Immunodiagnosis of latent tuberculosis : new answers to an old question?

Franken, W.P.J.

4

INTERFERON-GAMMA RELEASE ASSAYS DURING FOLLOW-UP OF TUBERCULIN SKIN TEST POSITIVE CONTACTS

Willeke P.J. Franken[1], Sandra M. Arend[1], Steven F.T. Thijsen[2], John J.M. Bouwman[2], Ben F.P.J. Koster[3] , Jaap T. van Dissel[1], Ailko W.J. Bossink[4]

[1]Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

[2] Department of Medical Microbiology and Immunology, Diakonessenhuis Utrecht/Zeist, The Netherlands

[3] Department of Tuberculosis Control, Municipal Health Authority, Utrecht, The Netherlands

[4] Department of Pulmonology and Tuberculosis, Diakonessenhuis, Utrecht, The Netherlands

INTERNATIONAL JOURNAL OF TUBERCULOSIS AND LUNG DISEASE
2008;12(11):1286-94

ABSTRACT

Following a large scale contact investigation, individuals with a positive tuberculin skin test (TST) result were offered preventive tuberculosis treatment. This allowed us to investigate both the effect of isoniazid (INH) treatment as well as the effect of time on interferon gamma release assays (IGRA) results during follow-up.

TST positive subjects (N=122) detected during the large scale contact investigation were included. During 2 years blood was obtained every six months to perform both blood tests.

Preventive INH treatment was completed by 36/122 (29.5%) of the individuals, 71/122 (58.2%) were followed with 6-monthly X-ray screening and 15/122 (12.3%) did not complete INH treatment. The overall percentage of individuals with a positive result remained stable during the 2 years at approximately 45-50%, but individual responses could vary over time. The majority of initially low IGRA results remained below the cut-off value, initially high IGRA results remained positive while initially intermediate IGRA results were followed by more dynamic patterns.

This study showed a highly variable pattern of IGRA responses over time and suggests limited value for their use during follow-up of latently-infected individuals. However, the significance of different kinetic patterns observed among subjects with initially intermediate IGRA results warrants further study.

INTRODUCTION

Since *Mycobacterium tuberculosis* (MTB) specific interferon gamma release assays (IGRA) QuantiFERON-TB Gold in-tube (QFT-GIT, Cellestis Ltd., Carnegie, Australia) and T-SPOT.*TB* (Oxford Immunotec, Abingdon, Oxon UK) have become available, numerous studies using these tests have been published (1-9). These have clarified the main characteristics of these assays, but also raised new questions regarding their use in daily practice (10-14). The main advantage of IGRA over the tuberculin skin test (TST) is the use of MTB-specific antigens ESAT-6 and CFP-10 (and additionally TB7.7 in QFT-GIT) (10;15;16). The TST, in contrast, uses purified protein derivative (PPD) which is a crude mixture of TB antigens and may cause a false-positive TST response due to vaccination with Bacillus Calmette Guérin (BCG) or exposure to nontuberculous mycobacteria (NTM). The lack of false-positive responses is the main advantage of IGRA. One of the unclear issues relates to their value for follow-up during treatment, as was pointed out previously (14;17). Thus far, reports on follow-up data during treatment mainly considered patients with active TB, and these have been inconsistent. Thus, it remains unclear whether IGRA can be used to monitor success of treatment and what conversions and/or reversions signify (18).

During a large-scale contact investigation in 2005, more than 400 BCG unvaccinated supermarket customers who had been exposed to a highly contagious employee had a positive TST result (19;20). They were offered preventive treatment, consisting of six months of isoniazid (INH) therapy or, in persons declining treatment, two years radiographic follow-up as an alternative. In the present study, we aimed to follow patients who were diagnosed with LTBI (TST ≥ 15 mm) in order to compare the effect of INH treatment as well as the effect of time on IGRA results. Because most subjects had also participated in the study that took place during the large scale contact investigation in 2005, pretreatment IGRA results of these individuals were available (19).

SUBJECTS AND METHODS

Study design

Individuals with a positive TST (defined as ≥ 15mm as explained in (19)) during the large scale contact investigation in February 2005 were invited to participate in this study. Blood was drawn half-yearly for two years starting September 2005 and ending April 2007. Time points are referred to as 0M (time of the previous study during the contact investigation), 6M (6 months later at the start of the

present follow-up study), 12M, 18M and 24M (figure 1). Participants completed a questionnaire regarding frequency of visits to the supermarket and potential exposure to NTM.

The Ethical Review Board of the Leiden University Medical Center approved the study protocol (Protocol number P05.53). All participants provided informed consent.

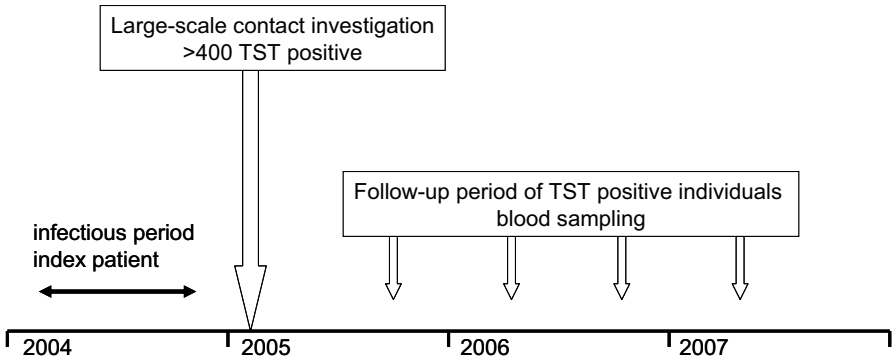

Figure 1. Time schedule
Time line of events of this study.

Procedures

Blood was drawn at the Municipal Health Authority and transported to Diakonessenhuis in Utrecht for T-SPOT.*TB* and to Leiden University Medical Center for QFT-GIT. Assays were performed following the manufacturers instructions as described in the previous study (19).

Statistics

The agreement and disagreement between assays were investigated with kappa statistics and McNemar's test, respectively. ANOVA for repeated measurements was used to determine whether individual IGRA responses varied over time. Paired T-Test was used to compare results in the T-SPOT.*TB* panels for 6M and 24M. Two-sided P values <0.05 were considered statistically significant.

RESULTS

Study population

In total, 122 individuals participated in this study. Preventive INH treatment was completed by 36/122 (29.5%) individuals, 71/122 (58.2%) were followed during 2 years by X-ray and 15/122 (12.3%) had started INH treatment but stopped prematurely because of side effects. When comparing the cumulative shopping time, as measure of exposure to the index patient, there were no significant differences between INH treated individuals and those with radiographic follow-up (P=0.44). Table 1 shows the characteristics of individuals with or without INH treatment. As of September 2007, none of the participants of this study had developed active TB. The first blood sample was obtained either at 0M or at 6M. Seventy-eight (63.9%) individuals had participated at 0M (19). A total of 109 (89.3%) participants donated blood at 6M, 70 (57.4%) at 12M, 49 (40.2%) at 18M and 87 (71.3%) at 24M. Blood was obtained at all four follow-up time points 6M, 12M, 18M as well as 24M in 23 (18.9%) individuals, of whom 15 (12.3%) had also participated during the contact investigation (0M).

Table 1. Characteristics of the study population.

	INH		Follow-up
	completed	stopped	Chest radiography
	N=36	N=15	N=71
TST result (mm)	17.8 ± 3.9	19.1 ± 3.4	18.1 ± 5.1
Age (y)	36.9 ± 13.1	43.4 ± 11.8	47.2 ± 12.2
No. (%) of men	9 (25.0)	3 (20.0)	40 (56.8)
Cumulative shopping time (range in minutes)			
1-300	7 (21.2)	5 (38.5)	15 (27.7)
301-600	5 (15.2)	1 (7.7)	4 (7.4)
601-1200	9 (27.3)	1 (7.7)	14 (25.9)
1201-2400	10 (30.3)	6 (46.2)	14 (25.9)
> 2400	2 (6.1)	0 (0.0)	7 (13.0)

Abbreviations; TST: Tuberculin Skin Test.
Data are expressed as mean ± SD or No (%)

QuantiFERON-TB Gold in-tube

The overall percentage of individuals with a positive QFT-GIT result remained stable during the 2 years at around 45% (Table 2). Using ANOVA for repeated measurements for the 15 individuals with QFT-GIT results at all time points and for the 23 with results from 6M until 24M, no significant relationship between follow-up time and QFT-GIT responsiveness could be distinguished. Also, when applying McNemar's test comparing initial test result with the result at 24M, no significant difference was observed. Results were not different between subjects of whom a 0M value was available compared to those who were first included at 6M. All available individual QFT-GIT results are shown in Table 3.

When individual patterns of QFT-GIT results were evaluated, all possible variations were observed. However, common patterns were distinguished when the initial QFT-GIT result was divided into three categories. When the initial QFT-GIT result was low (< 0.25 IU/ml) the majority of follow-up results stayed below the cut-off value of 0.35 IU/ml. When the initial QFT-GIT result was > 4 IU/ml, results remained positive and well above the cut-off value. When the initial QFT-GIT result was ≥ 0.25 and ≤ 4 IU/ml more dynamic patterns were distinguished (Figure 2). Moreover, these patterns were similar for the two patient groups with INH and radiographic follow-up.

Table 2. IGRA results

	Assay	No. of positive results/ No. tested (%)			
		INH completed	INH stopped	Chest radiography	ALL
		N=36*	N=15*	N=71*	N=122*
0M	QFN	11/22 (50.0)	4/11 (36.4)	17/45 (37.8)	32/78 (41.0)
	TSPOT	12/22 (54.5)	5/10 (50.0)	21/45 (46.7)	38/77 (49.4)
6M	QFN	11/29 (37.9)	7/15 (46.7)	30/65 (46.2)	48/109 (44.0)
	TSPOT	17/29 (58.6)	7/15 (46.7)	32/65 (49.2)	56/109 (51.4)
12M	QFN	7/16 (43.8)	5/11 (45.4)	21/43 (48.8)	33/70 (47.1)
	TSPOT	9/16 (56.2)	6/11 (54.5)	20/42 (47.6)	35/69 (50.7)
24M	QFN	11/27 (40.7)	5/11 (45.4)	24/49 (49.0)	40/87 (46.0)
	TSPOT	13/27 (48.1)	4/9 (44.4)	23/48 (47.9)	40/84 (47.6)

*Abbreviations; M: months; QFN: Quantiferon-TB Gold in-tube; TSPOT: T-SPOT.*TB*; INH: isoniazid*

** Total number of participants in each group. The number of participants differs for each time point.*

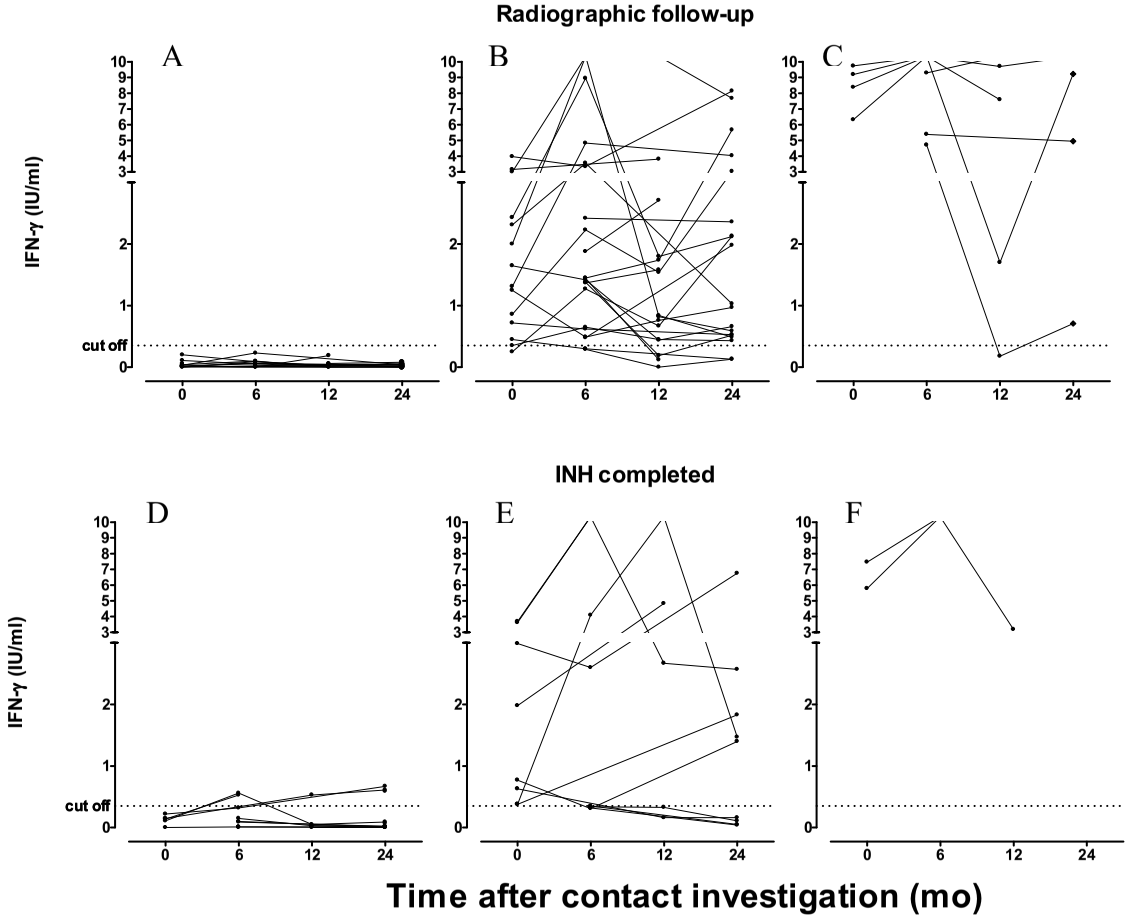

Figure 2. Kinetics of QFT-GIT results during radiographic follow-up or INH treatment

Number on X-axis indicates the number of months after the initial contact investigation in February 2005. The figures show the time course of QFT-GIT results in IU of IFN-γ/ml in subjects during radiographic follow-up and INH treatment whose initial QFT-GIT result was < 0.25 IU/ml (Figure 2A respectively 2D); ≥ 0.25 and ≤ 4.0 IU/ml (Figure 2B; respectively 2E) or > 4.0 IU/ml (Figure 2C respectively 2F). QFT-GIT values exceeding 10 IU/mL are shown as 10 IU/mL.

Table 3. Individual QuantiFERON TB Gold in-tube and T-SPOT.*TB* results in all study participants.

No.	Assay	Cat. *	0 M		6 M		12 M		24 M	
1	QFT-GIT-GIT	CN	Neg	-0.01			Neg	0.00	Neg	0.00
	T-SPOT.*TB*	CN	Neg	1			Neg	0	Neg	2
2	QFT-GIT-GIT	CN	Neg	-0.01	Neg	0.00	Neg	-0.02	Neg	0.00
	T-SPOT.*TB*	CN	Neg	5	Neg	1	Neg	0	Neg	0
3	QFT-GIT-GIT	Rev			Pos	1.44	Neg	0.12		
	T-SPOT.*TB*	CP			Pos	30	Pos	11		
4	QFT-GIT-GIT	CP	Pos	2.99	Pos	2.60			Pos	6.75
	T-SPOT.*TB*	CP	Pos	25	Pos	40			Pos	13
5	QFT-GIT-GIT	CN	Neg	-0.18			Neg	0.02		
	T-SPOT.*TB*	CN	Neg	2			Neg	1		
6	QFT-GIT-GIT	Rev	Pos	0.63			Neg	0.16	Neg	0.16
	T-SPOT.*TB*	Rev	Pos	9			Pos	8	Neg	2
7	QFT-GIT-GIT	Conv	Neg	0.15			Pos	0.53	Pos	0.61
	T-SPOT.*TB*	Conv	Neg	1			Pos	8	Pos	6
8	QFT-GIT-GIT	CN	Neg	-0.01	Neg	0.00				
	T-SPOT.*TB*	CN	Neg	0	Neg	0				
9	QFT-GIT-GIT	CN			Neg	-0.03	Neg	0.04	Neg	0.05
	T-SPOT.*TB*	CN			Neg	0	Neg	0	Neg	2
10	QFT-GIT-GIT	CN	Neg	0.00	Neg	0.01			Neg	-0.01
	T-SPOT.*TB*	CN	Neg	1	Neg	3			Neg	0
11	QFT-GIT-GIT	Conv	Neg	0.22	Neg	0.32			Pos	0.67
	T-SPOT.*TB*	CP	Pos	27	Pos	38			Pos	29
12	QFT-GIT-GIT	CP	Pos	0.38					Pos	1.83
	T-SPOT.*TB*	CP	Pos	16					Pos	42
13	QFT-GIT-GIT	No FU					Pos	4.71		
	T-SPOT.*TB*	No FU					Pos	59		
14	QFT-GIT-GIT	CN	Neg	0.11	Neg	0.05			Neg	0.04
	T-SPOT.*TB*	Rev	Pos	12	Neg	4			Neg	3
15	QFT-GIT-GIT	CP	Pos	3.16			Pos	3.81		
	T-SPOT.*TB*	Rev	Pos	67			Neg	2		
16	QFT-GIT-GIT	CP	Pos	0.72	Pos	0.62			Pos	0.53
	T-SPOT.*TB*	CP	Pos	13	Pos	50				
17	QFT-GIT-GIT	CP	Pos	5.78	Pos	10	Pos	10	Pos	10
	T-SPOT.*TB*	CP	Pos	162	Pos	72	Pos	46	Pos	56
18	QFT-GIT-GIT	CN	Neg	0.03	Neg	0.23			Neg	0.04
	T-SPOT.*TB*	CN	Neg	1	Neg	0			Neg	1
19	QFT-GIT-GIT	CP	Pos	1.98			Pos	4.83		
	T-SPOT.*TB*	CP	Pos	69			Pos	56		
20	QFT-GIT-GIT	CP	Pos	10	Pos	10	Pos	10	Pos	10
	T-SPOT.*TB*	CP	Pos	95	Pos	15	Pos	44	Pos	100
21	QFT-GIT-GIT	CP			Pos	10	Pos	10	Pos	10
	T-SPOT.*TB*	CP			Pos	63	Pos	75	Pos	100
22	QFT-GIT-GIT	CP					Pos	0.84	Pos	0.49
	T-SPOT.*TB*	CP					Pos	27	Pos	24
23**	QFT-GIT-GIT	CN					Neg	-0.01		
	T-SPOT.*TB*	CN					Neg	0		
24	QFT-GIT-GIT	CN	Neg	0.01	Neg	0.00				
	T-SPOT.*TB*	CN	Neg	1	Neg	0				
25	QFT-GIT-GIT	Conv	Neg	0.25	Pos	1.27	Pos	0.67	Pos	2.13
	T-SPOT.*TB*	CP	Pos	28	Pos	28	Pos	21	Pos	22
26**	QFT-GIT-GIT	CN					Neg	0.04		
	T-SPOT.*TB*	Conv					Neg	1		
27	QFT-GIT-GIT	CN	Neg	-0.03	Neg	0.02	Neg	0.02	Neg	-0.01
	T-SPOT.*TB*	Rev	Pos	16	Neg	0	Neg	2	Neg	0
28**	QFT-GIT-GIT	CN			Neg	-0.03				
	T-SPOT.*TB*	CN			Neg	0				
29	QFT-GIT-GIT	CP	Pos	1.65	Pos	1.42	Pos	0.44	Pos	0.66
	T-SPOT.*TB*	CP	Pos	20	Pos	40	Pos	7	Pos	46
30	QFT-GIT-GIT	CN			Neg	0.01			Neg	0.00
	T-SPOT.*TB*	CN			Neg	0			Neg	0

No.	Assay	Cat.	0 M		6 M		12 M		24 M	
31	QFT-GIT-GIT	CN	Neg	-0.02	Neg	-0.01	Neg	-0.01	Neg	0.01
	T-SPOT.*TB*	CN	Neg	0	Neg	0	Neg	0	Neg	2
32	QFT-GIT-GIT	CP			Pos	1.45	Pos	1.74	Pos	5.67
	T-SPOT.*TB*	Var			Pos	45	Neg	3	Pos	60
33	QFT-GIT-GIT	CN	Neg	0.04	Neg	0.10	Neg	0.00	Neg	-0.06
	T-SPOT.*TB*	CN	Neg	0	Neg	0	Neg	0	Neg	1
34	QFT-GIT-GIT	CN	Neg	-0.09	Neg	-0.04			Neg	0.01
	T-SPOT.*TB*	CN	Neg	0	Neg	0			Neg	0
35	QFT-GIT-GIT	CP			Pos	10	Pos	10		
	T-SPOT.*TB*	CP			Pos	100	Pos	100		
36	QFT-GIT-GIT	CN	Neg	-0.01	Neg	0.01			Neg	0.01
	T-SPOT.*TB*	CN	Neg	0	Neg	0			Neg	0
37	QFT-GIT-GIT	Rev	Pos	0.45	Neg	0.30			Neg	0.13
	T-SPOT.*TB*	Var	Pos	14	Neg	2			Pos	9
38	QFT-GIT-GIT	CP	Pos	1.25	Pos	0.49	Pos	0.76	Pos	0.97
	T-SPOT.*TB*	CP	Pos	19	Pos	26	Pos	19	Pos	24
39	QFT-GIT-GIT	CP			Pos	2.42			Pos	2.36
	T-SPOT.*TB*	CP			Pos	39			Pos	16
40	QFT-GIT-GIT	CN	Neg	0.01	Neg	0.06			Neg	0.03
	T-SPOT.*TB*	Rev	Pos	13	Pos	14			Neg	2
41	QFT-GIT-GIT	CP	Pos	2.43	Pos	8.95	Pos	1.80	Pos	2.12
	T-SPOT.*TB*	Conv	Neg	0	Pos	63	Pos	16	Pos	22
42	QFT-GIT-GIT	CN	Neg	-0.01	Neg	-0.02	Neg	0.00	Neg	0.01
	T-SPOT.*TB*	CN	Neg	0	Neg	0	Neg	0	Neg	0
43	QFT-GIT-GIT	CN			Neg	-0.02			Neg	0.03
	T-SPOT.*TB*	CN			Neg	0			Neg	0
44	QFT-GIT-GIT	CP	Pos	0.86	Pos	2.23	Pos	1.54	Pos	3.03
	T-SPOT.*TB*	CP	Pos	33	Pos	42	Pos	40	Pos	21
45	QFT-GIT-GIT	CP	Pos	3.69	Pos	10	Pos	2.67	Pos	2.57
	T-SPOT.*TB*	CP	Pos	58	Pos	47	Pos	14	Pos	47
46	QFT-GIT-GIT	CP	Pos	9.73	Pos	10				
	T-SPOT.*TB*	CP	Pos	42	Pos	83				
47	QFT-GIT-GIT	Var	Neg	0.11	Pos	0.56	Neg	0.05	Neg	0.09
	T-SPOT.*TB*	Var	Neg	5	Pos	9	Neg	5	Pos	7
48	QFT-GIT-GIT	CN	Neg	0.02	Neg	0.06	Neg	0.02	Neg	0.05
	T-SPOT.*TB*	CN	Neg	1	Neg	0	Neg	0	Neg	0
49	QFT-GIT-GIT	CP			Pos	10	Pos	10	Pos	10
	T-SPOT.*TB*	CP			Pos	100	Pos	92	Pos	100
50	QFT-GIT-GIT	CN	Neg	-0.06	Neg	0.01			Neg	0.02
	T-SPOT.*TB*	CN	Neg	1	Neg	0			Neg	-2
51**	QFT-GIT-GIT	CN			Neg	-0.01				
	T-SPOT.*TB*	CN			Neg	0				
52	QFT-GIT-GIT	CN	Neg	0.01	Neg	0	Neg	0.19		
	T-SPOT.*TB*	CN	Neg	0	Neg	0	Neg	-1		
53	QFT-GIT-GIT	CN			Neg	-0.03			Neg	0.00
	T-SPOT.*TB*	CN			Neg	0			Neg	0
54	QFT-GIT-GIT	CN			Neg	0.15	Neg	0.03	Neg	0.02
	T-SPOT.*TB*	CP			Pos	19	Pos	17	Pos	16
55	QFT-GIT-GIT	CN			Neg	-0.30			Neg	-0.01
	T-SPOT.*TB*	CN			Neg	0			Neg	0
56	QFT-GIT-GIT	CN	Neg	-0.02	Neg	-0.04	Neg	0.02		
	T-SPOT.*TB*	Conv	Neg	1	Neg	0	Pos	10		
57	QFT-GIT-GIT	CN	Neg	-0.01	Neg	-0.01	Neg	0.03	Neg	0.01
	T-SPOT.*TB*	CN	Neg	0	Neg	0	Neg	0	Neg	-1
58	QFT-GIT-GIT	CP	Pos	0.38	Pos	4.09	Pos	10	Pos	1.47
	T-SPOT.*TB*	CP	Pos	110	Pos	60	Pos	31	Pos	41
59	QFT-GIT-GIT	CP			Pos	1.88	Pos	2.71		
	T-SPOT.*TB*	CP			Pos	65	Pos	7		
60	QFT-GIT-GIT	CP	Pos	3.98	Pos	3.34			Pos	8.14
	T-SPOT.*TB*	CP	Pos	32	Pos	72			Pos	55

Table continued

No.	Assay	Cat.	0 M		6 M		12 M		24 M	
61	QFT-GIT-GIT	CP	Pos	7.46	Pos	10	Pos	3.19		
	T-SPOT.*TB*	CP	Pos	13	Pos	72	Pos	32		
62	QFT-GIT-GIT	Rev			Pos	0.35			Neg	0.05
	T-SPOT.*TB*	CN			Neg	4			Neg	0
63	QFT-GIT-GIT	CP	Pos	0.35	Pos	0.65	Pos	0.45	Pos	0.43
	T-SPOT.*TB*	Var	Pos	16	Neg	3	Pos	11	Neg	3
64	QFT-GIT-GIT	CP			Pos	4.95	Pos	2.64		
	T-SPOT.*TB*	CP			Pos	42	Pos	19		
65	QFT-GIT-GIT	CN			Neg	-0.01	Neg	-0.02		
	T-SPOT.*TB*	CN			Neg	0	Neg	2		
66	QFT-GIT-GIT	CN			Neg	0.29	Neg	0	Neg	0.13
	T-SPOT.*TB*	Var			Neg	1	Pos	7	Neg	3
67	QFT-GIT-GIT	Var			Pos	4.71	Neg	0.18	Pos	0.71
	T-SPOT.*TB*	CP			Pos	15	Pos	24	Pos	36
68	QFT-GIT-GIT	CP			Pos	8.28	Pos	6.42		
	T-SPOT.*TB*	CP			Pos	98	Pos	50		
69	QFT-GIT-GIT	CP	Pos	10	Pos	5.35			Pos	10
	T-SPOT.*TB*	CP	Pos	87	Pos	79				
70	QFT-GIT-GIT	CN	Neg	-0.02	Neg	-0.02	Neg	0.00		
	T-SPOT.*TB*	CN	Neg	6	Neg	-5	Neg	-1		
71	QFT-GIT-GIT	CP			Pos	0.48			Pos	1.98
	T-SPOT.*TB*	No FU			Pos	65				
72	QFT-GIT-GIT	CN	Neg	-0.04	Neg	-0.07	Neg	-0.01	Neg	0.01
	T-SPOT.*TB*	CN	Neg	2	Neg	2	Neg	-1	Neg	0
73	QFT-GIT-GIT	CN			Neg	0.34	Neg	0.33	Neg	0.11
	T-SPOT.*TB*	Rev			Pos	25	Neg	0	Neg	2
74	QFT-GIT-GIT	No FU			Neg	0.12				
	T-SPOT.*TB*	No FU			Pos	7				
75	QFT-GIT-GIT	CN	Neg	0.13	Neg	-0.23	Neg	-0.03	Neg	-0.01
	T-SPOT.*TB*	CN	Neg	4	Neg	1	Neg	-1	Neg	1
76	QFT-GIT-GIT	Conv			Neg	-0.21			Pos	0.59
	T-SPOT.*TB*	CN			Neg	-1			Neg	1
77	QFT-GIT-GIT	CN			Neg	-0.03			Neg	0.07
	T-SPOT.*TB*	CN			Neg	2			Neg	1
78	QFT-GIT-GIT	CN			Neg	0.10			Neg	0.01
	T-SPOT.*TB*	CN			Neg	0			Neg	0
79	QFT-GIT-GIT	CN			Neg	0.32			Neg	0.04
	T-SPOT.*TB*	CN			Neg	4			Neg	2
80	QFT-GIT-GIT	CN			Neg	-0.03	Neg	0.06	Neg	0.08
	T-SPOT.*TB*	Var			Pos	26	Neg	0	Pos	23
81	QFT-GIT-GIT	CN	Neg	-0.01	Neg	-0.02	Neg	0.03	Neg	0.00
	T-SPOT.*TB*	Var	Neg	5	Pos	13	Neg	1	Neg	5
82	QFT-GIT-GIT	CN			Neg	0.09			Neg	0.02
	T-SPOT.*TB*	CP			Pos	10			Pos	9
83	QFT-GIT-GIT	Conv	Neg	0.14	Pos	0.53				
	T-SPOT.*TB*	Conv	Neg	5	Pos	15				
84	QFT-GIT-GIT	CP	Pos	2.31	Pos	3.56			Pos	1.03
	T-SPOT.*TB*	CP	Pos	28	Pos	74			Pos	100
85	QFT-GIT-GIT	CN	Neg	0.01	Neg	-0.07				
	T-SPOT.*TB*	CN	Neg	0	Neg	0				
86	QFT-GIT-GIT	CP	Pos	8.38	Pos	10	Pos	9.7	Pos	10
	T-SPOT.*TB*	Var	Pos	69	Pos	88	Neg	5	Pos	56
87	QFT-GIT-GIT	CN	Neg	0.02	Neg	-0.15			Neg	-0.01
	T-SPOT.*TB*	CN	Neg	1	Neg	0			Neg	1
88	QFT-GIT-GIT	CP	Pos	3.62	Pos	10				
	T-SPOT.*TB*	CP	Pos	68	Pos	78				
89	QFT-GIT-GIT	Conv	Neg	-0.08	Neg	0.19	Neg	0.24	Pos	0.42
	T-SPOT.*TB*	Var			Neg	5	Pos	6	Neg	5
90	QFT-GIT-GIT	CN	Neg	0.00	Neg	0.09	Neg	0.02	Neg	0.05
	T-SPOT.*TB*	Rev	Pos	10	Neg	1	Neg	0	Neg	3
91	QFT-GIT-GIT	CP			Pos	10	Pos	1.70	Pos	9.22
	T-SPOT.*TB*	CP			Pos	41	Pos	14	Pos	38

No.	Assay	Cat.	0 M		6 M		12 M		24 M	
92	QFT-GIT-GIT	CN	Neg	0.06	Neg	-0.03				
	T-SPOT.*TB*	CN	Neg	1	Neg	1				
93	QFT-GIT-GIT	CN	Neg	-0.01	Neg	0.02				
	T-SPOT.*TB*	CN	Neg	0	Neg	0				
94	QFT-GIT-GIT	CN	Neg	0.02	Neg	-0.04			Neg	0.01
	T-SPOT.*TB*	CN	Neg	0	Neg	0			Neg	0
95	QFT-GIT-GIT	CN	Neg	-0.02	Neg	-0.03	Neg	-0.01		
	T-SPOT.*TB*	Rev	Pos	6	Neg	1	Neg	0		
96	QFT-GIT-GIT	CP	Pos	9.19	Pos	10				
	T-SPOT.*TB*	Conv	Neg	0	Pos	86				
97	QFT-GIT-GIT	CN			Neg	-0.01			Neg	0.00
	T-SPOT.*TB*	CN			Neg	4			Neg	0
98	QFT-GIT-GIT	CN	Neg	-0.01	Neg	-0.02				
	T-SPOT.*TB*	Conv	Neg	1	Pos	8				
99	QFT-GIT-GIT	CP			Pos	1.37	Pos	1.58		
	T-SPOT.*TB*	Rev			Pos	27	Neg	1		
100	QFT-GIT-GIT	CN	Neg	0.20	Neg	0.09	Neg	0.02		
	T-SPOT.*TB*	Conv	Neg	1	Neg	4	Pos	8		
101	QFT-GIT-GIT	CP	Pos	3.01	Pos	10	Pos	0.82	Pos	0.59
	T-SPOT.*TB*	CP	Pos	48	Pos	39	Pos	13	Pos	16
102	QFT-GIT-GIT	CN	Neg	-0.02	Neg	-0.04	Neg	0.05	Neg	0.01
	T-SPOT.*TB*	CN	Neg	0	Neg	1	Neg	-2	Neg	0
103	QFT-GIT-GIT	Var			Pos	1.38	Neg	0.19	Pos	0.52
	T-SPOT.*TB*	Rev			Pos	63	Neg	0	Neg	1
104	QFT-GIT-GIT	CN	Neg	0.01	Neg	0.01	Neg	-0.05	Neg	0.00
	T-SPOT.*TB*	CN	Neg	1	Neg	0	Neg	0	Neg	0
105	QFT-GIT-GIT	CN	Neg	-0.01	Neg	-0.01				
	T-SPOT.*TB*	CN	Neg	1	Neg	1				
106	QFT-GIT-GIT	CN			Neg	-0.03			Neg	-0.01
	T-SPOT.*TB*	CN			Neg	1			Neg	0
107	QFT-GIT-GIT	CP	Pos	2.00	Pos	10	Pos	10	Pos	7.67
	T-SPOT.*TB*	CP	Pos	50	Pos	87			Pos	98
108	QFT-GIT-GIT	CP	Pos	7.82	Pos	8.53			Pos	4.46
	T-SPOT.*TB*	CP	Pos	18	Pos	88			Pos	34
109	QFT-GIT-GIT	CN	Neg	-0.01	Neg	0.08	Neg	0.00		
	T-SPOT.*TB*	CN	Neg	3	Neg	2	Neg	0		
110	QFT-GIT-GIT	CP	Pos	1.31	Pos	4.83			Pos	4.04
	T-SPOT.*TB*	CP	Pos	43	Pos	44			Pos	18
111	QFT-GIT-GIT	CP			Pos	5.39			Pos	4.95
	T-SPOT.*TB*	CP			Pos	78			Pos	92
112	QFT-GIT-GIT	CP	Pos	10	Pos	10	Pos	7.37	Pos	10
	T-SPOT.*TB*	CP	Pos	19	Pos	100	Pos	83	Pos	99
113	QFT-GIT-GIT	CN	Neg	0.06	Neg	0.08	Neg	0.00	Neg	0.09
	T-SPOT.*TB*	Rev	Pos	7	Pos	11	Neg	4		
114	QFT-GIT-GIT	Rev			Pos	0.95	Pos	0.63	Neg	0.07
	T-SPOT.*TB*	Conv			Neg	4	Pos	22	Pos	23
115	QFT-GIT-GIT	CP	Pos	6.32	Pos	10	Pos	7.59		
	T-SPOT.*TB*	Rev	Pos	18	Pos	94	Neg	5		
116	QFT-GIT-GIT	CN	Neg	0.00	Neg	0.02	Neg	0.04	Neg	0.00
	T-SPOT.*TB*	CN	Neg	1	Neg	0	Neg	0	Neg	0
117	QFT-GIT-GIT	CP	Pos	0.37	Pos	3.60	Pos	0.85	Pos	1.44
	T-SPOT.*TB*	CP	Pos	18	Pos	96	Pos	19	Pos	26
118	QFT-GIT-GIT	CP			Pos	9.28	Pos	10	Pos	10
	T-SPOT.*TB*	CP			Pos	64	Pos	56	Pos	50
119	QFT-GIT-GIT	CP			Pos	6.16			Pos	3.64
	T-SPOT.*TB*	CP			Pos	63			Pos	18
120	QFT-GIT-GIT	Var	Pos	0.77	Neg	0.31			Pos	1.40
	T-SPOT.*TB*	CP	Pos	18	Pos	21			Pos	13
121**	QFT-GIT-GIT	CN							Neg	-0.01
	T-SPOT.*TB*	CN							Neg	0
122	QFT-GIT-GIT	CN	Neg	-0.01					Neg	-0.01
	T-SPOT.*TB*	CN	Neg	5					Neg	0

Legend table 3
** Cat = category of kinetics of QFT-GIT and T-SPOT.TB results. Conversions were defined as a change from negative to positive according the manufacturer's instructions (cut-off for QFT-GIT at 0.35 IU/ml; cut-off for T-SPOT.TB at 6 spots). Reversions were defined as change from positive to negative. The variable category was appointed when conversion or reversion was not consistent.*
CN: consistent negative
CP: consistent positive
Conv: conversion
Rev: reversion
Var: variable
No FU: missing follow-up data
*** These individuals had a follow-up visit after 18M.*

T-SPOT.TB

The overall percentage of positive T-SPOT.*TB* results remained unchanged during follow-up (Table 2). Similar to QFT-GIT, no significant change in T-SPOT.*TB* results was observed among individuals with T-SPOT.*TB* results at all time points or among subjects with results from 6M until 24M (ANOVA for repeated measurements or McNemar's test). All available individual T-SPOT.*TB* results are shown in Table 3.

The three patterns that were observed for QFT-GIT were also found for T-SPOT.*TB* (Figure 3). Most individuals with low spot counts in the initial T-SPOT.*TB* (maximum spot count < 5) remained negative during the entire follow-up period (N=24/33), only 9/33 converted to a positive result at any later time point. If the first T-SPOT.*TB* result was high (maximum spot count exceeding 50 spots) the assay remained positive during the entire follow-up period in 8/10 subjects, although spot counts were usually lower at the last follow-up time point. Just 2/10 initially high-responding individuals reverted to a negative result at any later time point. Of 35 individuals with an initial T-SPOT.*TB* score between 5 and 50 spots, most had a stable number of responding T cells during the follow-up period, although there were some individuals with more dynamic patterns. Just as was observed for the QFT-GIT, no influence of INH treatment could be distinguished.

Next, responses to the different antigens of the T-SPOT.*TB* assay were analyzed. Of individuals with an initially positive T-SPOT.*TB* result, the majority (31/38) responded positively to both antigens and remained positive for both during follow-up. The response to panel A decreased significantly between 6M and 24M, (P=0.015), whereas the decrease in responses to panel B was of borderline significance (P=0.051).

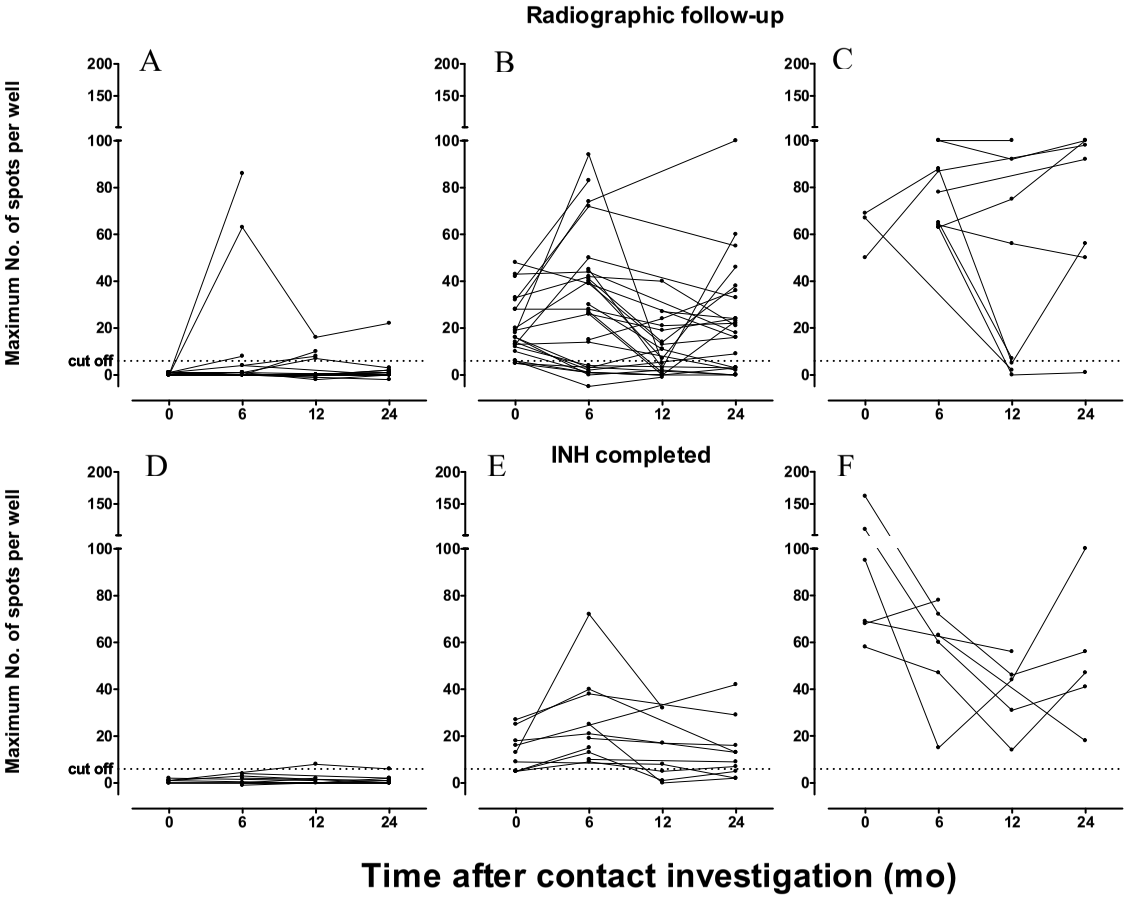

Figure 3. Kinetics of T-SPOT.TB results during radiographic follow-up or INH treatment
Number on X-axis indicates the number of months after the initial contact investigation in February 2005. The figures show the time course of T-SPOT.TB results expressed as the maximum spot count. The course of quantitative T-SPOT.TB results is shown for subjects during radiographic follow-up or INH treatment, whose initial response was < 5 spots (Figure 3A respectively 3D) ≥5 and >50 (Figure 3B respectively 3E) or ≥ 50 spots (Figure 3C respectively 3F).

Agreement between T.SPOT-TB and QuantiFERON-TB Gold

There was a strong correlation between T-SPOT.*TB* and QFT-GIT results at all different time points, with Cohen's kappa values between 0.7 and 0.8, which was independent of the time point and of preventive treatment. There was a positive correlation between the number of spots in the T-SPOT.*TB* assay and the ^{10}log of concentration of IFN-γ produced in the QFT-GIT (R=0.6, P <0.001). This was the case for panel A and panel B separately, but also when the individual maximum number of spots was analyzed.

DISCUSSION

This study showed three different kinetic patterns of IGRA response. T-SPOT.*TB* and QFT-GIT remained positive during at least the follow-up in 92% resp. 90% of the individuals with an initially strongly positive QFT-GIT or T-SPOT.*TB* result, irrespective of whether they had completed preventive INH treatment. This finding is in accordance with other studies reporting that responses remain positive in a proportion of treated individuals (4;21-23). Moreover, initially very low or very high responses mostly remained in the same range over the two-year follow-up period, with or without INH preventive treatment. Thus, overall IGRA appear to be of little clinical value for follow-up.

T-SPOT.*TB* was more frequently positive than QFT-GIT at all investigated time points. The cause of this difference is not known, but it has been suggested that T-SPOT.*TB* is more sensitive than the QFT-GIT for detection of TB infection (6;24). Alternatively, T-SPOT.*TB* could be more sensitive to detect infection that was not recently acquired, as most studies included populations with undocumented exposure history. The present study most likely included individuals with a positive TST due to past infection, because they were identified during mass screening. In the absence of a gold standard for the presence of LTBI, the only method to proof superiority would require follow-up of untreated TST positive persons with a positive or negative IGRA result, as is currently under way in several clinical-epidemiological settings (25;26).

An interesting observation was the strong correlation between the number of spots in the T-SPOT.*TB* and the log of the IFN-γ concentration produced in the QFT-GIT, for both antigens and at a low as well as high number of spots. This implies that cells responding to ESAT-6 or CFP-10 do not differ at a qualitative level. Since a linear increase of spots appears to be responsible for an exponential

increase in the concentration of IFN-γ, reflecting total production, this suggests a strong dependence of QFT-GIT result on the number of cells (PBMC's) present in the volume of whole blood used for QFT-GIT and may explain part of observed discrepancies between the two different IGRA formats. Participants of this study were all healthy, immuno-competent individuals and therefore it is unlikely that low cell counts had led to false negative results. However, in other settings with immuno-compromised patients or children, this could be problematic and false-negative or indeterminate results might be obtained (8).

Our study shows that it is possible to characterize different kinetic patterns of responses varying by the initial response. IGRA responses that were either negative or very high at the first measurement most often remained in the same category of responses during the entire follow-up period. In contrast, initially intermediate results were more variable over time and both conversions and reversions were observed, even within the same individual. The clinical significance of these patterns is unclear, as the positive or negative predictive value of IGRA results for later TB reactivation is as yet unproven. In that regard, the group with initially intermediate responses seems the most interesting category because the changes of responses suggest an ongoing dynamic interaction between host and pathogen. Follow-up of sufficiently large numbers of individuals in each category in a low prevalence TB setting will provide more definitive information with regard to the relation between IGRA kinetics and risk of progression to TB disease, which would provide a rational basis for therapeutic consequences.

All individuals included in this study had a TST induration of at least 15 mm, yet half of them were IGRA negative and remained negative during follow-up. Since none of these individuals has developed TB there are three possible explanations. The first option of false-positive TST results caused by NTM or undetected BCG vaccination was unlikely as TST results generally do not exceed 15 mm in that setting. Second, Hill et al showed that rapid ELISPOT reversion can occur after TB exposure. Because the infectious period of the index patient lasted from February until October 2004 while we first tested in February 2005, it is possible that participants with a positive TST and negative IGRA had already reverted to IGRA negative (27). Thirdly, since these individuals were detected during large scale contact screening the TST could be positive due to old infection in associated with a reverted IGRA result.

Before concluding that the added value of follow-up IGRA to guide clinical decisions may be limited, some potential pitfalls need to be taken into consideration. Firstly, data on all time points were available for a limited number of subjects, which was the result of the design and the voluntary nature of the study, precluding robust statistical analysis. Yet, the observed patterns were consistent and the agreement between the QFT-GIT and T-SPOT.*TB* was good at all time points with κ of 0.7, independent of preventive INH treatment, which is in agreement with previous comparative studies (6;19;24). Secondly, very few studies have addressed the issue of inter-assay variability and reproducibility of IGRA. The studies reported so far have analyzed the inter-assay variability of the QFT-GIT within a short period of time, i.e., with repeated measurements over a maximum interval of 3 months in a population not recently exposed to TB (28;29). These studies indicate that variability between measurements (i.e., positive or negative) of about 16% can be expected. In our study data were collected over a much longer period in a population recently exposed to TB, and consistent changes over time may reflect a change in immune reactivity rather than experimental variability of IGRA. Another factor of consideration with regard to repeated measurements is regression to the mean: when very high or low results are obtained first, on subsequent testing it is expected that such outliers are closer to the group average. This can be a cause for variations in test outcome but is without clinical significance. Taking the above into consideration together with the current lack of understanding the normal kinetics/variability of IGRA, it was not possible to differentiate between test variability and true conversions and/or reversions.

Based on the data of our study, IGRA do not seem to be useful during follow-up of patients with LTBI to decide when to stop treatment or identify those at risk of reactivation. The moderate effect of INH treatment on the percentage of positive IGRA responses cannot be regarded as relevant at the individual level for the determination of treatment success. In that regard, our findings were different from those of a follow-up study of patients with TB disease describing a consistent conversion to negative results at the end of successful treatment (30), but in part similar to those of another follow-up study using ELISPOT in recently exposed subjects that observed a decrease of responses among treated but not among untreated subjects (31). Based on our data, repeated testing in individuals with initially negative or very high responses will not yield additional information. Repeating IGRA in individuals with initially intermediate responses might provide useful information with regard to progression to TB disease. In animal models there was a clear correlation between high ESAT-6 responses and subsequent development of TB disease, as was the case in two human studies (25;32-35).

Because a number of individuals with initially negative results just below the cut-off (>0.25 and <0.35 IU/ML) had dynamic responses including conversions to positive, it could be useful to repeat the assay in these cases e.g. six months to one year later.

Acknowledgements
The authors wish to thank all the participants and the staff of the Municipal Health Authority in Utrecht.

REFERENCE LIST

(1) Franken WP, Koster BF, Bossink AW, Thijsen SF, Bouwman JJ, van Dissel JT et al. Follow-up study of tuberculosis-exposed supermarket customers with negative tuberculin skin test results in association with positive gamma interferon release assay results. Clin Vaccine Immunol 2007; 14(9):1239-1241.

(2) Franken WP, Timmermans JF, Prins C, Slootman EJ, Dreverman J, Bruins H et al. Comparison of Mantoux and QuantiFERON TB Gold tests for diagnosis of latent tuberculosis infection in Army personnel. Clin Vaccine Immunol 2007; 14(4):477-480.

(3) Pai M, Joshi R, Dogra S, Mendiratta DK, Narang P, Kalantri S et al. Serial testing of health care workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med 2006; 174(3):349-355.

(4) Pai M, Joshi R, Dogra S, Mendiratta DK, Narang P, Dheda K et al. Persistently elevated T cell interferon-gamma responses after treatment for latent tuberculosis infection among health care workers in India: a preliminary report. J Occup Med Toxicol 2006; 1(1):7.

(5) Pai M, Gokhale K, Joshi R, Dogra S, Kalantri S, Mendiratta DK et al. Mycobacterium tuberculosis infection in health care workers in rural India: comparison of a whole-blood interferon gamma assay with tuberculin skin testing. JAMA 2005; 293(22):2746-2755.

(6) Lee JY, Choi HJ, Park IN, Hong SB, Oh YM, Lim CM et al. Comparison of two commercial interferon-gamma assays for diagnosing Mycobacterium tuberculosis infection. Eur Respir J 2006; 28(1):24-30.

(7) Kang YA, Lee HW, Yoon HI, Cho B, Han SK, Shim YS et al. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005; 293(22):2756-2761.

(8) Ferrara G, Losi M, Meacci M, Meccugni B, Piro R, Roversi P et al. Routine hospital use of a new commercial whole blood interferon-gamma assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2005; 172(5):631-635.

(9) Diel R, Nienhaus A, Lange C, Meywald-Walter K, Forssbohm M, Schaberg T. Tuberculosis contact investigation with a new, specific blood test in a low-incidence population containing a high proportion of BCG-vaccinated persons. Respir Res 2006; 7(1):77.

(10) Pai M, Riley LW, Colford JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 2004; 4(12):761-776.

(11) Richeldi L. An update on the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2006; 174(7):736-742.

(12) Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. Expert Rev Mol Diagn 2006; 6(3):413-422.

(13) Dheda K, Udwadia ZF, Huggett JF, Johnson MA, Rook GA. Utility of the antigen-specific interferon-gamma assay for the management of tuberculosis. Curr Opin Pulm Med 2005; 11(3):195-202.

(14) Menzies D, Pai M, Comstock G. Meta-analysis: New Tests for the Diagnosis of Latent Tuberculosis Infection: Areas of Uncertainty and Recommendations for Research. Ann Intern Med 2007; 146(5):340-354.

(15) Lalvani A, Richeldi L, Kunst H. Interferon gamma assays for tuberculosis. Lancet Infect Dis 2005; 5(6):322-324.

(16) Rothel JS, Andersen P. Diagnosis of latent Mycobacterium tuberculosis infection: is the demise of the Mantoux test imminent? Expert Rev Anti Infect Ther 2005; 3(6):981-993.

(17) Pai M, Dheda K, Cunningham J, Scano F, O'Brien R. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. Lancet Infect Dis 2007; 7(6):428-438.

(18) Pai M, O'Brien R. Serial testing for tuberculosis: can we make sense of T cell assay conversions and reversions? PLoS Med 2007; 4(6):e208.

(19) Arend SM, Thijsen SF, Leyten EM, Bouwman JJ, Franken WP, Koster BF et al. Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. Am J Respir Crit Care Med 2007; 175(6):618-627.

(20) Borgen K, Koster B, Meijer H, Kuyvenhoven V, van der SM, Cobelens F. Evaluation of a large scale tuberculosis contact investigation in The Netherlands (2005). Eur Respir J 2008.

(21) Wu-Hsieh BA, Chen CK, Chang JH, Lai SY, Wu CH, Cheng WC et al. Long-lived immune response to early secretory antigenic target 6 in individuals who had recovered from tuberculosis. Clin Infect Dis 2001; 33(8):1336-1340.

(22) Dheda K, Pooran A, Pai M, Miller RF, Lesley K, Booth HL et al. Interpretation of Mycobacterium tuberculosis antigen-specific IFN-gamma release assays (T-SPOT.TB) and factors that may modulate test results. J Infect 2007; 55(2):169-173.

(23) Chee CB, Khinmar KW, Gan SH, Barkham TM, Pushparani M, Wang YT. Latent tuberculosis infection treatment and T-cell responses to Mycobacterium tuberculosis-specific antigens. Am J Respir Crit Care Med 2007; 175(3):282-287.

(24) Ferrara G, Losi M, D'Amico R, Roversi P, Piro R, Meacci M et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. Lancet 2006; 367(9519):1328-1334.

(25) Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Nienhaus A. Predictive Value of a Whole-blood IFN-{gamma} Assay for the Development of Active TB Disease. Am J Respir Crit Care Med 2008.

(26) Andersen P, Doherty TM, Pai M, Weldingh K. The prognosis of latent tuberculosis: can disease be predicted? Trends Mol Med 2007; 13(5):175-182.

(27) Hill PC, Brookes RH, Fox A, Jackson-Sillah D, Jeffries DJ, Lugos MD et al. Longitudinal assessment of an ELISPOT test for Mycobacterium tuberculosis infection. PLoS Med 2007; 4(6):e192.

(28) Veerapathran A, Joshi R, Goswami K, Dogra S, Moodie EE, Reddy MV et al. T-cell assays for tuberculosis infection: deriving cut-offs for conversions using reproducibility data. PLoS ONE 2008; 3(3):e1850.

(29) Perry S, Sanchez L, Yang S, Agarwal Z, Hurst P, Parsonnet J. Reproducibility of QuantiFERON-TB gold in-tube assay. Clin Vaccine Immunol 2008; 15(3):425-432.

(30) Carrara S, Vincenti D, Petrosillo N, Amicosante M, Girardi E, Goletti D. Use of a T cell-based assay for monitoring efficacy of antituberculosis therapy. Clin Infect Dis 2004; 38(5):754-756.

(31) Ewer K, Millington KA, Deeks JJ, Alvarez L, Bryant G, Lalvani A. Dynamic antigen-specific T-cell responses after point-source exposure to Mycobacterium tuberculosis. Am J Respir Crit Care Med 2006; 174(7):831-839.

(32) Doherty TM, Demissie A, Olobo J, Wolday D, Britton S, Eguale T et al. Immune responses to the Mycobacterium tuberculosis-specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. J Clin Microbiol 2002; 40(2):704-706.

(33) Dietrich J, Aagaard C, Leah R, Olsen AW, Stryhn A, Doherty TM et al. Exchanging ESAT6 with TB10.4 in an Ag85B fusion molecule-based tuberculosis subunit vaccine: efficient protection and ESAT6-based sensitive monitoring of vaccine efficacy. J Immunol 2005; 174(10):6332-6339.

(34) Buddle BM, Parlane NA, Keen DL, Aldwell FE, Pollock JM, Lightbody K et al. Differentiation between Mycobacterium bovis BCG-vaccinated and M. bovis-infected cattle by using recombinant mycobacterial antigens. Clin Diagn Lab Immunol 1999; 6(1):1-5.

(35) Vordermeier HM, Chambers MA, Cockle PJ, Whelan AO, Simmons J, Hewinson RG. Correlation of ESAT-6-specific gamma interferon production with pathology in cattle following Mycobacterium bovis BCG vaccination against experimental bovine tuberculosis. Infect Immun 2002; 70(6):3026-3032.