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

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COMPARISON OF TWO INTERFERON-G ASSAYS AND TUBERCULIN SKIN TEST FOR TRACING TUBERCULOSIS CONTACTS

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ABSTRACT

Background:

The tuberculin skin test (TST) has low specificity. QuantiFERON-TB Gold (QFT-G) and T-SPOT.TB are based on interferon (IFN)-γ responses to *Mycobacterium tuberculosis*–specific antigens. A novel in-tube format of QFT-G (QFT-GIT) offers logistical advantages.

Objective:

To compare TST, QFT-GIT, and T-SPOT.*TB* in bacillus Calmette-Gue´rin unvaccinated contacts and correlate results with measures of recent exposure.

Methods:

When a supermarket employee with smear-positive tuberculosis had infected most close contacts, a contact investigation among more than 20,000 customers was performed. We recruited subjects randomly on the day of TST administration (n = 469) and subjects with TST of more than 0 mm on the day of TST reading (n = 316). QFT-GIT and T-SPOT.*TB* were performed. Demographic data and measures of exposure were collected. TST results were analyzed at a cutoff of 10 or 15 mm. Blood tests were interpreted following the manufacturers' criteria and by varying cutoff levels.

Results:

Among 785 study participants, TST results were associated with age, whereas positive IFN-γ responses were significantly associated with cumulative shopping time, most markedly for QFT-GIT. Among participants with a TST of 15 mm or greater, sensitivity of QFT-GIT and T-SPOT.*TB* was 42.2 and 51.3%, respectively. Interassay agreement was 89.6% (κ = 0.59). By varying cutoff values, agreement between the IFN-γ assays was optimal at 93.6% (κ = 0.71) using a cutoff of 0.20 IU/ml for QFT-GIT and 13 spots for T-SPOT.TB.

Conclusions:

Blood test results were associated with exposure, whereas the TST was not. A possible lack of sensitivity of IFN-γ assays in detecting individuals with TST of 15mm or greater, despite negative bacillus Calmette-Gue´rin vaccination status, warrants further investigation into alternative cutoff values.

Keywords: contact tracing; ELISPOT; interferon-γ; latent tuberculosis infection; tuberculosis; tuberculin skin test

INTRODUCTION

Most cases of tuberculosis (TB) disease arise as reactivation TB in latently infected individuals. One-third of the world's population is believed to harbor latent TB infection (LTBI) (1). Approximately 5 to 15% of immunocompetent persons with LTBI will ever develop TB disease. In countries with a low incidence of TB, the tracing and targeted treatment of individuals with LTBI constitutes a major pillar of TB control (2, 3). Until recently, the detection of LTBI was based exclusively on tuberculin skin testing, which has low specificity after vaccination with bacillus Calmette-Gue´rin (BCG) or exposure to environmental mycobacteria, due to cross-reactive immune responses. The treatment of LTBI is effective when treatment is sustained (4), However, effectiveness tends to be decreased when compliance is low (5). These facts underscore the need for more accurate methods for detection of LTBI and targeting treatment.

The search for improved tools for detection of LTBI has led to the development of *in vitro* assays based on interferon (IFN)-γ production in response to enzyme early-secreted antigenic target 6-kDa protein (ESAT-6) and culture filtrate protein 10 (CFP-10), antigens that are highly specific for *Mycobacterium tuberculosis* (6–8). Various formats of such IFN-γ release assays (IGRAs) showed a high sensitivity and nearly complete specificity (9–14). Two IGRAs have thus far been marketed. QuantiFERON-TB Gold (QFT-G; Cellestis, Carnegy, Australia) is a whole-blood assay that uses ELISA for detection of IFN-γ responses (13, 15, 16). It has been approved for use in Europe and received approval from the U.S. Food and Drug Administration in 2005. Recent guidelines that were issued by the Centers for Disease Control and Prevention (CDC) recommend that QFT-G may be used in all circumstances in which the tuberculin skin test (TST) is currently used (17). A novel in-tube version of QFT-G that contains a third *M. tuberculosis*–specific antigen (TB7.7) has been approved for use in Europe (subsequently referred to as QFT-GIT). T-SPOT.*TB* (Oxford Immunotec, Oxford, UK) is based on the enzyme-linked immunospot technique (ELISPOT) and has been marketed in Europe. Both tests are included in the U.K. guidelines issued by the National Institute for Health and Clinical Excellence, recommending a two-stage strategy of TST testing followed by an IGRA to confirm a positive TST result, although there are no studies that have demonstrated the validity of this approach (18). A recent review reported comparable specificity of QFT-GIT and T-SPOT.*TB* (19). The sensitivity of these tests for detection of TB disease varied between studies (1, 13, 20). With regard to LTBI, test results in lowincidence settings were significantly correlated with measures of exposure, whereas the TST was not (10, 12, 16, 21–23). However, the sensitivity for detection of presumed LTBI varied widely among studies (9, 10,

12, 16, 20–24). Differences in study populations plus the lack of a gold standard for LTBI impeded the interpretation of these differences.

Recently, two publications reported on a direct comparison between the QFT-G and T-SPOT.*TB* (25, 26). In the first study among a heterogeneous population of 393 consecutive hospitalized patients with suspected active TB disease or LTBI, including many immunosuppressed patients, T-SPOT.*TB* produced significantly more positive results and less indeterminate results than did QFT-G (25). The second study, among 218 subjects in Korea suspected of having active TB, showed higher sensitivity of T-SPOT.*TB* compared with TST and QFT-G, whereas QFT-G showed superior specificity over TST and T-SPOT.*TB* (26). The clinical relevance of discordant blood test results was not known. However, the agreement between both blood tests was higher than between the TST and either assay. To assess the diagnostic value of these assays in various clinicoepidemiologic settings, further studies are needed. In the present study, we aimed to compare QFT-GIT and T-SPOT.*TB* results in relation to TST responses and measures of exposure among BCG-unvaccinated and predominantly immunocompetent contacts in a large-scale contact investigation in a population with an estimated background prevalence of LTBI of 1.4% (27).

Part of the data was presented at the American Thoracic Society 2006 conference in San Diego, California (28, 29).

METHODS

Study Design

Nested within a large-scale contact investigation (*see* the online supplement), we aimed to recruit 500 subjects on the 2 days of TST administration by random selection (pre-TST inclusion group). To include sufficient numbers of subjects with probable LTBI, we aimed to also include 500 subjects on the reading days who had a TST result of 1 mm or greater (post-TST inclusion group). In the pre-TST group and post-TST group, blood was collected, respectively, immediately after and 72 ± 8 hours after the TST was administered. Written, informed consent was obtained from all participants. The ethical review board of the Hospital Diakonessenhuis Utrecht/Zeist, The Netherlands, approved the study (protocol no. 2004.23).

Inclusion Criteria

Eligible for inclusion were BCG-unvaccinated subjects aged 17 years or older who had visited the supermarket at least once monthly within the period of infectiousness of the index case and in whom a TST was indicated.

Questionnaire

Demographic data and data reflecting the amount of exposure were obtained by questionnaire (*see* the online supplement).

Tuberculin Skin Testing

Tuberculin skin testing was performed according to the Mantoux method using 2 tuberculin units (TU) of tuberculin RT-23 (Statens Serum Institute, Copenhagen, Denmark) according to standard protocol. This tuberculin is bioequivalent to the international standard of 5 TU PPD-S. (30). TSTs were administered and read by experienced staff from the Municipal Health Authority. Indurations were measured at 72 ± 8 hours by two independent readers, and the average was used as final result. In case of a discrepancy exceeding 2 mm, a third person made the final reading.

Blood Sampling and Laboratory Procedures

In total, 10 ml of blood was collected in three tubes: one 8-ml cell preparation tube (Vacutainer citrate CPT; BD, Franklin Lanes, NJ), for the isolation of peripheral blood mononuclear cells for use in T-SPOT.*TB*, and two heparinized tubes of 1 ml each for QFT-GIT. During the investigation, a positive control tube was not available. Both assays were processed according to the manufacturer's instructions (*see* the online supplement).

Statistical Analysis

Statistical analyses were performed using SPSS version 11.5.0 (SPSS Benelux, Gorinchem, The Netherlands) and Stata version 8 (StataCorp, College Station, TX). Proportions were compared by the Pearson's chisquare test or Fisher's exact test as appropriate. Associations between test result and exposure were assessed by univariate and multivariate case-control analysis using logistic regression. To make maximum use of records with complete data, we combined the pre-and post-TST inclusion groups, including as cases all study subjects with a positive test result and as controls all study subjects with a negative test result. Because this approach might introduce selection bias, we checked its validity in two ways. First, in the multivariate analysis, we adjusted for inclusion group, and assessed interactions between inclusion group and other variables in the model. Second, we compared the results of our univariate analyses with those of restricted analyses in which cases were only selected from subjects included post-TST and the subjects included pre-TST served as control subjects. This was done separately after including and excluding subjects with a positive test result from the control group.

Analyses of associations between test result and exposure were restricted to subjects with complete data on exposure in the supermarket. The assumption of linearity was checked by plotting the log odds and by comparing model likelihoods with categorical and the scale variables using the likelihood ratio (LR) test. Concordance between test results was assessed using κ coefficients. p values less than 0.05 were considered significant. All reported p values were two-sided. TST results were analyzed in millimeters as categorical values (category 0: 0 mm; category 1: 1–4 mm; category 2: 5–9 mm; category 3: 10–14 mm; category 4: ≥15 mm) or as binary values using 15 mm or greater as the cutoff for a positive response following Dutch guidelines (31). For the present study, data were also analyzed using 5 mm or greater and 10 mm or greater as cutoffs.

RESULTS

Characteristics of the Study Population

Between January 31 and February 4, 2005, a total of 15,515 TSTs were administered and 14,128 (92%) were read at 72 ± 8 hours. In the present study, 878 subjects gave informed consent. Both a blood sample and the TST result were available for 785 unvaccinated subjects (Figure 1). Of 31 subjects with two blood samples obtained, only IGRA results of the first blood sample were used for the analyses.

Characteristics that were observed more frequently in subjects who were included in the post-TST group were as follows: origin from a TB-endemic country or a history of occupational exposure and travel to TB-endemic regions (data not shown). We found no significant interaction in any of the models between day of inclusion and age or cumulative shopping time. For the remainder of the analysis, both groups were combined. Although it is tempting to extrapolate the results to the whole population screened by the Municipal Health Authority, we caution against it because we selected subjects with any kind of induration in the post-TST group and we excluded participants younger than 17 years.

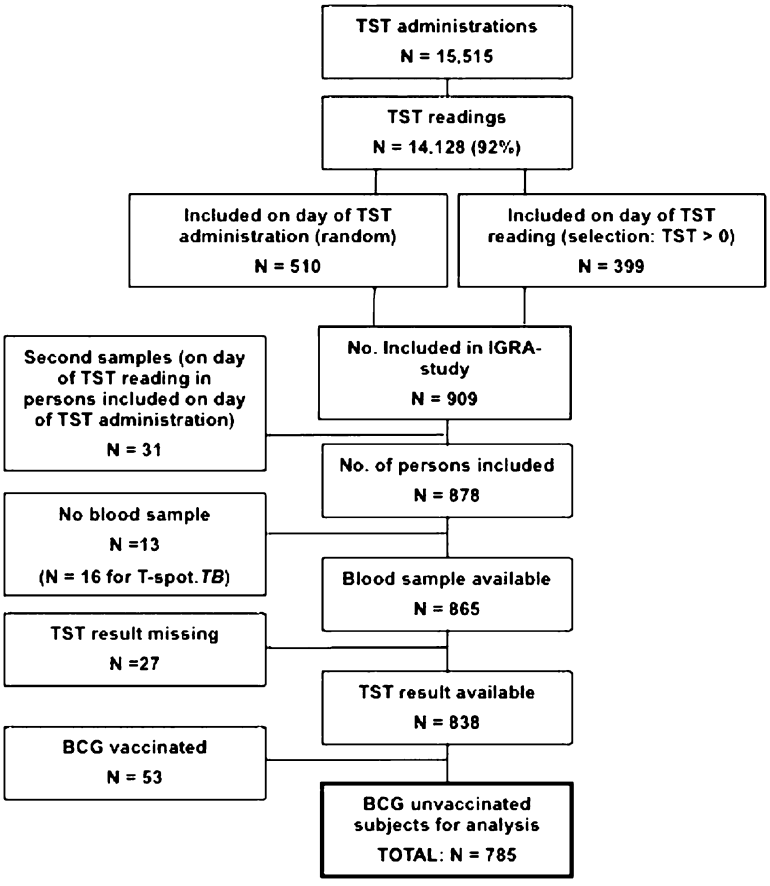

Figure 1. Flow diagram of the study population.
BCG = bacillus Calmette-Guérin; IGRA = IFN-γ release assay; TST = tuberculin skin test.

TST Results

Of 469 persons included in the pre-TST group, 90.6, 1.3, 1.9, 1.9, and 4.3% were in TST categories 0, 1, 2, 3, and 4, respectively. These values were not significantly different from the distribution of TST results among all 14,128 individuals for whom TST results were read in the complete contact investigation (B.F.P.J.K., unpublished data). The corresponding percentages in the post-TST group (n = 316) were 2.8, 2.8, 21.1, 28.5, and 44.6%. The distribution of TST results is shown in Figure 2A.

Complete data on exposure were available for 712 subjects. Age was the only characteristic associated with a TST result of 15 mm or greater (odds ratio [OR] for a positive TST result per step increase in 10-yr age category, 1.40; 95% confidence interval [CI], 1.13–1.74; p = 0.002; Table 1).

TABLE 1. UNIVARIATE AND MULTIVARIATE ANALYSIS OF PREDICTORS OF A TUBERCULIN SKIN TEST OF 10 mm OR GREATER OR 15 mm OR GREATER

	TST ⩾ 10 mm			TST ⩾ 15 mm			TST ⩾ 10 mm		TST ⩾ 15 mm	
	Cases (%)	Controls (%)	p Value*	Cases (%)	Controls (%)	p Value*	Adj. OR (*95% CI*)	p Value*	Adj. OR (*95% CI*)	p Value*
Sex										
Male	95 (41.0)	194 (41.0)	0.99	60 (41.1)	229 (41.0)	0.98	1	0.66	1	0.98
Female	137 (59.0)	279 (59)		86 (58.9)	330 (59.0)		0.90 (0.56–1.45)		1.01 (0.63–1.60)	
Age, yr										
< 35	50 (21.4)	134 (28.0)	0.09	27 (18.4)	157 (27.8)	0.01	1.34 (1.08–1.66)	0.008	1.40 (1.13–1.74)	0.002
35–44	57 (24.4)	130 (27.2)		37 (25.2)	150 (26.6)					
45–54	80 (34.2)	142 (29.7)		47 (32.0)	175 (31.0)					
⩾ 55	47 (20.1)	72 (15.1)		36 (24.5)	83 (14.7)					
Birth in high TB prevalence country										
No	225 (97.8)	462 (98.1)	0.82	144 (99.3)	543 (97.7)	0.16	1	0.2	1	0.07
Yes	5 (2.2)	9 (1.9)		1 (0.7)	13 (2.3)		0.41 (0.11–1.61)		0.14 (0.02–1.17)	
Work in health care†										
No	198 (87.6)	388 (84.2)	0.23	126 (88.1)	460 (84.6)	0.28	1	0.06	1	0.16
Yes	28 (12.4)	73 (15.8)		17 (11.9)	84 (15.4)		0.53 (0.27–1.03)		0.62 (0.32–1.21)	
Travel to high prevalence countries										
No	135 (58.4)	283 (59.7)	0.19	87 (59.6)	331 (59.2)	0.93	1	0.73	1	0.22
⩽ 3 mo (cumulative)	76 (32.9)	167 (35.2)		49 (33.6)	194 (34.7)		0.85 (0.53–1.38)		0.98 (0.61–1.56)	
> 3 mo (cumulative)	20 (8.7)	24 (5.1)		10 (6.9)	6 (6.1)		1.11 (0.46–2.67)		0.78 (0.33–1.83)	
History of TB in household										
No	226 (98.3)	453 (95.8)	0.07	141 (97.9)	538 (96.2)	0.30	1	0.051	1	0.27
Yes	4 (1.7)	20 (4.2)		3 (2.1)	21 (3.8)		0.27 (0.07–1.00)		0.46 (0.11–1.83)	

Duration of exposure (mo)											
0–3	15 (6.4)	17 (3.6)	0.13	8 (5.4)	24 (4.3)	0.20					
4–6	8 (3.4)	24 (5.0)		3 (2.0)	29 (5.1)						
7–9	26 (11.1)	38 (8.0)		17 (11.6)	47 (8.3)						
⩾ 10	185 (79.1)	399 (83.5)		119 (81.0)	465 (82.3)						
Frequency of shopping											
⩽ 1×/mo	47 (20.1)	58 (12.1)	0.01	27 (18.4)	78 (13.8)	0.36					
> 1×/mo and < 1×/wk	43 (18.4)	119 (24.9)		27 (18.4)	135 (23.9)						
1×/wk	63 (26.9)	154 (32.2)		46 (31.3)	171 (30.3)						
> 1×/wk	81 (34.6)	147 (30.8)		47 (32.0)	181 (32.0)						
Average shopping time, min											
1–15	67 (28.6)	131 (27.4)	0.33	46 (31.3)	152 (26.9)	0.12					
16–30	104 (44.4)	216 (45.2)		58 (39.5)	262 (46.4)						
31–60	54 (23.1)	123 (25.7)		36 (24.5)	141 (25.0)						
> 60	9 (3.9)	8 (1.7)		7 (4.8)	10 (1.8)						
Cumulative exposure time, min											
1–300	59 (25.2)	103 (21.6)	0.07	36 (24.5)	126 (22.3)	0.51	1.12 (0.94–1.34)	0.20	1.05 (0.89–1.25)	0.54	
301–600	35 (15.0)	94 (19.7)		23 (15.7)	106 (18.8)						
601–1,200	53 (22.7)	124 (25.9)		34 (23.1)	143 (25.3)						
1,201–2,400	70 (29.9)	107 (22.4)		43 (29.3)	134 (23.7)						
> 2,400	17 (7.3)	50 (10.5)		11 (7.5)	56 (9.9)						

Definition of abbreviations: CI = confidence interval; OR = odds ratio; TB = tuberculosis; TST = tuberculin skin test.

* p values based on likelihood ratio test in logistic regression.

† Work including direct patient contact.

TST results were not associated with any measure of exposure to the index case at the supermarket (Table 1). When 10 mm was applied as the cutoff value for a positive TST (Table 1), we observed a similar association with age (OR, 1.34; 95% CI, 1.08–1.66; p = 0.008) and no association with any measure of exposure. With a cutoff of 5 mm, findings were similar (see the online supplement), except for a significantly higher prevalence of a TST of 5 mm or greater among subjects born in high-prevalence countries (OR, 12.8; 95% CI, 1.87–87.0; p = 0.009). We observed no significant interaction between inclusion group and any of the variables in these models. Restricted case-control analyses yielded similar associations; the ORs for associations with age and exposure in the supermarket rarely differed by more than 5% from those in the primary analyses (data not shown).

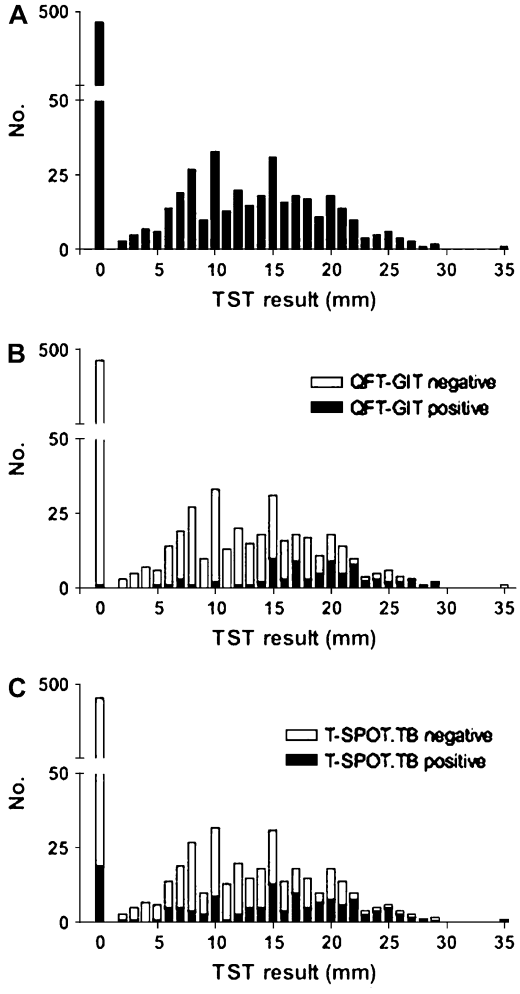

Figure 2. Distribution of TST results.
(A) Distribution of all 785 TST results. (B) Distribution of positive QuantiFERON Gold In-Tube (QFT-GIT) results in relation to TST result among 785 participants. (C) Distribution of positive T-SPOT. TB results in relation to TST result among 759 participants (there were three missing blood samples and 23 indeterminate test results).

QFT-GIT and T-SPOT.*TB*

QFT-GIT results were obtained for all 785 participants, none of whom could have been determined to have indeterminate results because the tube with positive controls was not available. For T-SPOT.TB, 23 (2.9%) of 782 blood samples yielded indeterminate test results due to insufficient responses to the positive control. None of these 23 subjects reported use of immunosuppressive drugs and 4 (17.4%) had a TST result of 15 mm or greater, compared with 164 of 759 (20.6%) participants with valid T-SPOT.TB results (p = 0.628). The agreement between independent readers of T-SPOT.TB results was 94.9% (κ = 0.84). The agreement between visual and automated readings was 97.5% (κ =0.923). Overall, positive QFT-GIT responses were observed in 81 of 785 (10.3%) subjects, compared with 142 of 759 (18.7%) for T-SPOT.TB (p = 0.001). A positive QFTGIT result was observed in 0.2, 0, 7.9, 6.1, and 42.2% of subjects withaTSTresult of0 mm,1to 4 mm,5to 9mm,10 to14 mm,and 15 mm or greater, respectively (Figure 3A). The corresponding percentages of positive T-SPOT.TB results were higher in each TST category, being 4.6, 13.3, 23.7, 23.5, and 51.3%, respectively (Figure 3A).

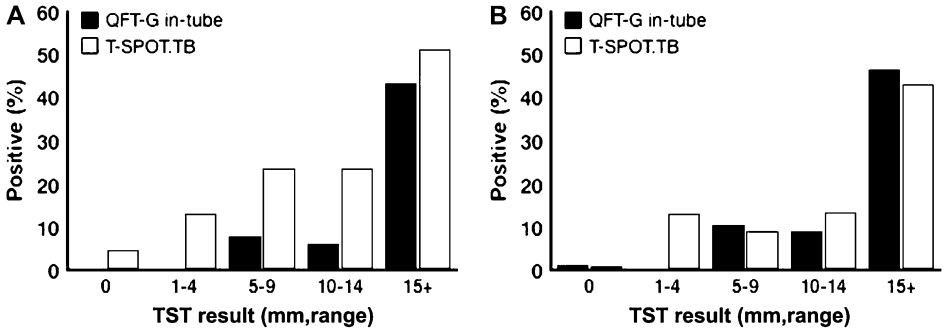

Figure 3. Proportion of positive results of QuantiFERON Gold and T-SPOT.*TB* by category of TST results among 785 BCG unvaccinated study participants (n = 759 T-SPOT.*TB* results).
(A) Using the cutoff values for a positive test result as provided by the manufacturer. (B) Using cutoff values for a positive test result that yielded the highest agreement between both tests (see also Table 6).

Complete data on exposure were available for 712 subjects with complete QFT-GIT results, and for 691 subjects with complete T-SPOT.*TB* results. QFT-GIT and T-SPOT.*TB* results were not associated with age, sex, occupational exposure, or country of origin in univariate analysis (Table 2). The probability of a positive QFT-GIT increased significantly in association with the frequency of shopping as well as with the cumulative shopping time (Table 2).

TABLE 2. UNIVARIATE AND MULTIVARIATE ANALYSIS OF PREDICTORS OF A POSITIVE IFN-γ TEST RESULT

	QFT-GIT Positive			T-SPOT.*TB* Positive			QFT-GIT Positive		T-SPOT.*TB* Positive	
	Cases (%)	Controls (%)	p Value*	Cases (%)	Controls (%)	P Value*	Adj. OR (*95% CI*)	P Value*	Adj. OR (*95% CI*)	p Value*
Sex										
Male	22 (30.6)	267 (42.2)	0.054	47 (37.6)	240 (42.9)	0.27	1	0.18	1	0.85
Female	50 (69.4)	366 (57.8)		78 (62.4)	319 (57.1)		1.50 (0.83–2.71)		1.04 (0.66–1.65)	
Age, yr										
< 35	14 (18.9)	170 (26.7)	0.52	27 (21.4)	151 (26.7)	0.60	1.10 (0.84–1.43)	0.50	1.07 (0.87–1.31)	0.52
35–44	22 (29.7)	165 (25.9)		35 (27.8)	143 (25.3)					
45–54	25 (33.8)	197 (30.9)		40 (31.8)	179 (31.7)					
⩾ 55	13 (17.6)	106 (16.6)		24 (19.0)	92 (16.3)					
Birth in high TB prevalence country										
No	73 (98.7)	614 (97.9)	0.66	121 (97.6)	546 (97.9)	0.76			1	0.85
Yes	1 (1.3)	13 (2.1)		3 (2.4)	11 (2.0)				1.89 (0.35–10.3)	
Work in health care†										
No	64 (88.9)	522 (84.9)	0.35	105 (86.1)	467 (85.9)	0.95	1	0.24	1	0.71
Yes	8 (11.1)	93 (15.1)		17 (13.9)	77 (14.2)		0.60 (0.26–1.40)		0.89 (0.47–1.67)	
Travel to high prevalence countries										
No	44 (60.3)	374 (59.2)	0.70	73 (58.4)	331 (59.2)	0.90	1	0.51	1	0.25
⩽ 3 mo (cumulative)	26 (35.6)	217 (34.3)		45 (36.0)	192 (34.4)		1.03 (0.59–1.81)		1.07 (0.68–1.68)	
> 3 mo (cumulative)	3 (4.1)	41 (6.5)		7 (5.6)	36 (6.4)		0.46 (0.13–1.65)		0.65 (0.26–1.62)	
History of TB in household										
No	71 (98.6)	608 (96.4)	0.26	119 (96.8)	539 (96.4)	0.86	1	0.35	1	0.91
Yes	1 (1.4)	23 (3.6)		4 (3.2)	20 (3.6)		0.36 (0.05–3.04)		0.93 (0.28–3.11)	

Duration of exposure, mo										
0–3	0	32 (5.0)	0.09	4 (3.2)	27 (4.8)	0.17				
4–6	1 (1.4)	31 (4.9)		2 (1.6)	29 (5.1)					
7–9	4 (5.4)	60 (9.4)		10 (7.9)	53 (9.4)					
⩾ 10	69 (93.2)	515 (80.7)		110 (87.3)	456 (80.7)					
Frequency of shopping										
⩽ 1×/mo	8 (10.8)	97 (15.2)	0.005	19 (15.1)	83 (14.7)	0.04				
> 1×/mo and < 1×/wk	7 (9.5)	155 (24.3)		17 (13.5)	139 (24.6)					
1 ×/wk	28 (37.8)	189 (29.6)		42 (33.3)	168 (29.7)					
> 1×/wk	31 (41.9)	197 (30.9)		48 (38.1)	175 (31.0)					
Average shopping time, min										
1–15	20 (27.0)	178 (27.9)	0.17	34 (27.0)	161 (28.5)	0.12				
16–30	27 (36.5)	293 (45.9)		49 (39.9)	263 (46.6)					
31–60	23 (31.1)	154 (24.1)		37 (29.4)	130 (23.0)					
> 60	4 (5.4)	13 (2.0)		6 (4.8)	11 (1.9)					
Cumulative exposure time, min										
1–300	8 (10.8)	154 (24.1)	0.007	21 (16.7)	137 (24.3)	0.17	1.48 (1.19–1.84)	< 0.001	1.30 (1.10–1.53)	0.002
301–600	11 (14.9)	118 (18.5)		20 (15.9)	107 (18.9)					
601–1,200	17 (23.0)	160 (25.1)		31 (24.6)	138 (24.4)					
1,201–2,400	30 (40.5)	147 (23.0)		39 (31.0)	132 (23.4)					
> 2,400	8 (10.8)	59 (9.3)		15 (11.9)	51 (9.0)					

Definition of abbreviations: CI = confidence interval; OR = odds ratio; QFT-GIT = QuantiFERON-TB Gold In-Tube; TB = tuberculosis.

* p values based on likelihood ratio test in logical regression.

† Work including direct patient contact.

The probability of a positive T-SPOT.*TB* was significantly associated only with the monthly number of visits to the supermarket (Table 2). In multivariate analysis, adjusting for day of inclusion and all variables in Table 2, except duration of exposure, frequency of shopping, and average shopping time per visit to the supermarket, both QFT-GIT and T-SPOT.*TB* were significantly associated with the cumulative shopping time. Per increase of category of the latter parameter, the average OR (95% CI) of a positive IGRA result was 1.48 (1.19–1.84, p = 0.001) for QFT-GIT and 1.30 (1.10–1.53, p = 0.002) for T-SPOT.*TB*, implicating a 4.8-times (1.48^4) and 2.9-times (1.30^4) increased risk in the highest exposure category compared with the baseline category for QFT-GIT and T-SPOT.*TB*, respectively. Significant interactions between inclusion group and any of the variables in these models were not observed. In addition, restricted case-control analyses yielded similar associations, with small differences in ORs compared with the primary analyses (data not shown).

Of 23 participants who reported the use of immunosuppressive drugs, 3 (13%) had a TST result of 15 mm or greater, compared with 20.8% of participants who did not report the use of immunosuppressive drugs (p = 0.370). Positive QFT-GIT results were observed in 2 of 23 (8.7%) and positive T-SPOT.*TB* results in 6 of 23 (26.1%) participants. These percentages were not significantly different from those observed in the complete study population (p = 0.801 and p = 0.374, respectively).

Using 5 mm as the TST cutoff, the sensitivity and specificity of QFT-GIT were 80 of 333 (23.8%) and 448 of 449 (99.8%), respectively, and the agreement was 67.3% (κ = 0.26; Table 3). The sensitivity and specificity of T-SPOT.*TB* compared with TST at 5 mm or greater were 121 of 330 (36.7%) and 408 of 429 (95.1%), respectively, and the agreement was 69.7% (κ = 0.34; Table 3).

Among 70 subjects with a TST result of 5 to 9 mm, 5 (7.1%) had a positive QFT-GIT result, and 16 (22.9%) had a positive T-SPOT.*TB* result. Neither a positive QFT-GIT result nor a positive T-SPOT.*TB* result in this group was significantly associated with exposure to the index case at the supermarket (data not shown).

Using 10 mm as the TST cutoff, the sensitivity and specificity of QFT-GIT were 74 of 260 (28.5%) and 518 of 525 (98.7%), respectively, and the agreement was 75.4% (κ = 0.33; Table 3). The sensitivity and specificity of T-SPOT.*TB* compared with TST at 10 mm or greater were 103 of 254 (40.6%) and 466 of 505 (92.3%), respectively, and the agreement was 75.0% (κ = 0.37; Table 3). Using the TST at a cutoff of 15 mm or greater as a reference, the sensitivity and specificity of QFT-GIT were 68 of 161 (42.2%) and 611 of 624 (97.9%), respectively. The agreement between the TST at a cutoff of 15 mm or greater and QFT-GIT was 86.5% (κ = 0.49; Table 3). The sensitivity and specificity of T-SPOT.*TB* compared with TST at

15 mm or greater were 80 of 156 (51.3%) and 541 of 603 (89.7%), respectively. The agreement between the TST at 15 mm or greater and T-SPOT.*TB* was 81.8% (κ = 0.42; Table 3). Thus, agreement between each IGRA and TST increased with a higher TST cutoff. Furthermore, in a two-stage approach with TST being used to screen contacts and those with a "positive TST result" being assayed by IGRA to define likely LTBI, an increasing proportion of positive IGRA test results is found for both QFTGIT and T-SPOT.*TB* (Table 3).

T-SPOT.*TB* versus QFT-GIT

Among 759 persons with valid results of both IGRAs, results were concordant negative in 608 (80.1%), concordant positive in 72 (9.5%), and discordant in 79 (10.4%; overall agreement, 89.6%; κ = 0.59, p = 0.0001). Of the discordant results, 70 (88.6%) were T-SPOT.TB positive, and 9 (11.4%) QFT-GIT positive. The agreement between QFT-GIT and T-SPOT.TB increased with each TST category (Table 4).

We assessed the characteristics of subjects with positive T-SPOT.TB but negative QFT-GIT by comparing them in multivariate analyses with two control groups of the subjects with concordant-negative and those with concordant-positive results, respectively (Table 5). Compared with the concordant-negative control group, a discordant-positive T-SPOT.TB was significantly associated with the TST result (p = 0.001) with similar ORs (6.0–6.9) for TST categories of 5 mm or greater. Compared with the concordant-negative control group, a discordant-positive T-SPOT.TB was near-significantly associated with immunosuppression (OR 3.94; 95% CI, 0.93–16.6), but not with cumulative exposure time. A significant univariate association with increasing age disappeared after adjustment for TST result (data not shown). Assuming a causal relationship, 4.3% of all discordant-positive TSPOT.TB results were directly attributable to immune suppression (population attributable fraction; 95% CI, 0–5.4%). Compared inversely, with TST result (p = 0.001), but not with immunosupwith the concordant-positive control group, a discordant-positive pression or exposure. T-SPOT.TB was significantly associated with increasing age The observed discrepancies between QFT-GIT and (average OR per 10-yr increase, 1.88; 96% CI, 1.14–3.11) and, T-SPOT.TB prompted us to reanalyze the interassay agreement at varying cutoff values of both assays (Table 6). Among all subjects for whom both IGRA results were available, agreement between both IGRA was maximized at IFN-γ of 0.20 IU/ml or greater for QFT-GIT and 13 spots or more for T-SPOT.TB.At these optimum cutoff values, the absolute number of results that were positive in both assays was very similar to the number of concordant positive results when using the manufacturers' cutoff values, but with a different distribution in relation to the TST categories (Figure 3B).

TABLE 3. AGREEMENT BETWEEN IFN-γ ASSAYS AND TUBERCULIN SKIN TEST RESULTS

	TST		TST		TST	
	< 5 mm	⩾ 5 mm	< 10 mm	⩾ 10 mm	< 15 mm	⩾ 15 mm
QuantiFERON-TB Gold In-Tube (n = 785)						
Negative (n = 704)	448* (63.6)	256 (36.4)	518 (73.6)	186 (26.4)	611 (86.8)	93 (13.2)
Positive (n = 81)	1 (1.2)	80 (98.8)	7 (8.6)	74 (91.4)	13 (16.0)	68 (84.0)
Agreement, %	67.3		75.4		86.5	
OR (95% CI)	140.0 (19.4–1,012.1)		29.4 (13.3–65.1)		34.4 (18.3–64.7)	
κ	0.26		0.33		0.49	
T-SPOT.*TB* (n = 759)†						
Negative (n = 617)	408 (66.1)	209 (33.9)	466 (75.5)	151 (24.5)	541 (87.7)	76 (12.3)
Positive (n = 142)	21 (14.8)	121 (85.2)	39 (27.5)	103 (72.5)	62 (43.7)	80 (56.3)
Agreement, %	69.7		75		81.8	
OR (95% CI)	11.25 (6.87–18.41)		8.15 (5.40–12.30)		9.19 (6.10–13.8)	
κ	0.34		0.37		0.42	

Definition of abbreviations: CI = confidence interval; OR = odds ratio; TST = tuberculin skin test.

* Data are expressed as number (%).

† For T-SPOT.*TB*, there were three missing blood samples and 23 (2.9%) indeterminate test results.

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

TST Category	No.			T-SPOT.*TB* Negative	T-SPOT.*TB* 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.

TABLE 5. MULTIVARIATE ANALYSIS OF DETERMINANTS OF A DISCORDANT IFN-γ TEST RESULT (T-SPOT.*TB* POSITIVE/QFT-GIT NEGATIVE, n = 70) COMPARED WITH A CONCORDANT NEGATIVE AND A CONCORDANT POSITIVE CONTROL GROUP

	Compared with T-SPOT.*TB* Negative/ QFT-GIT Negative (*n = 608*)		Compared with T-SPOT.*TB* Positive/ QFT-GIT Positive (*n = 72*)	
	Adj. OR (*95% CI*)	p Value*	Adj. OR (*95% CI*)	p Value*
Age (per 10 yr increase)	1.15 (0.87–1.51)	0.330	1.88 (1.14–3.11)	0.010
Cumulative exposure time†	1.16 (0.93–1.44)	0.177	0.86 (0.58–1.27)	0.450
TST result, mm		< 0.001		< 0.001
0–4	1		—	
5–9	6.86 (3.04–15.5)		—	
0–9	—		1	
10–14	6.33 (2.95–13.6)		0.47 (0.09–2.54)	
15+	6.05 (2.74–13.3)		0.02 (0.00–0.08)	
Immunocompromised‡	3.94 (0.93–16.6)	0.089	0.85 (0.07–9.80)	0.894

For the analysis of TST results, 0–4 mm was used as the reference category for the comparison with T-SPOT.*TB* negative/QFT-GIT negative subjects and 0–9 mm was used as the reference category for the comparison with T-SPOT.*TB* positive/QFT-GIT positive subjects.

Definition of abbreviations: CI = confidence interval; OR = odds ratio; QFT-GIT = QuantiFERON-TB Gold In-Tube; TST = tuberculin skin test.

* p values based on likelihood ratio test in logistic regression.

† Average increase in OR per step increase in exposure category (*see* Table 1).

‡ Compared with immunocompetent (reference category).

TABLE 6. AGREEMENT BETWEEN T-SPOT.*TB* AND QUANTIFERON TB GOLD IN 759 SAMPLES AT VARYING CUTOFF VALUES FOR A POSITIVE TEST

QuantiFERON TB Gold Cutoff Value (*IU/ml IFN-γ*)	T-SPOT.*TB* Cutoff Value (no. of spots)													
	⩾ 4	⩾ 5	⩾ 6	⩾ 7	⩾ 8	⩾ 9	⩾ 10	⩾ 11	⩾ 12	⩾ 13	⩾ 14	⩾ 15	⩾ 16	⩾ 17
	0.454	0.515	0.556	0.587	0.606	0.615	0.623	0.640	0.646	**0.649**	0.619	0.634	0.649	0.650
⩾ 0.40	83.6	87.5	89.1	90.3	91.2	91.6	91.9	92.4	92.9	**93.2**	92.9	93.4	93.8	93.8
	67	67	67	67	65	64	63	63	61	**58**	54	53	53	52
	0.480	0.543	0.585	0.617	0.638	0.647	0.656	0.673	0.680	**0.685**	0.657	0.673	0.675	0.662
⩾ 0.35	85.2	88.0	89.7	90.8	91.7	92.1	92.5	93.0	93.3	**93.7**	93.3	93.9	94.0	93.9
	72	72	72	72	70	69	68	68	66	**63**	59	58	57	55
	0.499	0.554	0.597	0.629	0.639	0.648	0.657	0.674	0.681	**0.686**	0.659	0.662	0.663	0.651
⩾ 0.30	85.6	88.2	89.8	91.0	91.6	91.6	92.3	92.8	93.3	**93.6**	93.3	93.5	93.6	93.5
	76	75	75	75	72	71	70	70	68	**65**	61	59	58	56
	0.513	0.569	0.612	0.644	0.655	0.664	0.674	0.691	0.699	**0.704**	0.678	0.682	0.683	0.672
⩾ 0.25	85.9	88.3	90.0	91.2	91.9	92.2	92.6	93.1	93.5	**92.9**	93.5	93.7	93.9	93.7
	79	78	78	78	75	74	73	73	71	**68**	64	62	61	59
	0.525	**0.582**	**0.615**	**0.648**	**0.658**	**0.668**	**0.667**	**0.694**	**0.702**	*0.708*	**0.683**	**0.686**	**0.688**	**0.677**
⩾ 0.20	**86.0**	**88.6**	**89.9**	**91.0**	**91.7**	**92.1**	**92.5**	**93.0**	**93.1**	*93.8*	**93.3**	**93.6**	**93.8**	**93.6**
	83	**82**	**81**	**81**	**78**	**77**	**76**	**76**	**74**	*71*	**67**	**65**	**64**	**62**
	0.535	0.583	0.616	0.648	0.649	0.657	0.667	0.683	0.690	**0.696**	0.672	0.676	0.678	0.667
⩾ 0.15	85.9	88.1	89.6	90.7	91.1	91.4	91.8	92.3	92.7	**93.1**	92.8	93.0	93.1	93.0
	89	87	86	86	82	81	80	80	78	**75**	71	69	68	66
	0.573	0.605	0.621	0.643	0.644	0.643	0.652	0.667	0.664	**0.669**	0.647	0.650	0.652	0.643
⩾ 0.10	86.5	88.2	89.2	90.0	90.4	90.6	90.9	91.4	91.6	**92.0**	91.6	91.8	92.0	91.6
	100	96	93	92	88	86	85	85	82	**79**	75	73	72	70

Values in each cell represent κ value, % agreement, and number of tests that were positive in both assays, respectively. Values in boldface represent the optimal values for either test at varying cutoffs of the other test. The agreement was optimal in the cell with values shown in boldface italics. Manufacturers' cutoff values are ⩾ 0.35 IU/ml for QFT-GIT and ⩾ 6 spots for T_SPOT.*TB* (*see* the online supplement).

DISCUSSION

This study describes a direct comparison between the TST and two commercially available IGRA, QFT-GIT and T-SPOT.*TB*, for detection of LTBI in a large contact investigation. The setting of our study was unique as more than 20,000 mainly BCGunvaccinated individuals from an area with low TB endemism were potentially exposed to *M. tuberculosis* repeatedly for as long as 10 months. In our study, in which BCG-vaccinated subjects were excluded, a TST cutoff of 15 mm or greater was regarded to reliably indicate LTBI. Among participants in the pre-TST group, the rate of positive TST results was 4.3%, reflecting the infection risk of the contact investigation at large (B.F.P.J.K., unpublished data). TST results of 15 mm or greater were significantly associated with age but not with measures of exposure at the supermarket, suggesting that positive TST responses reflected largely delayed-type hypersensitivity due to remote infection with *M. tuberculosis* acquired before the source case at the supermarket became infectious. Using the cutoff of 10 mm resulted in similar but less pronounced associations, likely reflecting bias due to the less specific outcome measure, but this did not affect our conclusions.
In contrast, results of QFT-GIT and those of T-SPOT.TB were not associated with age but were significantly associated with the cumulative shopping time in the supermarket, which was most marked for QFT-GIT.

In this large contact investigation, it was not possible to document actual face-to-face contact with the source case and we therefore used the number of months that a customer frequented the supermarket during the infectious period of the source case, the shopping frequency, and average time of each shopping visit as proxy indicators. Even though we used only the cumulative exposure time as a variable in our multivariate models, we observed similar patterns of association with IGRA responses for various individual proxy indicators in the univariate analysis, suggesting that our findings were robust to the way exposure was estimated. For reasons of study efficiency, we enriched our sample by including not only a random sample of supermarket customers who reported for skin testing but also a nonrandom sample of customers who had a TST reaction of more than 0 mm. This could in theory affect the observed associations with age or exposure. We corrected for this by adjustment for inclusion group in the multivariate analysis. Moreover, we found no significant interactions—that is, the size of observed associations did not differ between both inclusion groups.
The observed association of IGRA results with exposure is in accordance with previous studies using either ELISPOT or whole blood–based IGRA (10, 12, 16,

21–23). Our results confirm these findings and, in addition, demonstrate that IGRAs, in particular QFT-GIT, correlate better with the level of exposure than the TST even in a BCG-unvaccinated population.

Sensitivity of IGRA for Detection of LTBI

In recent CDC guidelines (17), several cautions and potential limitations of QFT-G were discussed, among which the determination of the sensitivity of IGRA for detection of LTBI was a key issue, a concern that had previously been expressed (32–34). Our study provides important new data in this regard. In our study, the high agreement between both IGRAs that were performed independently at different laboratories and the significant association of IGRA results with exposure argue against technical problems with the IGRA as an explanation of the low sensitivity. False-positive TST results were also unlikely because the study population was BCG unvaccinated. Moreover, a cutoff value of 15 mm has a specificity for LTBI exceeding 97% in the Dutch population, suggesting that cross-reactive TST responses due to previous nontuberculous mycobacterial infections rarely exceed 15 mm (35). The lower sensitivity of IGRA compared with TST must therefore be related to intrinsic differences between blood and skin tests. A positive TST result after infection with *M. tuberculosis* often remains positive during a lifetime ("once positive, no longer useful"), with waning being infrequent in those younger than 55 years (36).

In persons who were actually infected at the supermarket, the infection could have been acquired as long ago as 1 year before the study because the source case had been contagious since February 2004 and the large-scale contact investigation was performed at the end of January 2005. Although there are no definitive data of the kinetics of IGRA responses, we think that the decay kinetics of IGRA responses in relation to the interval between infection and blood sampling provides a hypothesis for the observed difference in sensitivity of IGRAs between studies, as has been suggested earlier (16, 24). In this regard, IGRAs are highly sensitive for detection of recent infection, but test responses can revert to negative if the antigen is cleared when the infection is adequately controlled and activated T cells are no longer required. Memory T cells may remain undetected during the short incubation period of 16 to 24 hours of IGRA, whereas the TST measures infiltration of the skin by immune cells 72 hours after injection of tuberculin.

In support of decreasing IGRA responses over time was the observation that results of an ELISPOT-based IGRA reverted to negative in patients treated successfully for TB disease (37, 38). Another study reported increased ELISPOT responses after 4 weeks of treatment for LTBI followed by a decrease (39). Follow-up with IGRAs of treated and untreated TST-positive individuals in our study is currently ongoing and may provide further clarification of this issue.

Clinical Significance of IGRA Test Result

Although IGRAs are now considered more specific and show a better correlation with exposure than the TST, it has not been demonstrated whether they provide a valid basis for therapeutic decisions regarding treatment. The risk of TB disease in the presence of a positive test result has not been established. Notably, positive IGRA results were observed in a significant proportion of recently exposed contacts with a negative TST result (15, 22, 23, 40). The clinical significance of this finding merits further study if IGRAs are to replace the TST and be used for therapeutic decisions (41).

If a positive IGRA result reflects an ongoing immune response against *M. tuberculosis*, it is possible that IGRAs will have a higher prognostic value with regard to the risk of progression to TB disease than the TST. This would allow better targeting of preventive treatment of LTBI cases found in outbreak investigations. Thus far, only one study reported an increased risk of TB disease within 2 years among ESAT-6–responsive contacts (42). More follow-up studies of the natural kinetics of IGRAs in both immunocompetent and immunocompromised hosts and the development of TB disease after infection are needed.

We therefore agree with Mazurek and coworkers that negative results of an IGRA must be interpreted with caution and should always be regarded in the light of all other available clinical and epidemiologic data (17).

Discordances between QFT-GIT and T-SPOT.*TB* Results

The agreement between QFT-GIT and T-SPOT.TB was 89.5% (κ = 0.59). Nevertheless, there were important discrepancies between the results of both IGRAs. A positive result of T-SPOT.*TB* in combination with a negative result of QFT-GIT was observed eightfold more frequently than the reverse discrepant combination. The difference in percentage of positive results of both assays varied from 4.6 to 14.5% in different TST categories, the difference being most pronounced with reaction sizes of less than 5 mm. The reported percentage of positive ELISPOT results in association with a negative TST in contact investigations was even higher in several earlier studies (22, 23). Among contacts with a TST result of smaller than 5 mm, 30 of 205 (14.6%) were positive using ELISPOT (40). Finally, positive T-SPOT.*TB* results were observed in comparable frequency in association with negative TST results in a heterogeneous cohort of patients suspected of TB disease or LTBI (25). The consistency of these findings suggests that this is an inherent characteristic of ELISPOT.

With QFT-GIT or QFT-G, positive results were observed in association with a TST result of less than 10 mm in 62 of 421 (15%) highly exposed health care workers in India (15), in 13 of 372 (3.5%) United States jail inmates (24), and a similar

proportion of the above-mentioned heterogeneous cohort (25). As yet, no cases of TB disease have been reported in persons with such discrepant results and the clinical significance is therefore unclear, but this information becomes essential if the TST is replaced by IGRA since there would be no discrepancy, just a positive or negative IGRA result to act on.

Our data suggest that the specificity of T-SPOT.*TB* could be improved by increasing the cutoff value, whereas the sensitivity of QFT-G could be improved by decreasing the cutoff. Using bidirectional variation of the cutoff values of both IGRAs, the interassay agreement was found to be optimal at cutoff values of IFN-γ of 0.20 IU/ml or greater for QFT-GIT and 13 or more spots for T-SPOT.*TB*. At these optimized cutoff points, the proportion of positive test results in each TST category was comparable for both tests (Figure 3B), whereas these were significantly different when results were based on the manufacturers' cutoff values (Figure 3A). In general, cutoff points are determined by the aims of a study, which are different for comparative studies, prevalence assessments, or those concerning patient management. Further study is needed to evaluate whether cutoff values different from those advocated by the manufacturers may provide a better basis for decision making in specific clinical or epidemiologic settings. Furthermore, it remains speculative what the results of our study would be if indeterminate QFT-GIT results were known.

CONCLUSIONS

In conclusion, in this study among 785 BCG-nonvaccinated Dutch adults who had been exposed to a patient with smearpositive TB, IGRA results related to measures of the level of exposure better than did the TST. In relation to each other, QFT-GIT was more closely associated with exposure than was T-SPOT.*TB*. However, a possible lack of sensitivity for both assays in detecting individuals with a TST of 15 mm or greater, despite negative BCG vaccination status, requires further investigation. Optimum agreement between both IGRAs was reached after lowering the cutoff value for QFT-GIT and increasing the cutoff value for T-SPOT.*TB*. Despite the higher correlation between T-SPOT.*TB* and QFT-GIT than between the TST and either assay, the discrepancies between both IGRAs await clarification. Important subjects for future research are the sensitivity of IGRAs in relation to the interval since infection, the evaluation of different cutoff levels, and the predictive value of an IGRA result for development of TB disease.

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