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Effect of tuberculin skin testing on a *Mycobacterium tuberculosis*-specific interferon-γ assay

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ABSTRACT: Recently, interferon-γ release assays (IGRA) for specific diagnosis of *Mycobacterium tuberculosis* infection have become available. In recent UK tuberculosis (TB) guidelines, it has been advised to screen for latent *M. tuberculosis* infection using the tuberculin skin test (TST), followed by IGRA if the TST is positive. Since TST can boost immune responses to tuberculin, the present authors evaluated whether TST administration affects the result of QuantiFERON®-TB Gold in-tube (QFT-GIT), a whole blood-based IGRA.

QFT-GIT was performed on the day of TST administration and the day of reading in 15 TST-negative subjects, 46 TST-positive subjects with recent or remote exposure to *M. tuberculosis* and five cured TB patients.

No systematic boosting of QFT-GIT responses from negative to positive was observed. Only in a few TST-positive persons did TST enhance pre-existing QFT-GIT responses.

Screening for latent *Mycobacterium tuberculosis* infection using tuberculin skin testing followed by interferon-γ release assays on the day of reading is a reliable approach, as the specificity of QuantiFERON®-TB Gold in-tube is not affected by prior tuberculin skin test administration.

KEYWORDS: Diagnosis, immunoassays, interferon, *Mycobacterium tuberculosis*, skin test, tuberculosis

During the past century, the tuberculin skin test (TST) was the only available diagnostic tool for detection of latent infection with *Mycobacterium tuberculosis*. The most important limitation of this test is that the specificity is impaired by cross-reactive immune responses to *Mycobacterium bovis* bacille Calmette–Guérin (BCG) and environmental mycobacteria. Recently, new *in vitro* immunodiagnostic assays have been developed that were specifically designed to overcome this problem. These assays measure interferon (IFN)-γ production in whole blood or production of peripheral blood mononuclear cells (PBMC) in response to antigens that are specific for *M. tuberculosis* and absent from BCG and most environmental mycobacteria. Herein, these assays will be referred to as IFN-γ release assays (IGRA). The two most frequently used antigens are early secreted antigen of 6 kDa (ESAT-6; Rv3875) and culture filtrate protein (CFP)-10 (Rv3874); T-cell responses to these antigens were found to be sensitive as well as specific for the detection of tuberculosis (TB) [1, 2]. The QuantiFERON®-TB Gold (QFT-G) is a commercial IGRA based on an overnight culture of whole blood with peptides of ESAT-6 and CFP-10. The reported sensitivity for detection of active TB infection was 85–90%, with a specificity of 98% [3, 4]. In a contact investigation in a Danish school, mainly among BCG unvaccinated individuals, a modified QFT assay using recombinant antigens of ESAT-6 and CFP-10, was significantly correlated with exposure to the index case and showed excellent agreement with the TST [5]. Recently, a novel in-tube format of QFT-G (QFT-GIT) has been marketed, which, apart from peptides of ESAT-6 and CFP-10, contains an additional peptide of the *M. tuberculosis*-specific antigen TB7.7 (Rv 2654) [6].

Although the novel IGRAs are highly specific for *M. tuberculosis*, the TST still remains a valuable assay, as its positive predictive value for the risk of development of active TB has been well documented [7]. Moreover, the documented beneficial effect of isoniazid (INH) prophylaxis for the reduction of secondary TB cases is based

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on studies using TST results as the indicator for latent TB infection [8–10]. According to recently issued guidelines by the National Institute for Health and Clinical Excellence (NICE) in the UK, a two-step procedure using TST followed by an IGRA in case the TST is positive, is advocated as the method of choice to screen for latent *M. tuberculosis* infection.

From the two-step TST, it is known that an increase in tuberculin reaction can be observed, which is believed to result from immunological recall of pre-existing delayed-type hypersensitivity response to mycobacterial antigens [11]. Tuberculin contains fragments of the *M. tuberculosis*-specific antigens that are also used in QFT-G(IT). It is therefore conceivable that TST administration could also boost *in vitro* immune responses to peptides of these antigens. This would be relevant if TST and IGRA are used sequentially, as suggested in the above-mentioned NICE guidelines for TB. Implementation of sequential use of TST and IGRA would be facilitated if it can be demonstrated that IGRA can be reliably performed on the day of TST reading. The present study aimed to evaluate the effect of TST administration on the result of a commercially available IGRA, QFT-GIT, on the day of reading of the TST.

MATERIALS AND METHODS

Study setting and study subjects

Study subjects

Persons aged ⩾18 yrs with a documented TST induration of ⩾10 mm after known exposure to *M. tuberculosis* and TST negative (0 mm) individuals were eligible for the study. Study subjects were recruited with the aim of covering a wide spectrum of ages and intervals since exposure or last TST, in order to allow evaluation of TST effect in relation to the time elapsed since infection with *M. tuberculosis.* The minimum interval since the last TST was 6 months in order to avoid hyperresponsive TST reactions. Individuals with a known immune defect due to HIV infection or treatment with immunosuppressive drugs were excluded from participation.

Study setting

The study protocol (P04-183) was approved by the Institutional Review Board of the Leiden University Medical Center, Leiden, The Netherlands. Oral and written informed consent was obtained from all study subjects. Prior to the TST, 2 mL of blood was obtained for QFT-GIT. The following data were collected by questionnaire: 1) demographic data; 2) medical history; 3) BCG vaccination status; 4) exposure to smear-positive TB patients; 5) indication for previous TST; 6) date of previous TST; and 7) previous TST results. All participants underwent a TST for the purpose of this study (day 0). The TST was read 72 h later (day 3) and, at the same time, a second blood sample of 2 mL was obtained for QFT-GIT.

Tuberculin skin testing

The TST was performed by trained personnel following standard procedures. In brief, 0.1 mL (2 TU) of purified protein derivative (PPD, RT23; Statens Serum Institute, Copenhagen, Denmark) was injected intradermally into the dorsal side of the left forearm. Transverse induration at the TST site was measured after 72 h by experienced personnel.

Whole blood IGRA, QFT-GIT

Blood samples of 1 mL were collected in two special tubes for the QFT-GIT (Cellestis Ltd. Carnegie, Victoria, Australia): one coated with *M. tuberculosis*-specific peptides of ESAT-6, CFP-10 and TB7.7 (Rv2654, only peptide 4) and one without antigen as negative control. Within 8 h of blood sampling, tubes were incubated for 24 h at 37°C, followed by centrifugation and cold storage until testing as specified by the manufacturer. The concentration of IFN-γ in plasma was measured using the commercial QFT-GIT ELISA. The test result was determined as either negative or positive (cut-off at 0.35 IU·mL^{-1}), using the software of the manufacturer (qualitative test result), and as the IFN-γ concentration in IU·mL^{-1} (quantitative test result).

Statistical analysis

For comparison of qualitative QFT-GIT results of each individual before and after the TST, McNemar's test was used and a Wilcoxon signed rank test was performed to compare the strength of the IFN-γ response. Patient characteristics between QFT-GIT-positive and -negative individuals were compared using a Chi-squared or a Student's unpaired t-test as appropriate. A p-value <0.05 was considered statistically significant.

RESULTS

Characteristics of study subjects and tuberculin skin test results

In all, 66 Dutch subjects were included (table 1). Fifteen healthy subjects were used as TST-negative controls. Out of a total of 46 TST-positive individuals, 23 had been exposed to a case of smear-positive pulmonary TB between 6 months and 1 yr before participating in the study (table 1). Of the remaining 23 TST-positive subjects (table 1), 10 had been exposed >3 yrs ago (median 4.5 yrs, range 3–53 yrs) and 13

TABLE 1 Characteristics of study subjects

	Subjects n	Age yrs	Male sex	BCG	INH	TST mm
TST negative	15	44 (25–77)	1 (7)	1 (6.6)		0
TST positive	46	48 (23–62)	24 (52)	9 (19)	9 (19)	16.8±0.7 (8–29)
Recent exposure	23	47 (23–60)	12 (52)	1 (4)	3 (13)	18.0±0.9 (10–25)
Remote exposure/screening	23	49 (24–62)	12 (52)	8 (35)	6 (26)	15.7±1 (8–29)
Cured TB	5	51 (36–66)	4 (80)	0 (0)		19.2±2.7 (10–27)

Data are presented as mean (range), n (%) or mean±SEM (range), unless otherwise stated. BCG: bacille Calmette–Guérin; INH: isoniazid; TST: tuberculin skin test; TB: tuberculosis.

had become TST positive >2 yrs ago and were found during routine screening because of a professionally related increased risk of exposure to TB patients. Five additional study subjects had been successfully treated for active TB 1.5–52 yrs before the study.

All 15 control subjects had a TST of 0 mm. In the total group of TST-positive individuals, the mean TST result during the study was 16.8 mm. The mean TST in 23 recently exposed individuals was 18 mm. In the latter group, only one subject was BCG vaccinated (TST of 18 mm) and three had been treated with INH. The remaining 23 TST-positive individuals had a mean TST of 15.7 mm. In this group, eight subjects were BCG vaccinated (mean TST 16 mm) and six had received treatment with INH. Among five cured TB patients, the mean TST was 19.2 mm.

QFT-GIT before tuberculin skin testing

QFT-GIT was negative in all TST-negative controls (table 2). Out of the five cured TB patients, four had a strongly positive QFT-GIT results and in one subject, who had suffered TB 48 yrs earlier, the IFN-γ response was just below the cut-off level of the assay. QFT-GIT results were positive in 17 (37%) out of the 46 TST-positive individuals. Out of the 37 BCG-unvaccinated individuals, 15 (40.5%) had a positive test result. Among the 23 TST-positive individuals with recent exposure, 11 (48%) had a positive QFT-GIT result (median IFN-γ of 1.06 IU·mL^{-1} among subjects with a positive test), while six out of 23 (26%) of TST-positive individuals with a more remote exposure had a positive test result (median IFN-γ of 0.76 IU·mL^{-1} among positive responders; table 2). However, these differences were not statistically significant. In QFT-GIT-positive subjects, the mean interval between TST conversion and blood testing was shorter than in QFT-GIT-negative subjects (4.2 and 7.5 yrs, respectively) but this did not reach a level of significance.

When considering a possible effect of earlier treatment, it was found that only one (6%) QFT-GIT-positive subject had been treated with INH, compared with eight (28%) QFT-GIT negative individuals (p=0.07). No differences in age were observed between QFT-GIT-positive and -negative individuals.

Influence of tuberculin skin test on QFT-GIT

QFT-GIT was repeated at the time of reading of the TST (table 2). QFT-GIT results on days 0 and 3 are presented in figure 1. Due to logistical reasons, in two subjects (fig. 1) the blood sample for the second QFT-GIT was taken on days 10 and 11 after the TST. In the TST-negative group, QFT-GIT results remained negative. Overall, QFT-GIT results before and after TST were concordant in 95.5%. The percentage of individuals with test result that converted from negative to positive (two out of 45; 4.4%) was equal to those with a test result that changed from positive to negative (one out of 21; 4.8 %). If the analysis was restricted to those subjects who were re-evaluated precisely on day 3, these percentages were 2.3% (one out of 44) and 5.0% (one out of 20), respectively. Thus, no significant boosting of QFT-GIT results by TST was observed. Interestingly, in the two cases in which QFT-GIT converted from negative to positive, IFN-γ responses on day 0 were just below the cut-off of the assay. Latent infection with *M. tuberculosis* was likely in these subjects, one of whom was a Dutch BCG-unvaccinated individual with a TST of 25 mm observed during a contact investigation and the other was a Dutch, BCG-unvaccinated healthcare worker with a TST conversion. It is noteworthy that in the latter person, the blood sample for the second QFT-GIT was taken on day 10 after TST instead of on day 3. In a few *M. tuberculosis*-infected individuals, quantitative boosting of a positive IFN-γ response was seen following TST administration but responses remained at a similar level in most cases (fig. 1). No significant rise in IFN-γ responses between day 0 and day 3 could be observed in any of the study groups (table 2).

DISCUSSION

The present study reports the first evaluation of the effect of TST administration on *in vitro* immune responses to *M. tuberculosis*-specific peptides, as measured by QFT-GIT on the day of reading of TST. The results demonstrated that boosting of QFT-GIT response was rare and did not jeopardise the

TABLE 2 QuantiFERON®-TB Gold in-tube (QFT-GIT) results before and after tuberculin skin test (TST)

	Study subjects	QFT-GIT results			Discordant QFT-GIT results	
		Before TST[#]	After TST[#,¶]	Difference[+]	Negative to positive	Positive to negative
TST negative	15	0 (0)	0 (0)	NS	0	0
TST positive[§]	46	17 (37)	18 (39)	NS	2	1
Recent exposure	23	11 (48)	11 (48)	NS	1	1
Remote exposure/screening	23	6 (26)	7 (30)	NS	1[f]	0
Cured TB	5	4 (80)	4 (80)	NS	0	0
Total	66	21	22	NS	2 (4.4)	1 (4.8)

Data are presented as n or n (%). TB: tuberculosis; NS: nonsignificant. [#]: Number of positive results; [¶]: the second QFT-GIT was taken on day 3 at the time of reading of the TST (n=64) and in two subjects on days 10 and 11, respectively; [+]: mcNemar's test for comparison of qualitative QFT-GIT results and a Wilcoxon signed rank test for quantitative interferon-γ responses before and after TST; [§]: defined as documented TST ⩾10 in the past after known exposure to TB; [f]: second QFT-GIT was taken on day 10.

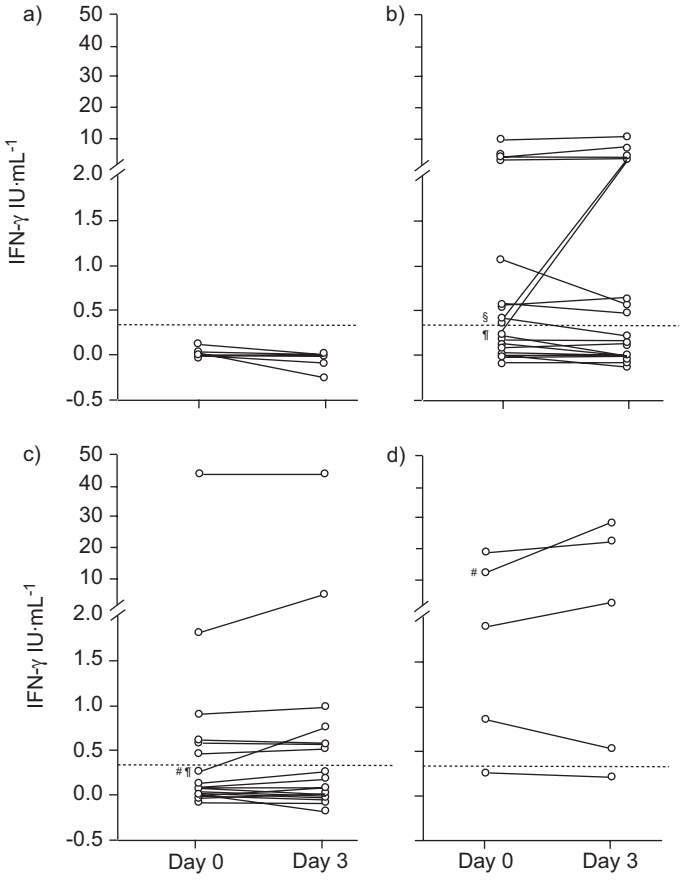

FIGURE 1. Effect of tuberculin skin test (TST) on QuantiFERON®-TB Gold in-tube (QFT-GIT) response in a) controls, b) recent TST converters, c) remote TST converters and d) tuberculosis (TB) patients. QFT-GIT was carried out before TST administration (day 0) and 72 h later (day 3), at the time of reading of the TST in 15 TST-negative subjects, 23 TST-positive subjects with recent exposure to *Mycobacterium tuberculosis*, 23 TST-positive subjects with a more remote exposure and five cured TB patients. ------: The cut-off for a positive QFT-GIT response (interferon (IFN)-γ of 0.35 IU·mL^{-1}). #: in two persons, a second QFT-GIT was done on day 10/11 instead of on day 3. In two subjects, QFT-GIT response was boosted from negative to positive (¶), and in one the opposite was seen (§).

specificity of the QFT-GIT assay for the detection of *M. tuberculosis* infection.

To reach these conclusions, QFT-GIT was performed just before TST and on the day of reading of TST in 66 individuals, including TST-negative controls, TST-positive subjects with known exposure to TB and cured TB patients. Participants underwent a TST for the purpose of the present study only and sequential QFT-GIT samples were processed identically. However, the present study also has several possible limitations. First, the effect of TST on QFT-GIT was evaluated only 3 days after TST administration. From a two-step TST, it is known that maximal boosting of TST responses were found after an interval of 1–5 weeks [12, 13]. Therefore, boosting of QFT-G(IT) after a longer interval cannot be excluded. In the two cases where QFT-GIT was repeated on days 10 and 11 after TST, a clear rise in IFN-γ response was observed; however, this could be caused by chance alone. In a recent study of 48 individuals, no boosting of the QFT-TB (based on PPD) was observed when the blood test was repeated 3 months after the TST [14]. Although the present results are only applicable when the QFT-GIT is carried out on the day of TST reading, the information is very relevant, as this is the most practical moment to perform an IGRA when a two-step procedure (using a TST followed by an IGRA) is used for the screening of latent *M. tuberculosis* infection.

Secondly, the relatively small number of study individuals represents another possible limitation. However, individuals in whom boosting was most likely to occur were specifically selected. With repeated TST, the phenomenon of boosting is associated with a prior BCG vaccination, sensitisation to nontuberculous mycobacteria and remote *M. tuberculosis* infection [15–18]. Since the peptides used in QFT-G(IT) are absent from BCG and from most environmental mycobacteria, except for *Mycobacterium kansasii*, *Mycobacterium marinum* and *Mycobacterium szulgai* [19, 20], a boosted QFT-G(IT) response, if present, would be most likely to be expected in the group with a remote infection. Therefore, a substantial number of individuals with well-defined remote *M. tuberculosis* infection was specifically included in the present study. These data show that there was no systematic change of QFT-GIT results 3 days after TST administration, also in the subgroup with remote *M. tuberculosis* infections. Similarly, no significant change in IFN-γ response was observed when evaluating quantitative QFT-GIT results. Only in a few cases did TST enhance pre-existing immune responses to *M. tuberculosis*-specific peptides, and it rarely caused a negative-to-positive change in QFT-G results. In the TST-negative control group, no rise in IFN-γ response was seen in any of the cases.

Previous studies indicated that *M. tuberculosis*-specific IGRAs, such as QFT-G and ELISPOT, are both specific and sensitive for the detection of latent *M. tuberculosis* infection [21–24]. In the present study, however, only 37% of TST-positive subjects had a positive QFT-G result. This group consisted mainly of non-BCG-vaccinated individuals with a documented TST of $\geqslant$10 mm after exposure to a case of smear-positive pulmonary TB. In others, a TST conversion was observed during routine screening because of a professionally related increased risk of exposure to TB patients. Furthermore, data available from another study in the present authors' laboratory (S.M. Arend, Dept of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands; personal communication) comparing short *versus* long incubation IGRAs showed that 22 out of 24 (92%) of the TST-positive study subjects had IFN-γ responses to *M. tuberculosis*-specific peptides of ESAT-6, CFP-10 and/or TB 7.7 in a 6-day cell culture. Since these antigens are highly specific for *M. tuberculosis* [25], also when tested in a 6-day cell culture [26–28], these data indicated that a latent *M. tuberculosis* infection was most likely in TST-positive subjects in the present study. Two other recent studies have also found a lower sensitivity of QFT-G than TST for detection of remote *M. tuberculosis* infection. In a recent study among jail inmates, more than half of the US-born TST-positive subjects were QFT-G negative [29]. In a mostly BCG-vaccinated Korean control population, 51% was TST positive and only 4% was QFT-G positive, while the expected prevalence of *M. tuberculosis* infection was 33% [30]. Thus, differences in sensitivity of QFT-G(IT) might be related to differences in study population,

such as recent *versus* remote exposure, high- *versus* low-risk population, BCG background and prior treatment.

In conclusion, the present study demonstrated that systematic boosting of QuantiFERON-TB® Gold in-tube responses from negative to positive following tuberculin skin test administration does not occur and therefore shows that tuberculin skin test administration does not jeopardise the complete specificity of the assay. Thus, sequential use of tuberculin skin test for initial screening, followed by a QuantiFERON®-TB Gold (in-tube) on the day of tuberculin skin test reading is a reliable approach with which to screen for latent tuberculosis.

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REFERENCES

1 Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 2004; 4: 761–776.
2 Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000; 356: 1099–1104.
3 Mori T, Sakatani M, Yamagishi F, *et al.* Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. *Am J Respir Crit Care Med* 2004; 170: 59–64.
4 Ravn P, Munk ME, Andersen AB, *et al.* Prospective evaluation of a whole-blood test using *Mycobacterium tuberculosis*-specific antigens ESAT-6 and CFP-10 for diagnosis of active tuberculosis. *Clin Diagn Lab Immunol* 2005; 12: 491–496.
5 Brock I, Weldingh K, Lillebaek T, Follmann F, Andersen P. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. *Am J Respir Crit Care Med* 2004; 170: 65–69.
6 Brock I, Weldingh K, Leyten EM, Arend SM, Ravn P, Andersen P. Specific T-cell epitopes for immunoassay-based diagnosis of *Mycobacterium tuberculosis* infection. *J Clin Microbiol* 2004; 42: 2379–2387.
7 Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 1974; 99: 131–138.
8 Comstock GW, Baum C, Snider DE Jr. Isoniazid prophylaxis among Alaskan Eskimos: a final report of the bethel isoniazid studies. *Am Rev Respir Dis* 1979; 119: 827–830.
9 Comstock GW, Ferebee SH, Hammes LM. A controlled trial of community-wide isoniazid prophylaxis in Alaska. *Am Rev Respir Dis* 1967; 95: 935–943.
10 Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc* 1970; 26: 28–106.
11 Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. *Am J Respir Crit Care Med* 1999; 159: 15–21.
12 Cauthen GM, Snider DE Jr, Onorato IM. Boosting of tuberculin sensitivity among Southeast Asian refugees. *Am J Respir Crit Care Med* 1994; 149: 1597–1600.
13 Thompson NJ, Glassroth JL, Snider DE Jr, Farer LS. The booster phenomenon in serial tuberculin testing. *Am Rev Respir Dis* 1979; 119: 587–597.
14 Nguyen M, Perry S, Parsonnet J. QuantiFERON-TB predicts tuberculin skin test boosting in U.S. foreign-born. *Int J Tuberc Lung Dis* 2005; 9: 985–991.
15 Menzies R, Vissandjee B, Rocher I, St Germain Y. The booster effect in two-step tuberculin testing among young adults in Montreal. *Ann Intern Med* 1994; 120: 190–198.
16 Richards NM, Nelson KE, Batt MD, Hackbarth D, Heidenreich JG. Tuberculin test conversion during repeated skin testing, associated with sensitivity to nontuberculous mycobacteria. *Am Rev Respir Dis* 1979; 120: 59–65.
17 Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. *Am J Respir Crit Care Med* 1999; 159: 15–21.
18 Thompson NJ, Glassroth JL, Snider DE Jr, Farer LS. The booster phenomenon in serial tuberculin testing. *Am Rev Respir Dis* 1979; 119: 587–597.
19 Arend SM, de Haas P, Leyten E, *et al.* ESAT-6 and CFP-10 in clinical *versus* environmental isolates of *Mycobacterium kansasii*. *J Infect Dis* 2005; 191: 1301–1310.
20 Arend SM, van Meijgaarden KE, de Boer K, *et al.* Tuberculin skin testing and *in vitro* T cell responses to ESAT-6 and culture filtrate protein 10 after infection with *Mycobacterium marinum* or *M. kansasii*. *J Infect Dis* 2002; 186: 1797–1807.
21 Shams H, Weis SE, Klucar P, *et al.* Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis infection. *Am J Respir Crit Care Med* 2005; 172: 1161–1168.
22 Ewer K, Deeks J, Alvarez L, *et al.* Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 2003; 361: 1168–1173.
23 Lalvani A, Pathan AA, Durkan H, *et al.* Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Lancet* 2001; 357: 2017–2021.
24 Brock I, Weldingh K, Lillebaek T, Follmann F, Andersen P. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. *Am J Respir Crit Care Med* 2004; 170: 65–69.
25 Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 2004; 4: 761–776.
26 Ravn P, Demissie A, Eguale T, *et al.* Human T cell responses to the ESAT-6 antigen from *Mycobacterium tuberculosis*. *J Infect Dis* 1999; 179: 637–645.
27 Munk ME, Arend SM, Brock I, Ottenhoff TH, Andersen P. Use of ESAT-6 and CFP-10 antigens for diagnosis of extrapulmonary tuberculosis. *J Infect Dis* 2001; 183: 175–176.
28 Arend SM, Andersen P, van Meijgaarden KE, *et al.* Detection of active tuberculosis infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10. *J Infect Dis* 2000; 181: 1850–1854.
29 Porsa E, Cheng L, Seale MM, *et al.* Comparison of a new ESAT-6/CFP-10 peptide-based gamma interferon assay and a tuberculin skin test for tuberculosis screening in a moderate-risk population. *Clin Vaccine Immunol* 2006; 13: 53–58.
30 Kang YA, Lee HW, Yoon HI, *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: 2756–2761.