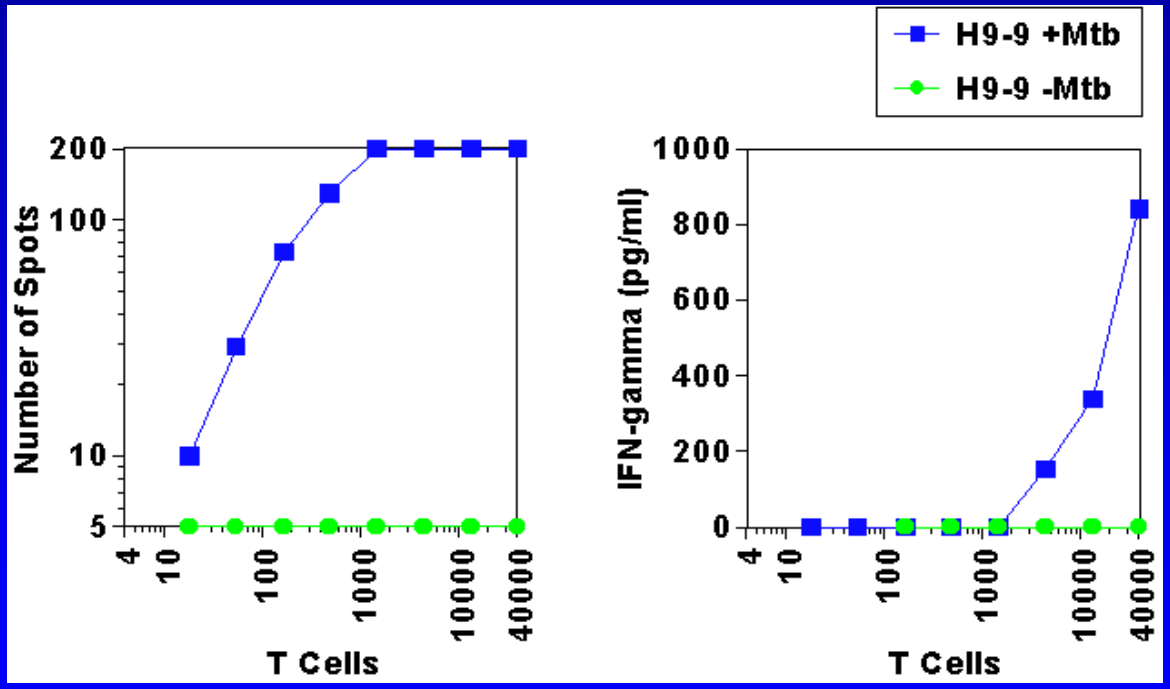
Interpretation of T-SPOT Results

ESAT6 (A) CFP10 (B)	Nil	Mitogen	T-SPOT result	Comment
(A or B) -nil ≥ 6 spots	<6 spots	Any	Positive	6-7 spots may be considered indeterminate
A or B ≥ 2 x nil	≥ 6 spots	Any	Positive	
< 6 spots < 2 x nil	<6 spots ≥ 6 spots	≥ 20 spots ≥ 20 spots	Negative	5 spots may be considered indeterminate

All other results may be considered indeterminate

ELISPOT versus ELISA for detecting T cell-derived interferon-gamma

ELISPOT ELISA



Comparing the sensitivity of ELISPOT (left) vs. ELISA (right) using T cell clones.
Data courtesy of David Lewinsohn, Oregon Health and Science University, Portland.

Effect of HIV-1 infection on T-cell based and skin test detection of tuberculosis infection

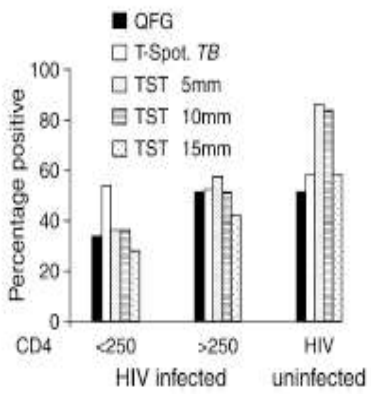


Figure 4. Proportion positive in each test stratified by HIV status and CD4+ cell count. Although the proportion scored positive in lower CD4+ cell count strata tended to be consistently higher for T-SPOT.*TB* compared with QFT-G, this effect was not statistically significant for any CD4+ cell count cutoff. When including the HIV-uninfected group for comparison, the proportion positive clearly fell for the TST ($p < 0.001$ for 5 and 10 mm, and $p = 0.053$ for 15 mm) but remained relatively constant for the IFNGRA (p = nonsignificant, regardless of whether indeterminate results were factored as negative).

Rangaka et al. AJRCCM 2007; 175:514-20.

IGRA META-ANALYSIS

	Sensitivity	Specificity
QFT-G	78% (73%-82%)	
QFT-IT	70% (63%-78%)	
Pooled-no BCG		99%(98%-100%)
Pooled- BCG		96%(94%-98%)
T-SPOT	90% (86%-93%)	93%(86%-100%)

Pai et al. Ann Intern Med 2008;149:1

Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts

TABLE 4. AGREEMENT BETWEEN QUANTIFERON TB GOLD IN-TUBE AND T-SPOT.*TB*

				T-SPOT.*TB*				
TST Category	No.			Negative	Positive	Agreement (%)	OR (95% CI)	κ
0 (0 mm)	414	QFT-GIT	Neg	394 (99.7)*	19 (100)	95.2	0	−0.005
			Pos	1 (0.3)	0			
1 (1–4 mm)	15	QFT-GIT	Neg	13 (100)	2 (100)	86.7	0	NA
			Pos	0	0			
2 (5–9 mm)	76	QFT-GIT	Neg	56 (96.6)	14 (77.8)	78.9	8.0 (1.5–∞)	0.24
			Pos	2 (3.4)	4 (22.2)			
3 (10–14 mm)	98	QFT-GIT	Neg	74 (98.7)	18 (78.3)	74.1	20.0 (2.9–∞)	0.27
			Pos	1 (1.3)	5 (21.7)			
4 (≥ 15 mm)	156	QFT-GIT	Neg	71 (93.4)	17 (21.3)	85.9	52.6 (18.8–146.4)	0.72
			Pos	5 (6.6)	63 (78.8)			
All categories	759†	QFT-GIT	Neg	608 (98.5)	70 (49.3)	89.6	69.5 (33.3–145.0)	0.59
			Pos	9 (1.5)	72 (50.7)			

Definition of abbreviations: CI = confidence interval; NA = not applicable; neg = negative; OR = odds ratio; pos = positive; QFT-GIT = QuantiFERON-TB Gold In-Tube; TST = tuberculin skin test.

* Data are expressed as number (%).

† For T-SPOT.*TB*, there were three missing blood samples and 23 indeterminate results.

71/156 (46%) of patients with a skin test ≥ 15 mm had both a negative QFT-IT and a negative T-SPOT

Arend. AJRCCM 2007; 175:618-27.

Detection of TB Infection in US Navy Recruits Using the TBST or IGRA

Table 3. Results of the tuberculin skin test (TST) versus the QuantiFERON-TB Gold assay (QFT-G) for all recruits, recruits at low risk for *Mycobacteria tuberculosis* infection, and recruits at high risk for *M. tuberculosis* infection.

TST induration, recruit group	QFT-G result				
	Negative	Positive	Indeterminate	Incomplete	All
<5 mm					
All	758	0	16	11	785
Low risk	517	0	10	8	535
High risk	241	0	6	3	250
5–9 mm					
All	9	0	1	0	10
High risk	6	0	1	0	7
Low risk	3	0	0	0	3
10–14 mm					
All	19	0	0	0	19
Low risk	4	0	0	0	4
High risk	15	0	0	0	15
⩾15 mm					
All	19	5	0	0	24
Low risk	4	1	0	0	5
High risk	15	4	0	0	19
Not completed					
All	18	0	0	0	18
Low risk	12	0	0	0	12
High risk	6	0	0	0	6
All					
All	823	5	17	11	856
Low risk	543	1	11	8	563
High risk	280	4	6	3	293

NOTE. Data are no. of recruits.

15/19 (79%) high risk patients with a skin test ≥15 had a negative QFT

Mazurek et al. CID 2007; 45:826

Prospective Comparison of the TBST and 2 IGRA Assays in Suspected TB

Table 5. Tuberculin skin test (TST) results versus QuantiFERON-TB Gold assay (QFT-G) results for subjects with culture-confirmed tuberculosis (TB), subjects with culture-negative TB, and subjects without TB disease.

TST induration, subject group	QFT-G result			
	Negative	Positive	Indeterminate	All
<5 mm				
Culture-confirmed TB	8	6	4	18
Culture-negative TB	4	0	0	4
No TB	27	3	3	33
5–9 mm				
Culture-confirmed TB	0	2	0	2
Culture-negative TB	0	0	0	0
No TB	0	0	0	0
10–14 mm				
Culture-confirmed TB	0	6	0	6
Culture-negative TB	1	1	0	2
No TB	7	2	0	9
≥15 mm				
Culture-confirmed TB	6	32	5	43
Culture-negative TB	5	15	1	21
No TB	4	5	1	10
All				
Culture-confirmed TB	14	46	9	69
Culture-negative TB	10	16	1	27
No TB	38	10	4	52

NOTE. Data are no. of subjects.

14/69 (20%) of patients with culture confirmed tuberculosis had a negative QFT

Mazurek et al. CID 2007; 45:837

Progression to Active Disease in 903 Untreated Close Contacts (2 year follow up)

TABLE 4. RATE OF PROGRESSION TO ACTIVE TUBERCULOSIS FOR THOSE QUANTIFERON-TB GOLD IN-TUBE ASSAY POSITIVE OR POSITIVE BY THE TUBERCULIN SKIN TEST AT VARIOUS INDURATION CUTOFFS AMONG THE 903 UNTREATED CONTACT PERSONS

	No. of Untreated Contacts	Progressed to Active TB	Progression Rate (%)
QFT			
Positive	147	19	12.9
Negative	756	0	0
TST, mm			
0–5	348*	2	0.6
>5	555	17	3.1
>10	207	10	4.8
>15	63	2	3.2

Definition of abbreviations: QFT = QuantiFERON-TB Gold in-tube assay; TB = tuberculosis; TST = tuberculin skin test.

*Two TST-negative but QFT-positive contact persons received preventive chemotherapy.

Diel AJRCCM 2011; 183:88-95

Progression to Active Disease in Close Contacts (2 year follow up)

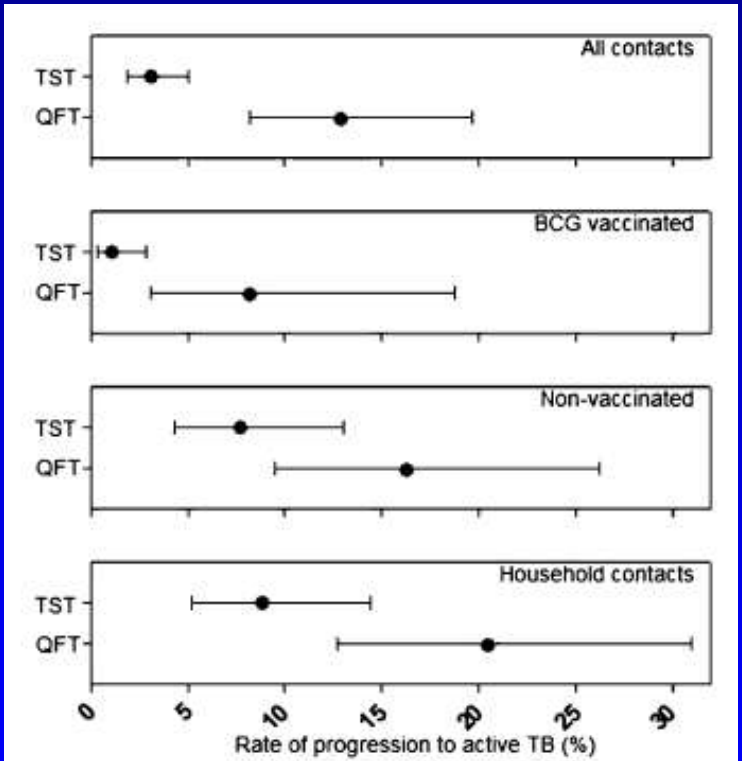


Figure 1. Rates of progression to active tuberculosis (TB) for untreated, test-positive contacts (% with 95% confidence intervals).

16.3% (14/86) vs 7.7% (13/170) in Non-vaccinated

Diel AJRCCM 2011; 183:414-5

IGRA Preferred

- Individuals that are unlikely to return for a skin test reading
- Individuals who have received BCG

MMWR 2010; 59 (No. RR-5)

TST Preferred

- Children < 5 yo

MMWR 2010; 59 (No. RR-5)

TST or IGRA

- Recent contacts
- Potential occupational exposures

- (there might be more “conversions” with IGRAs if the test is borderline the first time due to the sharp cutoff, as opposed to a 10 mm increase with the skin test)

MMWR 2010; 59 (No. RR-5)

Both TST and IGRA

- When the initial test is negative
  - in high-risk situations – high risk of infection, progression, or poor outcome (e.g., HIV, < 5 yo)
- When the initial test is positive
  - to encourage compliance with treatment
  - in healthy persons with low risk for infection and progression
- When the IGRA is indeterminate, borderline or invalid
  - can do a TST or repeat the IGRA

MMWR 2010; 59 (No. RR-5)

QUESTIONS