Interferon-γ assays in the immunodiagnosis of tuberculosis: a systematic review

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A major challenge in tuberculosis control is the diagnosis and treatment of latent tuberculosis infection. Until recently, there were no alternatives to the tuberculin skin test (TST) for diagnosing latent tuberculosis. However, an alternative has now emerged in the form of a new in-vitro test: the interferon-γ assay. We did a systematic review to assess the performance of interferon-γ assays in the immunodiagnosis of tuberculosis. By searching databases, contacting experts and test manufacturers, we identified 75 relevant studies. The results suggest that interferon-γ assays that use *Mycobacterium tuberculosis*-specific region of difference 1 (RD1) antigens (such as early secretory antigenic target 6 and culture filtrate protein 10) may have advantages over the TST, in terms of higher specificity, better correlation with exposure to *M tuberculosis*, and less cross-reactivity due to BCG vaccination and non-tuberculous mycobacterial infection. However, interferon-γ assays that use RD1 antigens in isolation may maximise specificity at the cost of sensitivity. Assays that use cocktails of RD1 antigens seem to overcome this problem, and such assays have the highest accuracy. RD1-based interferon-γ assays can potentially identify those with latent tuberculosis who are at high risk for developing active disease, but this requires confirmation. There is inadequate evidence on the value of interferon-γ assays in the management of immunocompromised individuals, children, patients with extrapulmonary or non-tuberculous mycobacterial disease, and populations in countries where tuberculosis is endemic. Current evidence suggests that interferon-γ assays based on cocktails of RD1 antigens have the potential to become useful diagnostic tools. Whether this potential can be realised in practice remains to be confirmed in well designed, long-term studies.

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Infection with *Mycobacterium tuberculosis*, in most individuals, is contained by the host immune defences, and the infection remains latent.[1–3] In latent tuberculosis infection, the *M tuberculosis* bacilli that persist in symptom-free individuals can reactivate and cause active disease in about 10% of those infected over a lifetime.[1–4] An estimated one-third of the world's population is latently infected, and it is from this enormous reservoir that new cases emerge.[5] Currently, it is difficult to predict exactly who among the latently infected will develop the disease and when. A major challenge in tuberculosis control, therefore, is to be able to diagnose, predict, and treat those with latent tuberculosis before they develop active disease.

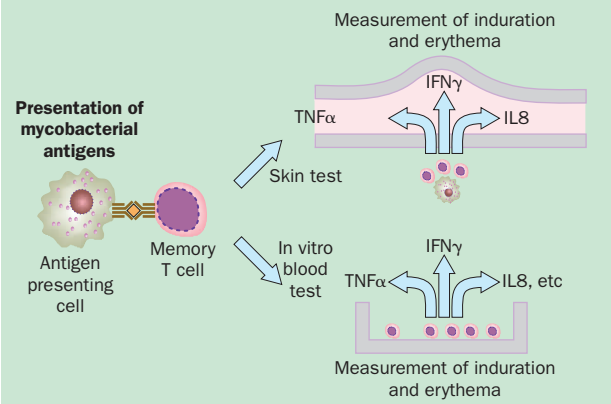

Figure 1. Biological basis of the tuberculin skin test and interferon-γ assay. TNFα=tumour necrosis factor α; IFNγ=interferon γ; IL8=interleukin 8. Reproduced with permission from Elsevier.[8]

For decades, we have had to rely on the tuberculin skin test (TST) to diagnose latent tuberculosis. First introduced in 1890, it is the oldest diagnostic test in use.[6,7] The TST attempts to measure cell-mediated immunity in the form of a delayed-type hypersensitivity response to the purified protein derivative (PPD). The PPD is a crude mixture of antigens, many of which are shared among *M tuberculosis*, *Mycobacterium bovis* BCG, and several non-tuberculous mycobacteria (NTM).[6–8] As a result, the TST has lower specificity in populations with high BCG coverage and NTM exposure.[2,6,8,9] The sensitivity may be low in individuals with depressed immunity (eg, AIDS and other immunosuppressive conditions, advanced tuberculosis, malnutrition).[2,6,8,9] The administration and reading of this test is not easy as variability between and within readers is a concern, trained personnel are required, and patients have to be seen a second time so that test results can be read.[6,9] Furthermore, there is some evidence that the TST might act

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as a microvaccination and elicit a systemic effect on the anti-tuberculosis T-cell immune response.[10]

Despite these limitations, the TST is still widely used because of its ability to predict active disease in latently infected individuals, and the fact that trials have shown that treatment of latent tuberculosis, diagnosed on the basis of TST results, reduces the risk of active disease by about 60%.[2,11] This strong experimental evidence has resulted in targeted skin testing and latent tuberculosis treatment programmes in developed countries.[2,11] A major advantage of the TST is its low material cost, and the fact that it does not require any laboratory infrastructure.

For the first time, an alternative to the TST has emerged in the form of a new type of in-vitro T-cell-based assay: the interferon-γ assay.[8,12–15] interferon-γ assays are based on the principle that T cells of individuals sensitised with tuberculosis antigens produce interferon γ when they re-encounter mycobacterial antigens (figure 1).[8] A high level of interferon-γ production, therefore, is presumed to be indicative of tuberculosis infection. Whereas initial research focused on interferon-γ assays that used PPD as the stimulating antigen, newer assays use antigens specific to *M tuberculosis*, such as the early secretory antigenic target 6 (ESAT6) and culture filtrate protein 10 (CFP10).[8,12] These proteins, encoded by genes located within the region of difference 1 (RD1) of the *M tuberculosis* genome, are significantly more specific to *M tuberculosis* than PPD, as they are not shared with any BCG substrains or most NTM species (with the exception of *Mycobacterium kansasii*, *Mycobacterium marinum*, and *Mycobacterium szulgai*).[8,16] The development of these specific RD1 antigens has been reviewed elsewhere.[8,12,14]

Research over the past decade[8,14,17] has resulted in the development of two commercial interferon-γ assays, the QuantiFERON-TB assay (Cellestis Limited, Carnegie, Victoria, Australia), and the T SPOT-TB assay (Oxford Immunotec, Oxford, UK). Both tests measure cell-mediated immunity by measuring interferon γ released from T cells in response to tuberculosis antigens, using methods such as ELISA and enzyme-linked immunospot (ELISPOT) assay (figure 2). The first-generation QuantiFERON-TB is a whole-blood assay that measures interferon-γ response to PPD with ELISA. This test is approved by the US Food and Drug Administration (FDA), and is commercially available in many countries.[18] The enhanced QuantiFERON-TB Gold assay (which uses ESAT6 and CFP10) is awaiting FDA approval.

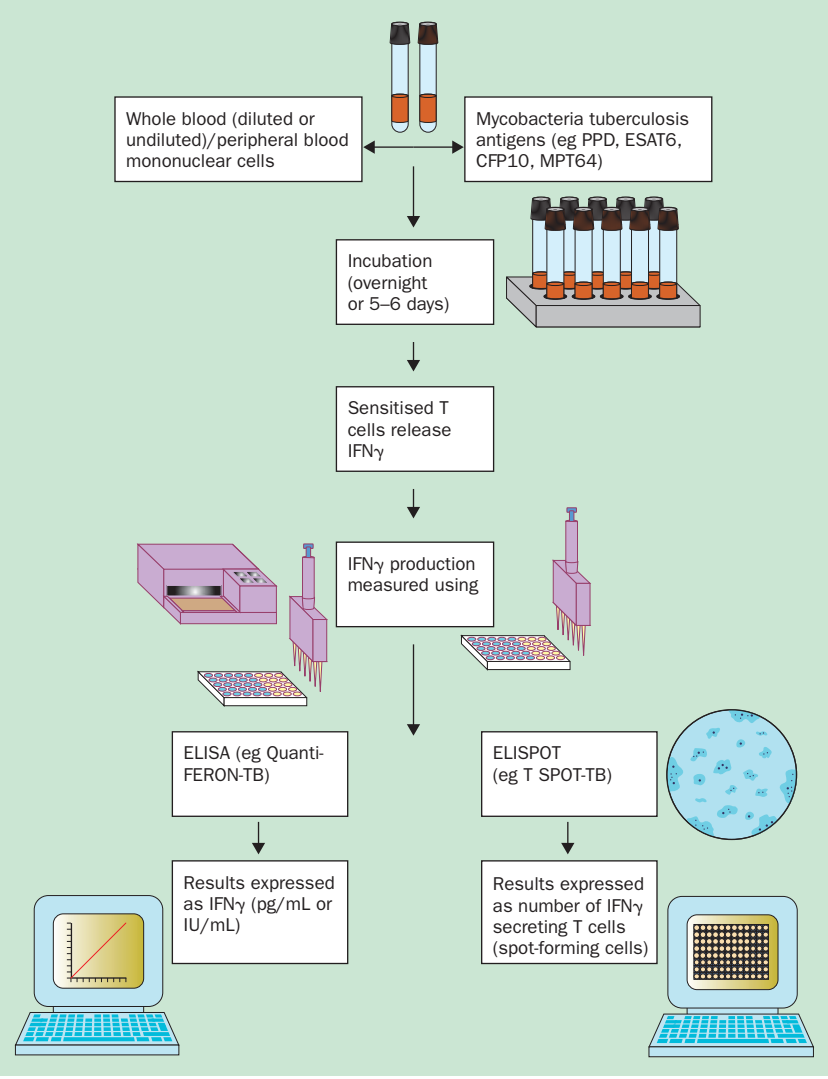

Figure 2. Overview of the interferon-γ (IFNγ) assay technology.

The T SPOT-TB assay, which uses peripheral blood mononuclear cells (PBMCs), uses ESAT6 and CFP10, and detects (by use of ELISPOT) the number of T cells producing interferon γ.[14] This test, currently approved for use in Europe, is awaiting FDA approval.[14] In addition to these two commercial assays, in-house assays (laboratory-developed tests that are not commercially available) have also been assessed.[8,13,15]

Although all interferon-γ assays are cellular immune-based tests that quantify interferon-γ response, the operational characteristics of these assays are quite variable. For example, the incubation periods used vary from short (eg, 24–48 h in QuantiFERON-TB, and T SPOT-TB) to long (5–6 days in several in-house assays). Some assays use whole blood (eg, QuantiFERON-TB), whereas others use PBMCs (eg, T SPOT-TB). PPD are used as antigens in some assays (eg, first-generation QuantiFERON-TB), whereas RD1-based antigens are used in others (eg, QuantiFERON-TB Gold, and T SPOT-TB). Finally, most

assays use the ELISA format (eg, QuantiFERON-TB), whereas some use the ELISPOT format (eg, T SPOT-TB) for interferon-γ measurement.

It has been suggested that interferon-γ assays have several advantages over the TST.[8,12,14,18,19] Because the test is done in vitro and does not involve measurements such as skin induration, the results are less subjective, and a single visit by the patient is adequate. Newer, RD1-based interferon-γ assays are also thought to be more specific than the PPD-based TST.[8,14] Although initial research largely focused on the diagnosis of latent tuberculosis, recent studies have assessed interferon-γ assays for various applications, such as (1) diagnosis of active tuberculosis, (2) distinguishing between NTM and *M tuberculosis* infection, (3) differentiating between *M tuberculosis* infection and previous BCG vaccination, (4) serving as a correlate of protective immunity and for the assessment of vaccine efficacy, (5) prediction of reactivation disease among those with latent tuberculosis, and (6) monitoring treatment response. We did a systematic review to summarise the evidence on the use of interferon-γ assays for the immunodiagnosis of tuberculosis.

Methods

Search strategy

We searched PubMed (1990 to August, 2004), Embase (1997 to 2003), Web of Science (1990 to 2003), BIOSIS (1990 to 2003), and the Cochrane Library (2003, issue 3). The search terms used included the following: "tuberculosis", "*Mycobacterium tuberculosis*", "tuberculin", "interferon-gamma", "cytokines", "Quantiferon", "ELISPOT", "T-cell response", "ESAT6", "CFP10", "interferon gamma assay", "ELISA", "accuracy", "sensitivity", and "specificity". We identified additional studies by contacting experts in the field, test manufacturers, and searching the reference lists from existing articles.

Study selection

Our search strategy was formulated to identify all available studies, published in English, on any of the following aspects of interferon-γ assays: (1) assessment among patients with active tuberculosis and healthy controls, (2) assessment among those with a high likelihood of latent tuberculosis, (3) direct comparison (eg, agreement) between TST and interferon-γ assays, (4) indirect comparison between TSTs and interferon-γ assays (eg, correlation with exposure to tuberculosis), (5) studies on the specificity of interferon-γ assays in BCG-vaccinated and NTM patients, (6) follow-up studies on the ability of interferon-γ assays to predict active tuberculosis, (7) effect of treatment on interferon-γ response, and (8) reproducibility of interferon-γ assays. The following studies were excluded: (1) animal studies, (2) reviews, (3) case reports, (4) studies on isolation of new antigens, and (5) studies designed to assess the efficacy of BCG vaccines.

Data extraction and synthesis

All articles included were assessed by a reviewer (MP), who extracted data that included the study design, participants, interferon-γ assay methods (eg, antigen, incubation time, interferon-γ measurement, cut-off point used), TST methods (eg, PPD dose, cut-off point), and outcome data (eg, sensitivity, specificity, agreement between TST and interferon-γ assays). In studies in which outcomes such as sensitivity, specificity, agreement, and kappa were not reported, these measures were calculated using the data presented in the primary studies. Study characteristics and results are shown as tables and graphs. For clarity of presentation, studies that reported results stratified by subgroups are shown more than once in tables or figures. Because the included studies varied greatly in their design, execution, and outcomes, and because meta-analysis methods are still not well defined for such heterogeneous diagnostic studies, we did not do a meta-analysis.

Results

Of the 1882 citations identified after literature searches, 75 articles met our eligibility criteria.[20–94] Table 1 shows only the main clinical questions of interest, and, therefore, includes only a subset of the 75 studies. The sections that follow address each of the questions in table 1.

What is the sensitivity of interferon-γ assays in populations with active tuberculosis?

We identified 29 studies that assessed the sensitivity of interferon-γ assays (table 2). Sensitivity is the proportion of patients with active tuberculosis who are positive by interferon-γ assays. Many of the studies also determined the sensitivity of TST in the same populations (head-to-head comparisons). All studies included patients with bacteriologically confirmed (culture or smear positive) diagnoses of tuberculosis. Most studies included HIV-negative patients who were untreated or received minimum treatment. The sensitivity estimates varied widely across studies (table 2). In head-to-head comparisons against TST, many studies reported a higher sensitivity for TST. In almost all the studies with head-to-head comparisons, PPD-based interferon-γ assays had higher sensitivity than interferon-γ assays that used ESAT6 or CFP10 in isolation. Studies that used antigen cocktails with ESAT6 and CFP10 yielded sensitivity estimates that were similar to or higher than PPD-based assays.[21,29,30,34,59,60,65,68,85]

Because concerns have been raised about the validity of using previously treated, active tuberculosis as an immunological model for assessing latent tuberculosis tests,[95,96] we examined the sensitivity of interferon-γ assays in treated versus untreated patients. Studies among treated patients showed a lower sensitivity for interferon-γ assays than TST, compared with untreated patients.[25,56,58,63] Although disease severity can also affect interferon-γ response,[46,74] most studies did not explore the effect of disease severity on sensitivity. The effect of HIV infection on sensitivity was assessed by Chapman and colleagues (table 2).[34] They showed that an ELISPOT assay (using ESAT6 or CFP10) had a sensitivity of 100% in HIV-negative patients, and this sensitivity was maintained at 90% in HIV-positive patients. By contrast, Elliott and co-workers[40] found that the interferon-γ response to PPD and to CFP was strongly

Table 1. Clinical questions of interest and studies that have addressed them

Question of interest	Number of studies
What is the sensitivity of interferon-γ assays in populations with active tuberculosis and how does it compare with that of the TST?	29
What is the specificity of interferon-γ assays in low-risk populations without active tuberculosis and how does it compare with that of the TST?	22
What is the sensitivity of interferon-γ assays in populations with suspected latent tuberculosis?	11
What is the agreement between TST and interferon-γ assays, and what factors are associated with discordance?	16
Do interferon-γ assays correlate better with exposure to *M tuberculosis* than the TST?	5
Are interferon-γ assays less affected by BCG vaccination than is the TST?	12
Are interferon-γ assays less affected by non-tuberculous mycobacterial infection than is the TST?	4
Can interferon-γ assays predict active tuberculosis among those with latent infection?	1
What is the effect of anti-tuberculosis treatment on interferon-γ response?	11
Are interferon-γ assay results reproducible?	5

impaired among HIV-positive compared to HIV-negative individuals.

What is the specificity of interferon-γ assays in populations without active tuberculosis?

22 studies assessed the specificity of interferon-γ assays (table 3). Specificity is the proportion of individuals without active tuberculosis who are negative by the interferon-γ assay. Many studies also reported comparisons against the TST. Almost all studies included healthy participants from low-endemic settings with no history of tuberculosis, and no known exposure to *M tuberculosis*. The specificity estimates varied across studies (table 3). In head-to-head comparisons against TST, most studies reported specificity estimates that were similar for the two tests; a few reported higher specificity estimates for RD1-based interferon-γ assays. interferon-γ assays that used RD1-based antigens had higher specificity than PPD-based interferon-γ assays.

Figure 3 shows the effect of antigens used (PPD *vs* RD1 antigens) on sensitivity and specificity in a receiver operating characteristic (ROC) space. Assays with RD1 antigens used in isolation had high specificity but relatively lower and variable sensitivity, whereas PPD-based assays had higher sensitivity but lower and variable specificity (figure 3). Assays that used cocktails of RD1 antigens had the highest sensitivity and specificity estimates.

What is the sensitivity of interferon-γ assays in populations with suspected latent tuberculosis?

11 studies assessed the sensitivity of interferon-γ assays among populations with a high likelihood of latent tuberculosis (table 4). Although latent tuberculosis cannot be definitively confirmed, sensitivity here refers to the proportion of those presumed to be latently infected that are positive by the interferon-γ assay. These studies included symptom-free individuals exposed to *M tuberculosis* (eg, household contacts and individuals exposed in outbreaks), who were TST-positive with normal chest radiographs and with no evidence of active disease. Of the eight studies that assessed PPD-based interferon-γ assays, seven studies reported sensitivity estimates greater than 80%. Of the nine studies that used RD1 antigens, four studies reported sensitivity estimates greater than 80%. In head-to-head comparisons, PPD-based assays had higher sensitivity than RD1-based assays.[22,54,55,64,87,88]

What is the agreement between TST and interferon-γ assays?

In the absence of a gold standard for latent tuberculosis, 16 studies reported the agreement between TST and interferon-γ assays, rather than sensitivity and specificity (table 5). Most studies reported modest agreement (60–80%) between the two tests. The kappa (κ) statistics, however, were highly inconsistent, ranging from –0·03 to 0·87. Although most studies analysed the TST and interferon-γ assay results as dichotomous outcomes, some studies reported the correlation (eg, Spearman's *r*) between the TST results (expressed as mm of induration) and interferon-γ response (expressed as IU/mL or pg/mL).[22,24–28,35,37,43,51,63,80,91] All studies, except one,[51] reported a moderate to strong positive correlation (r=0·4–0·6) between the two tests results (data not shown).

Three studies explored the reasons for discordance between TST and interferon-γ assays. Mazurek and colleagues[58] assessed the QuantiFERON-TB assay (using PPD) in a multicentre study. 1226 adults with varying risks of infection underwent both tests. The overall agreement was 83% (κ=0·60). In a multivariate model, the odds of a discordant result (ie, positive TST but negative QuantiFERON-TB) was seven times higher for BCG-vaccinated than for unvaccinated individuals. Fietta and colleagues[43] also assessed QuantiFERON-TB (using PPD) among 258 individuals with varying risks. The overall agreement was 78% (κ=0·58). Agreement was poor (κ=0·25) among patients with active tuberculosis; whereas the interferon-γ assay detected 91% of the tuberculosis cases, the TST was positive in only 65%. Most of the patients who were positive on the interferon-γ assay but negative by TST had at least one factor that increases the risk of tuberculosis but also results in false-negative TST: old age, hepatitis C, alcoholism, renal failure, steroid therapy, and cancer.

Ewer and co-workers[41] investigated a school outbreak that resulted from one infectious index case, using the ELISPOT assay (with ESAT6 and CFP10) and the Heaf test. The overall agreement between the two tests was 89% (κ=0·72). The ELISPOT assay showed no significant relation to BCG status. By contrast, BCG-vaccinated children were significantly more likely to have higher Heaf grades than unvaccinated children. An isolated positive ELISPOT result (ELISPOT-positive but TST-negative) was associated with exposure to *M tuberculosis*, whereas an isolated positive TST (TST-positive but ELISPOT-negative) result was not.

Table 2. Studies on sensitivity of interferon-γ assays in populations with active tuberculosis

Study (first author, year, country)	Design	Study population*	Treatment status	Antigen	Interferon-γ cut-off point	Sensitivity [true positives / (true positives + false negatives)] (%)	
						Interferon-γ assay	TST
QuantiFERON-TB assay (ELISA, whole blood, short incubation)							
Streeton, 1998, Australia[75]	Case-control	12 active TB and 41 treated TB patients	Active TB: NR Previous TB: fully treated	PPD	15% response ratio	Active: 10/12 (83%) Previous: 24/41 (59%)	..
Pottumarthy, 1999, New Zealand[63]	Case-control	60 patients (pulmonary and extra-pulmonary); HIV negative	Some patients were treated, duration NR	PPD	15% response ratio	46/60 (77%)	33/38 (87%)
Johnson, 1999, Australia[50]	Intervention	19 patients (pulmonary and extra-pulmonary); HIV negative	<12 days of treatment	PPD, ESAT6	15% response ratio	PPD: 12/19 (63%) ESAT6: 11/19 (58%)	..
Stuart, 2000, Australia[76]	Cohort	19 patients (pulmonary and extra-pulmonary)	Before treatment or <2 weeks of treatment	PPD	15% response ratio	12/18 (67%)	..
Mazurek, 2001, USA[58]	Cross-sectional	94 TB suspects; and 87 fully treated patients; HIV-negative	TB suspects: <6 weeks of treatment	PPD	15% response ratio	TB suspects: 66/94 (70%) Previous TB: 56/87 (64%)	TB suspects: 80/94 (85%) Previous TB: 83/87 (95%)
Brock, 2001, Denmark[29]	Case-control	18 patients (pulmonary and extra-pulmonary)	<1 month of treatment	PPD, ESAT6, CFP10	PPD: 15% response ratio; ESAT6/CFP10: 5% response ratio	PPD: 14/18 (78%); ESAT6/CFP10: 14/18 (78%)	..
Bellete, 2002, USA, Ethiopia[25]	Case-control	21 patients with treated, active TB	Fully treated	PPD	15% response ratio	15/21 (71%)	20/21 (95%)
Fietta, 2003, Italy[43]	Case-control	57 patients (pulmonary); HIV-negative	Untreated	PPD	15% response ratio	52/57 (91%)	37/57 (65%)
Mori, 2004, Japan[59]	Case-control	118 patients; HIV-negative	Untreated or treated <7 days	ESAT6, CFP10	>0.35 IU/mL	105/118 (89%)	50/76 (66%)
Ravn, 2004, Denmark[65]	Cross-sectional	48 patients (mostly pulmonary)	Untreated or treated <7 days	ESAT6, CFP10	>0.35 IU/mL	ESAT6: 31/48 (65%) CFP10: 32/48 (67%) ESAT6/CFP10:40/48 (83%)	..
Brock, 2004, Denmark[30]	Case-control	8 patients with active TB, and 13 individuals with latent TB	Untreated or treated <7 days	ESAT6, CFP10	ESAT6: 94 pg/mL CFP10: 80 pg/mL	ESAT6: 18/21 (86%) CFP10: 15/21 (71%) ESAT6/CFP10: 18/21 (86%)	..
T SPOT-TB assay (ELISPOT, PBMCs, short incubation)							
Lalvani, 2001, UK[55]	Case-control	47 patients (pulmonary and extra-pulmonary)	Untreated or treated for <1 month	PPD, ESAT6	5 SFC more than, and at least twice as many as, negative control wells	PPD: 47/47 (100%) ESAT6: 45/47 (96%)	18/26 (69%)
Lalvani, 2001, India[53]	Case-control	50 patients (mostly pulmonary); 12% HIV-positive	Untreated or treated for <1 month	ESAT6	5 SFC more than, and at least twice as many as, negative control wells	40/50 (80%)	..
Pathan, 2001, UK[62]	Case-control	25 patients (pulmonary); HIV negative	Untreated or treated for <1 month	ESAT6	5 SFC more than, and at least twice as many as, negative control wells	23/25 (92%)	..
Chapman, 2002, Zambia[34]	Case-control	50 patients; 78% HIV positive	Untreated or treated for <1 month	PPD, ESAT6, CFP10	5 SFC more than, and at least twice as many as, negative control wells	HIV negative: PPD 11/11 (100%) ESAT6/CFP10 11/11 (100%) HIV positive: PPD 28/39 (72%) ESAT6/CFP10 35/39 (90%)	..

(Continued on next page)

Table 2. Studies on sensitivity of interferon-γ assays in populations with active tuberculosis (continued)

Study (first author, year, country)	Design	Study population*	Treatment status	Antigen	Interferon-γ cut-off point	Sensitivity [true positives / (true positives + false negatives)] (%)	
						Interferon-γ assay	TST
In-house assays (ELISA, PBMCs, long incubation)							
Arend, 2000, Netherlands[21]	Case-control	37 patients (pulmonary extrapulmonary); HIV-negative	Untreated, on treatment, and or treated	ESAT6, CFP10	300 pg/mL	ESAT6/CFP10: 31/37 (84%)	..
Cardoso, 2002, Brazil[32]	Case-control	60 patients (mostly pulmonary); HIV-negative	>30 days of treatment	PPD, ESAT6, Ag85	>100 pg/mL	PPD: 57/60 (95%) ESAT6: 36/60 (60%) Ag85: 54/60 (90%)	..
Lein, 1999, USA[56]	Case-control	27 patients (pulmonary); HIV negative	On treatment or fully treated	PPD, ESAT6	Antigen-stimulated OD values divided by that of non-antigen control >2.0	PPD: 23/27 (85%) ESAT6: 16/27 (59%)	26/27 (96%)
Munk, 2001, Netherlands, Denmark[60]	Case-control	43 patients (pulmonary and extra-pulmonary); HIV negative	Untreated	PPD, ESAT6, CFP10	ESAT6/CFP10: 300 pg/mL PPD: 2700 pg/mL	Pulmonary TB: PPD: 18/21 (86%) ESAT6/CFP10: 16/21 (76%) Extrapulmonary TB: PPD: 14/22 (64%) ESAT6/CFP10: 18/22 (82%)	..
Mustafa, 1998, Kuwait[61]	Case-series	19 patients (mostly pulmonary); HIV negative	Untreated	ESAT6	>5 IU/ml	ESAT6: 16/19 (84%)	19/19 (100%)
Ravn, 1999, Ethiopia[64]	Case-control	34 patients (pulmonary); HIV negative	Untreated	PPD, ESAT6	>300 pg/mL	PPD: 21/34 (62%) ESAT6: 11/34 (32%)	..
Ravn, 1999, Denmark[64]	Case-control	25 patients treated for active TB	On treatment or fully treated	PPD, ESAT6	>300 pg/mL	PPD: 25/25 (100%) ESAT6: 14/25 (56%)	..
Rolinck-Werninghaus, 2003, Germany[67]	Case-control	30 patients; HIV-negative	Some patients on treatment	PPD, ESAT6	>300 pg/mL	PPD: 20/24 (83%) ESAT6: 11/24 (46%)	14/30 (47%)
van Pinxteren, 2000, Denmark[85]	Case-control	24 patients with minimum, active TB	Untreated	PPD, ESAT6, CFP10	>300 pg/mL	PPD: 10/11 (91%) ESAT6/CFP10: 8/11 (73%)	..
Wu-Hsieh, 2001, Taiwan[93]	Case-control	18 treated pulmonary TB; HIV-negative	Fully treated	PPD, ESAT6	45 pg/mL	PPD: 17/18 (94%) ESAT6: 13/18 (72%)	..
Vekemans, 2001, Gambia[87]	Case-control	30 patients; HIV negative	Untreated	PPD, ESAT6	Above the mean + 3 SD of the control sample	PPD: 25/30 (83%) ESAT6: 13/30 (43%)	27/29 (93%)
Vekemans, 2004, Gambia[88]	Case-control	33 patients (pulmonary)	Untreated	PPD, CFP10	>8 pg/mL	PPD: 30/33 (91%) CFP10: 20/33 (61%)	27/30 (90%)
In-house assays (ELISPOT, PBMCs, variable incubation)							
Ulrichs, 2000, Germany, USA[81]	Case-control	15 patients	Untreated	ESAT6	SFC exceeded the mean + 3 SD of antigen-free controls	8/15 (53%)	..
Vincenti, 2003, Italy[89]	Case-control	27 patients; 26% HIV-positive	<3 weeks of treatment	PPD, ESAT6	5 SFC more than, and at least twice as many as, negative control wells	PPD: 23/27 (85%) ESAT6: 20/27 (74%)	..
Scarpellini, 2004, Italy[68]	Cross-sectional	29 patients; 31% HIV-positive	..	ESAT6, CFP10	SFC exceeded the mean + 2 SD of antigen-free controls	27/29 (93%)	..

*In all studies, tuberculosis was confirmed bacteriologically. Ag85=Antigen 85, OD=optical density, NR=not reported, SFC=spot-forming cells, TB=tuberculosis.

Do interferon-γ assays correlate better with exposure to M tuberculosis *than do TSTs?*

Five studies compared TST and interferon-γ assays with respect to their correlation with exposure to *M tuberculosis*.[31,41,45,54,66] Exposure in these studies referred to duration and proximity of contact with an infectious tuberculosis patient. In an outbreak investigation, Ewer and colleagues[41] compared the correlation of an RD1-based ELISPOT and TST with degree of exposure to the index case. On the basis of proximity and shared activities at school, 535 students were classified into four exposure groups. Odds of a test result being positive for each increase across four exposure strata increased by 2·78 (95% CI 2·22–3·48) for ELISPOT and 2·33 (1·88–2·88) for TST. The ELISPOT assay correlated significantly better with exposure than did TST (p=0·03). In a similar study, Brock and colleagues[31] did a contact investigation of 125 contacts (40 were BCG vaccinated) of an index case in a high school in Denmark. Among the unvaccinated contacts, both the RD1-based QuantiFERON-TB Gold assay and the PPD-based interferon-γ assay discriminated between the high and low exposure groups. Among the BCG-vaccinated contacts, only the RD1-based QuantiFERON assay showed a high correlation with exposure.

In a study of 50 healthy contacts of infectious tuberculosis cases, Lalvani and co-workers[54] compared the correlation of the ELISPOT assay (using ESAT6) and TST with degree of exposure. The ELISPOT assay showed a strong positive correlation with increasing intensity of exposure (odds ratio [OR] 9·0, 95% CI 2·6–31·6, per unit increase in level of exposure), whereas TST results had a weak correlation with exposure (1·9, 1·0–3·5). Hill and colleagues[45] assessed the TST and two ELISPOT assays (using PPD, and ESAT6 or CFP10) among 735 household contacts of tuberculosis patients. Exposure was defined in terms of whether the contacts slept in the same bedroom as the patient, or elsewhere. Although all three tests showed a positive correlation with the exposure gradient, the TST showed the most marked change across exposure categories. The percentage of individuals who had positive TST results and negative ESAT6 or CFP10 ELISPOT results increased significantly with increasing exposure.

Richeldi and colleagues[66] investigated *M tuberculosis* transmission after nosocomial exposure to an infectious case of multidrug-resistant tuberculosis. In this study, after childbirth, a mother was diagnosed to have smear-positive multidrug-resistant tuberculosis. 41 neonates and 47 adults who were present during her admission in the maternity unit were screened with TST and a RD1-based ELISPOT assay. 17 individuals were ELISPOT positive, whereas only four were TST positive. The ELISPOT results correlated significantly with tuberculosis exposure. For each hour that room air was shared with the index case, the odds of a positive ELISPOT result increased by 1·05 (1·02–1·09). The TST results, by contrast, did not correlate with exposure.

Are interferon-γ assays less affected by BCG vaccination than is the TST?

Several studies examined the effect of BCG vaccination on interferon-γ assay performance. The specificity of RD1-based interferon-γ assays was much higher than the TST and PPD-based interferon-γ assays in every study that reported specificity stratified by BCG status (table 3). Other studies, including contact and outbreak investigations, have shown similar findings. Lalvani and co-workers[54] explored the correlation between ELISPOT (using PPD and ESAT6) and BCG status in a contact investigation study. The ESAT6-based ELISPOT showed no correlation with BCG status (1·5, 0·2–12·0), whereas TST results were significantly more likely to be positive in BCG-vaccinated contacts (12·1, 1·3–115·7). The PPD-based ELISPOT was also significantly associated with BCG status (9·7, 1·3–70·8).

Similar results were found in a school outbreak investigation, in which TST was significantly more likely to be positive in BCG-vaccinated individuals (p=0·002), whereas ELISPOT results were not (p=0·44).[41] A recent study from Denmark found that a RD1-based QuantiFERON-TB Gold assay was less influenced by BCG status of the contacts, than was a PPD-based assay.[31] Studies on QuantiFERON-TB assays with ESAT6 or CFP10 also showed higher specificity estimates in BCG-vaccinated individuals than TST and PPD-based assays.[29,50]

Are interferon-γ assays less affected by NTM infection than is the TST?

Four studies assessed the effect of NTM infection on the performance of interferon-γ assays. Lein and colleagues[56] compared an interferon-γ assay (using PPD and ESAT6) with the TST among patients with culture-confirmed *M avium* infection (table 3). The TST and ESAT6-based assay were negative in all patients (100% specificity). The PPD-based assay had a specificity of 75%. Another study among *M avium*-infected patients had a 100% specificity for an ESAT6-based interferon-γ assay, whereas the TST had a specificity of only 60%.[67] Mazurek and colleagues[58] found that reactivity to NTM may be the cause of a positive TST result in one-fifth of the non-BCG-vaccinated individuals who were TST positive but QuantiFERON-TB negative.

Although these studies showed that interferon-γ assays were less influenced than TST by *M avium* infection, Arend and co-workers[24] studied 12 patients infected with *M marinum* or *M kansasii*. Assays based on ESAT6, CFP10, and PPD were positive in 75%, 67%, and 90%, respectively, of the *M avium*-infected patients. This study showed that T-cell responses to ESAT6 and CFP10 were not completely specific for *M tuberculosis* infection but may result from infection with NTM such as *M marinum* and *M kansasii*. There is also limited evidence that genes for the ESAT6 and CFP10 antigens may be present in *Mycobacterium leprae*.[97] However, there are no data available on the effect of this on the specificity of interferon-γ assays in populations in which leprosy is endemic.

Can interferon-γ assays predict active tuberculosis among those with latent infection?

Doherty and colleagues[39] recruited 24 HIV-negative, healthy, household contacts of tuberculosis patients in Ethiopia. An interferon-γ assay using PPD and ESAT6 was done at baseline and repeated 2 years later, when the participants

Table 3. Studies on specificity of interferon-γ assays in low-risk populations without active tuberculosis

Study (first author, year, country)	Design	Study population*	BCG vaccinated (%)	Antigen	Interferon-γ cut-off point	Specificity [true negatives /(true negatives + false positives)] (%)	
						Interferon-γ assay	TST
QuantiFERON-TB assay (ELISA, whole blood, short incubation)							
Streeton, 1998, Australia[75]	Case-control	417 TST-negative volunteers	0%	PPD	15% response ratio	407/417 (98%)	100%
Johnson, 1999, Australia[50]	Intervention	60 medical students	0% at baseline	PPD, ESAT6	15% response ratio	Before BCG: PPD: 58/60 (97%) ESAT6: 50/50 (100%) After BCG: PPD: 43/54 (80%) ESAT6: 50/50 (100%)	Before BCG 60/60 (100%) After BCG 47/54 (87%) [10 mm] 53/54 (98%) [15 mm]
WRAIR, 2000, USA[92]	Cross-sectional	397 naval recruits with no latent TB risk factors	..	PPD	30% response ratio	389/397 (98%)	393/397 (99%)
Mazurek, 2001, USA[58]	Cross-sectional	98 volunteers with no latent TB risk factors	..	PPD	15% response ratio	90/98 (92%)	96/98 (98%)
Brock, 2001, Denmark[29]	Case-control	34 volunteers	56%	PPD, ESAT6, CFP10	PPD: 15% response ratio ESAT6/CFP10: 5% response ratio	Non-vaccinated: PPD: 15/15 (100%) ESAT6/CFP10: 15/15 (100%) BCG-vaccinated: PPD: 10/19 (53%) ESAT6/CFP10: 17/19 (89%)	..
Bellete, 2002, USA[25]	Case-control	52 volunteers	17%	PPD	15% response ratio	PPD: 44/52 (85%)	50/52 (96%)
Fietta, 2003, Italy[43]	Case-control	42 individuals for pre-employment or pre-school screening	0%	PPD	15% response ratio	39/42 (93%);	40/42(95%)
Mori, 2004, Japan[59]	Case-control	213 student nurses with no TB exposure	100%	ESAT6, CFP10	>0.35 IU/mL	209/213 (98%)	40/113 (35%), cut-off point 10 mm
Ravn, 2004, Denmark[65]	Cross-sectional	39 volunteers	100%	ESAT6, CFP10	>0.35 IU/mL	ESAT6: 39/39 (100%) CFP10: 39/39 (100%) ESAT6/CFP10: 39/39 (100%)	..
Brock, 2004, Denmark[30]	Case-control	22 volunteers	100%	ESAT6, CFP10	ESAT6: 94 pg/mL CFP10: 80 pg/mL	ESAT6: 22/22 (100%) CFP10: 22/22 (100%) ESAT6/CFP10: 22/22 (100%)	..
T SPOT-TB assay (ELISPOT, PBMCs, short incubation)							
Pathan, 2001, UK[62]	Case-control	32 volunteers	88%	ESAT6	5 SFC more than, and at least twice as many as, negative control wells	32/32 (100%)	..
Lalvani, 2001, UK[55]	Case-control	47 patients with pneumonia, sarcoidosis, and other non-TB diseases	77%	PPD, ESAT6	5 SFC more than, and at least twice as many as, negative control wells	PPD: 21/47 (45%) ESAT6: 43/47 (92%)	..
Chapman, 2002, UK[34]	Case-control	40 volunteers	83%	PPD, ESAT6, CFP10	5 SFC more than, and at least twice as many as, negative control wells	PPD: 7/40 (18%) ESAT6/CFP10: 40/40 (100%)	..

(Continued on next page)

Table 3. Studies on specificity of interferon-γ assays in low-risk populations without active tuberculosis (continued)

Study (first author, year, country)	Design	Study population*	BCG vaccinated (%)	Antigen	Interferon-γ cut-off point	Specificity [true negatives /(true negatives + false positives)] (%)	
						Interferon-γ assay	TST
In-house assays (ELISA, PBMCs, long incubation)							
Arend, 2000, Netherlands[21]	Case-control	8 TST-negative volunteers	0%	ESAT6, CFP10	>300 pg/mL	8/8 (100%)	8/8 (100%)
Lein, 1999, USA[56]	Case-control	8 patients with culture-positive MAC; 8 healthy volunteers with negative PPD and MAS skin tests and no exposure to TB	0%	PPD, ESAT6	Antigen-stimulated OD values divided by that of non-antigen control >2·0	MAC patients: PPD: 6/8 (75%) ESAT6: 8/8 (100%) Healthy controls: PPD: 3/8 (38%) ESAT6: 8/8 (100%)	MAC patients: 8/8 (100%) Healthy controls: 8/8 (100%)
Munk, 2001, Netherlands, Denmark[60]	Case-control	59 volunteers	56%	PPD, ESAT6, CFP10	ESAT6/CFP10: >300 pg/mL; PPD: >2700 pg/mL	Non-vaccinated: PPD: 25/26 (96%) ESAT6/CFP10: 26/26 (100%) BCG-vaccinated: PPD: 18/33 (55%) ESAT6/CFP10: 31/33 (94%)	..
Ravn, 1999, Denmark[64]	Case-control	37 volunteers	78%	PPD, ESAT6	>300 pg/mL	Non-vaccinated: PPD: 3/8 (38%) ESAT6: 8/8 (100%) BCG-vaccinated: PPD: 1/29 (3%) ESAT6: 27/29 (93%)	..
Rolinck-Werninghaus, 2003, Germany[67]	Case-control	10 patients with MAC; 21 TST-negative controls	..	PPD, ESAT6	>300 pg/mL	MAC patients: PPD: 7/9 (78%) ESAT6: 9/9 (100%) Healthy controls: PPD: 20/20 (100%) ESAT6: 20/20 (100%)	MAC patients: 6/10 (60%) Healthy controls: 20/20 (100%)
van Pinxteren, 2000, Denmark[85]	Case-control	14 volunteers	57%	PPD, ESAT6, CFP10	>300 pg/mL	PPD: 0/14 (0%) ESAT6/CFP10: 10/14 (71%)	..
In-house assays (ELISPOT, PBMCs, variable incubation)							
Ulrichs, 2000, Germany, USA[81]	Case-control	16 volunteers	50%	ESAT6	SFC exceeded the mean + 3 SD of antigen-free controls	16/16 (100%)	..
Vincenti, 2003, Italy[89]	Case-control	45 patients without active TB (9 were healthy controls; the rest had non-tuberculous diseases)	36%	PPD, ESAT6	5 SFC more than, and at least twice as many as, negative control wells	PPD: 19/45 (42%) ESAT6: 45/45 (100%)	..
Scarpellini, 2004, Italy[68]	Cross-sectional	32 TST-negative volunteers	..	ESAT6, CFP10	SFC exceeded the mean+2 SD of antigen-free controls	28/32 (88%)	..

*In all studies, unless specified, participants were healthy, with no previous tuberculosis or known exposure to tuberculosis. MAC=*Mycobacterium avium* complex, MAS=*M avium* sensitin, OD=optical density, SFC=spot-forming cells, TB=tuberculosis.

were re-examined for active tuberculosis. At follow up, seven of 24 (29%) contacts developed active tuberculosis. All seven contacts (100%) who developed active tuberculosis were positive by PPD-based interferon-γ assay at baseline, as were 14 of the 17 (83%) remaining contacts. By contrast, six of seven (86%) contacts who later developed active tuberculosis responded strongly to ESAT6, whereas only three of 17 (18%) contacts who did not develop tuberculosis responded to ESAT6. This study, although small, showed a strong association between interferon-γ response to ESAT6 and later progression to active tuberculosis.

What is the effect of antituberculosis treatment on interferon-γ response?

Several studies assessed the effect of tuberculosis treatment on interferon-γ response. Some studies (all using ELISPOT)

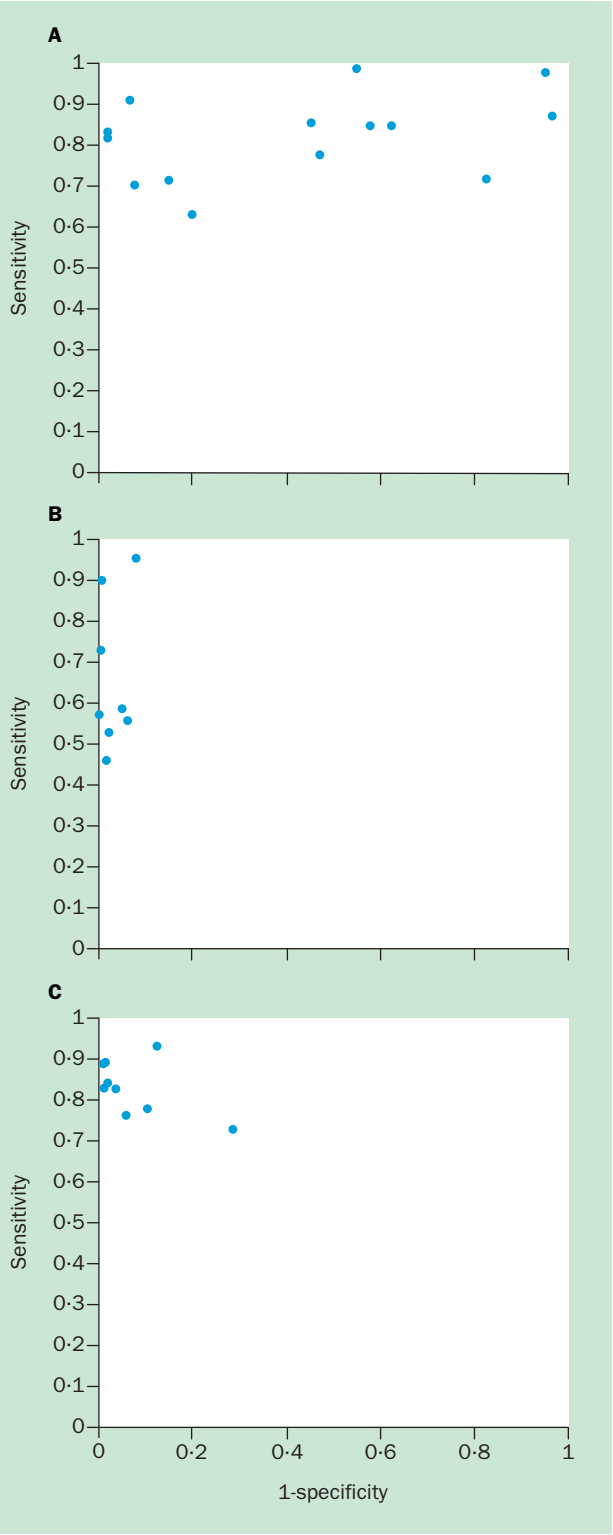

Figure 3. Effect of antigens on sensitivity and specificity of interferon-γ assays. (A) Interferon-γ assays with PPD (14 studies). (B) Interferon-γ assays with ESAT6 or CFP10 in isolation (eight studies). (C) Interferon-γ assays with ESAT6 and CFP10 in combination (cocktail) (nine studies). Receiver operating characteristic plots of estimates of sensitivity and specificity are shown, stratified by type of antigens (PPD vs *RD1) used. Each solid circle represents each study in the analysis.*

showed that interferon-γ response to ESAT6 decreased with treatment in patients with active tuberculosis.[33,53,62] Other studies showed that treatment resulted in an increased response to ESAT6.[20,82,87] Wu-Hsieh and colleagues[93] reported a study in which responses to ESAT6 persisted in individuals who have recovered from pulmonary tuberculosis after treatment. Some studies have shown that treatment for active tuberculosis made no difference in the response to the PPD-based QuantiFERON-TB assay[43,76] and an ESAT6-based in-house assay.[64] Hirsch and co-workers[46] showed that interferon-γ response to PPD was lower among patients with active tuberculosis, and the response remained low for 12 months after treatment.

Are interferon-γ assay results reproducible?

Four studies provided data on reproducibility of interferon-γ assays within individuals over time.[22,25,42,43] In three of these studies, the reproducibility was high.[22,42,43] By contrast, Bellete and colleagues[25] found the reproducibility of a PPD-based assay to be low in a small series of 11 individuals for whom TST and interferon-γ assay were repeated. However, concerns have been raised about the validity of this assessment, because the analysis did not account for the potential boosting of repeat interferon-γ assay results by previous TST administration.[95,96] One study provided data on reproducibility between testing sites.[42] In a blind study of two testing sites and 50 replicate blood samples, the agreement of the PPD-based QuantiFERON-TB assay results between sites was found to be greater than 98%.[42] A recent study assessed the correlation between a 72 h whole-blood ELISA and an overnight ELISPOT assay.[69] The interferon-γ response to ESAT6 and CFP10 in the two assays correlated well (r=0·69, p<0·0001).[69]

Discussion

Principal findings

Our review shows that the sensitivity and specificity of interferon-γ assays varies across studies. This may be due to variability in study populations (eg, disease spectrum, treatment, and HIV status), antigens (PPD *vs* RD1), incubation periods (short *vs* long), specimens used (whole blood *vs* PBMCs), assay formats (ELISA *vs* ELISPOT), and diagnostic cut-off points of the TST and interferon-γ assays. Despite this variability, certain trends are noticeable. In head-to-head comparisons against TST, most studies reported slightly higher sensitivity estimates for TST. This trend is supported by the finding that PPD-based interferon-γ assays yielded higher sensitivity than interferon-γ assays with RD1 antigens used in isolation. However, studies that explored the effect of antigen cocktails on sensitivity suggest that RD1-antigen combinations might increase the sensitivity by comparison with single antigens. The specificity of interferon-γ assays based on RD1 antigens was higher than TST and PPD-based assays. The data suggest a trade-off between sensitivity and specificity: assays with RD1 antigens in isolation had high specificity but relatively lower sensitivity, whereas PPD-based assays had higher sensitivity but lower specificity. The best combination of sensitivity and specificity was seen in

Table 4. Studies on sensitivity of interferon-γ assays in populations with suspected latent tuberculosis

Study (first author, year, country)	Design	Study population	Antigen	Interferon-γ cut-off point	N/total (%) positive by interferon-γ assay
QuantiFERON-TB assay (ELISA, whole blood, short incubation)					
Streeton, 1998, Australia[75]	Case-control	182 individuals with significant TST reaction, but no active TB	PPD	15% response ratio	163/182 (90%)
Stuart, 2000, Australia[76]	Longitudinal	119 health-care workers with TST >15 mm, normal CR, and likely exposure to TB	PPD	15% response ratio	73/119 (61%)
T SPOT-TB assay (ELISPOT, PBMCs, short incubation)					
Lalvani, 2001, UK[55]	Case-control	26 household contacts, symptom-free, normal CR, and positive TST	PPD, ESAT6	5 SFC more than, and at least twice as many as, negative control wells	PPD: 26/26 (100%) ESAT6: 22/26 (85%)
Lalvani, 2001, UK[54]	Contact investigation	50 adult contacts, almost all had no symptoms and normal CR; 47% were TST-positive	PPD, ESAT6	10 SFC more than, and at least twice as many as, negative control wells	PPD: 45/49 (92%) ESAT6: 19/49 (39%)
Pathan, 2001, UK[62]	Case-control	27 household contacts, symptom-free, normal CR, and strongly TST positive	ESAT6	5 SFC more than, and at least twice as many as, negative control wells	23/27 (85%)
Ewer, 2003, UK[41]	Outbreak investigation	128 children with positive TST and no evidence of active TB	ESAT6, CFP10	5 SFC more than, and at least twice as many as, negative control wells	97/128 (76%)
In-house assays (ELISA, PBMCs, long incubation)					
Ravn, 1999, Ethiopia[64]	Case-control	30 household contacts, normal CR and negative sputum smears	PPD, ESAT6	>300 pg/mL	PPD: 24/30 (80%) ESAT6: 14/30 (47%)
Arend, 2001, Netherlands[22]	Contact investigation	12 contacts, symptom-free, normal CR, positive TST	PPD, ESAT6, CFP10	PPD: >200 pg/mL ESAT6/CFP10: >60 pg/mL	PPD: 12/12 (100%) ESAT6/CFP10: 8/12 (67%)
Vekemans, 2001, Gambia[87]	Case-control	28 healthy household contacts; no evidence of active TB on follow up; 86% TST positive	PPD, ESAT6	Above the mean + 3 SD of the control samples	PPD: 23/28 (82%) ESAT6: 20/28 (71%)
Vekemans, 2004, Gambia[88]	Case-control	21 household contacts who had not had TB in the past; 89% were TST positive	PPD, CFP10	>8 pg/mL	PPD: 21/21 (100%) CFP10: 19/21 (90%)
Vekemans, 2004, Gambia[88]	Case-control	23 exposed, TST-positive health-care workers working with TB patients	PPD, CFP10	>8 pg/mL	PPD: 23/23 (100%) CFP10: 23/23 (100%)
In-house assays (ELISPOT, PBMCs, long incubation)					
Ulrichs, 2000, Germany, USA[81]	Case-control	12 unvaccinated, symptom-free, healthy recent TST converters; all treated for latent TB	ESAT6	SFC exceeded the mean + 3 SD of antigen-free controls	10/12 (83%)

CR=chest radiograph, SFC=spot-forming cells, TB=tuberculosis.

studies with cocktails of RD1 antigens (eg, QuantiFERON-TB Gold and T SPOT-TB assays). Studies among individuals with suspected latent tuberculosis show that interferon-γ assays detect about 80% of this population (sensitivity for latent tuberculosis).

Most studies reported modest agreement (60–80%) between the TST and the interferon-γ assays, and the correlation, when analysed as continuous data, seems to be moderate to strong. BCG vaccination was associated with a specific pattern of discordance (TST positive, interferon-γ negative) in some studies. interferon-γ assays with RD1 antigens correlated significantly better with increasing exposure than did the TST. By contrast, such interferon-γ assays showed no correlation with BCG status, whereas TST results were more likely to be positive in BCG-vaccinated individuals. interferon-γ assays that use RD1 antigens seem to be less influenced by *M avium* infection than are TST or PPD-based assays. However, T-cell responses to ESAT6 and CFP10 are not completely specific for infection with *M tuberculosis* complex, but they may result from some NTM infections. There is limited evidence of an association between interferon-γ response to ESAT6 and later progression to active tuberculosis among healthy contacts of tuberculosis patients. The data on the effect of treatment on interferon-γ response are limited and inconsistent. The available, albeit limited, data on reproducibility suggest that interferon-γ assays are fairly reliable.

Clinical implications

interferon-γ assays that use *M tuberculosis*-specific RD1 antigens may have several advantages over TST: higher specificity, better correlation with exposure to *M tuberculosis*, and relatively lower cross-reactivity due to previous BCG vaccination and NTM infection. interferon-γ assays that use cocktails of antigens seem to have the best combination of sensitivity and specificity. This finding has

Table 5. Studies on agreement between interferon-γ assays and tuberculin skin testing

Study (first author, year, country)	Study population	Interferon-γ assay	TST	Agreement	Kappa	Comments
QuantiFERON-TB assay (ELISA, whole blood, short incubation)						
Converse, 1997, USA[35]	67 IDU (52% HIV infected)	PPD	Mantoux, 5 TU PPD-S, 5 mm in HIV-positive, 10 mm in others	HIV-negative: 72% HIV-positive: 79%	HIV-negative: 0·41 HIV-positive: 0·59	Interferon-γ assay detected more reactors than TST
Pottumarthy, 1999, New Zealand[63]	51 patients with active TB; 237 immigrants from countries with a high prevalence of TB; 127 HCWs	PPD	Mantoux, risk-stratified cut-off points	Active TB: 84% Immigrants: 80% HCWs: 69%	Active TB: 0·65 Immigrants: 0·55 HCWs: 0·26	..
Kimura, 1999, USA[52]	467 IDU (300 HIV-negative and 167 HIV-positive)	PPD	Mantoux, 5 TU PPD-S, 5 mm in HIV-positive, 10 mm in HIV-negative	HIV negative: 59% HIV positive: 82%	HIV negative: 0·26 HIV positive: 0·28	Interferon-γ assay detected more reactors than TST
Mazurek, 2001, USA[58]	98 with low latent TB risk; 947 with high latent TB risk; 94 with suspected TB; 87 with active, treated TB	PPD	Mantoux, 5 TU PPD-S, risk-stratified cut-off points	Overall: 83% Low risk: 92% High risk: 85% TB suspects: 79% Active TB: 69%	Overall: 0·60 Low risk: 0·17 High risk: 0·55 TB suspects: 0·41 Active TB: 0·16	TST+/interferon-γ– results more likely in BCG-vaccinated
WRAIR, 2000, USA[92]	Healthy, naval recruits: 397 low latent TB risk, 1066 limited risk, and 232 high risk	PPD	Mantoux, 5 TU , PPD-S 15 mm for low and limited risk	High risk: 83% Low risk: 98% Limited risk: 98%	High risk: 0·27 Low risk: NR Limited risk: NR	..
Katial, 2001, USA[51]	48 HCWs with history of exposure and negative CR	PPD	Mantoux, 5 TU PPD-S, 15 mm	86%	0·73	..
Bellete, 2002, USA[25]	175 individuals (low risk, intermediate risk, treated TB, and TB suspects)	PPD	Mantoux, 5 TU PPD-S, 15 mm for low-risk, 10 mm in others	79%	0·68	..
Bellete, 2002, Ethiopia[25]	253 healthy volunteers and people screened for TB	PPD	Mantoux, 5 TU PPD-S, 5 mm	68%	0·35	..
Fietta, 2003, Italy[43]	57 active TB; 159 high-risk volunteers including household contacts; 42 volunteers with no TB exposure	PPD	Mantoux, 5 TU PPD, risk-stratified cut-off points	Overall: 78% Active TB: 70% High risk: 76% Low risk: 98%	Overall: 0·59 Active TB: 0·25 High risk: 0·58 Low risk: 0·79	TST–/interferon-γ+ results more likely in patients with conditions that impair TST response
Brock, 2004, Denmark[31]	85 BCG-unvaccinated contacts of an index TB case in a school	ESAT6, CFP10	Mantoux, 2 TU RT23, 10 mm	94%	0·87	..
T SPOT-TB assay (ELISPOT, PBMCs, short incubation)						
Lalvani, 2001, UK[54]	50 healthy adult contacts of cases with smear-positive active TB	ESAT6	Heaf test, grades 3 and 4 positive	69%	0·37	..
Chapman, 2002, Zambia[34]	49 healthy adults (14 HIV-positive) with no history of TB and normal CR	ESAT6, CFP10	Mantoux, 5 TU PPD RT23, 10 mm	HIV-negative: 60% HIV-positive: 64%	HIV-negative: –0·03 HIV-positive: 0·26	..
Ewer, 2003, UK[41]	535 students screened in a school outbreak	ESAT6, CFP10	Heaf grade 2 among unvaccinated, and grades 3 and 4, irrespective of BCG status	89%	0·72	TST more likely to be positive in BCG-vaccinated
Richeldi, 2004, Italy[66]	41 neonates and 47 adults who may have been exposed to a case of drug-resistant TB	ESAT6, CFP10	Mantoux, 5 TU PPD-S, 5 mm	82%	0·13	Interferon-γ assay detected more reactors than TST

(Continued on next page)

Table 5. Studies on agreement between interferon-γ assays and tuberculin skin testing (continued)

Study (first author, year, country)	Study population	Interferon-γ assay	TST	Agreement	Kappa	Comments
In-house assays (ELISA, PBMCs, long incubation)						
Lein, 1999, USA[56]	43 participants (27 active TB, 8 MAC disease, 8 healthy controls)	ESAT6	Mantoux, 5 TU PPD-S, 5 mm	74%	0·52	..
Arend, 2001, Netherlands[22]	44 contacts of a case of smear-positive TB	ESAT6, CFP10	Mantoux, 2 TU RT23, 10 mm	89%	0·73	..
In-house assays (ELISPOT, PBMCs, short incubation)						
Hill, 2004, Gambia[45]	735 household contacts of smear-positive TB patients	ESAT6, CFP10, PPD	Mantoux, 2 TU RT23,10 mm	PPD: 59% ESAT6/CFP10: 74%	PPD: 0·22 ESAT6/CFP10: 0·43	..

CR=chest radiograph, HCWs=health-care workers, IDU=injection drug users, MAC=*Mycobacterium avium* complex, NR=not reported, PPD-S=PPD Seibert, SFC=spot-forming cells, TU=tuberculin units.

important clinical implications: sensitive assays for tuberculosis infection will especially help those groups (eg, immunosuppressed patients) who are most prone to having false-negative TST results, and who, once infected, are at risk of progression to active tuberculosis. Two recent case reports suggest that among immunosuppressed patients who are at risk for tuberculosis, a positive interferon-γ result might help to uncover active tuberculosis, even if the TST is negative.[98,99]

There is some evidence to suggest that interferon-γ assays can potentially identify those with latent tuberculosis who are at high risk for developing active disease. The other advantages include the need for just one visit by the patient, avoidance of measurements such as skin induration, and the ability to perform repeat testing without inducing boosting. These findings, overall, suggest that RD1-based interferon-γ assays could be used to better target interventions for latent tuberculosis at those who are truly infected and, therefore, might benefit substantially from intervention. By reducing false-positive results, interferon-γ assays may help avoid unnecessary treatment of latent tuberculosis and its adverse effects. Unfortunately, there is inadequate evidence to determine the applicability of interferon-γ assays in immunocompromised individuals, children, patients with extrapulmonary or NTM disease, and high-risk populations in endemic countries. Finally, the role of interferon-γ assays in treatment monitoring is unclear as the available evidence is inconsistent. This could be, in part, because of variability in interferon-γ assay methods used.

Limitations of existing studies

In theory, interferon-γ assays have several advantages over TST. In practice, it is not easy to show the superiority of interferon-γ assays over TSTs. In the absence of a gold standard, direct estimation of sensitivity and specificity for latent tuberculosis is not possible. Although some studies (table 4) have assessed the sensitivity of interferon-γ assays in those presumed to have latent tuberculosis, these studies implicitly used the TST as one component of the gold standard, which is an approach fraught with problems, given the known limitations of the TST.[95,96] Research studies, therefore, have focused on the following hypotheses that allow an indirect ranking of the TST and interferon-γ assays: if interferon-γ assays are superior to the TST, then interferon-γ assays should (1) show higher sensitivity and specificity for active tuberculosis than the TST, (2) correlate better with exposure to *M tuberculosis* than the TST, (3) be less influenced by BCG vaccination, (4) be less influenced by NTM infection, and (5) be able to predict better who will develop active tuberculosis among those who are latently infected. Studies have also focused on measuring agreement between the TST and interferon-γ assays, and identifying factors associated with discordance. This approach avoids the use of the TST as the reference standard.

Existing studies on interferon-γ assays have several limitations. Most studies have used the case-control design in which test accuracy has been determined in patients with confirmed (and often advanced) active tuberculosis, and in healthy individuals with no history of tuberculosis exposure. Case-control studies, therefore, tend to overestimate diagnostic accuracy.[100] Many studies have included small numbers of participants, and few studies have reported design features such as independent and blinded interpretation of TST and interferon-γ assays. Lack of blinding can potentially result in overestimation of test accuracy.[100]

Although many studies reported agreement between the TST and interferon-γ assays, few identified reasons for discordance; such information can be more helpful than measures of agreement per se. Given the limitations of the TST, any comparison of a new test with high accuracy against the TST would most likely show poor agreement with the TST.[52,58] Some studies have used the TST as the gold standard—a potentially misleading approach.[58,95,96] Furthermore, studies have rarely addressed the issue of whether the TST could have affected (eg, boosted) the interferon-γ results in their studies.[95,96] Finally, the use of patients with advanced disease or who have completed treatment creates potential problems for the estimation of sensitivity, because both TST and interferon-γ results can be influenced by disease severity and treatment, and these can have unpredictable and dissimilar effects on the estimates on

the sensitivity of both tests.[46,58,95,96] Concerns have also been raised about the appropriateness of using treated, active tuberculosis as an immunological model for assessing diagnostic tools for latent tuberculosis.[95,96]

Implications for future research

Future studies on interferon-γ assays should prospectively recruit consecutive patients in whom the test is clinically indicated (rather than using the case-control design). The TST and the interferon-γ assay should be interpreted independently of each other. Studies should provide data on the reproducibility of interferon-γ assays. It is also important to avoid using the TST as the gold standard. Finally, studies on agreement between TST and interferon-γ assays should explore discordance between the test results.

With respect to applicability, very few studies on interferon-γ assays have been done in high-endemic countries with high prevalence of latent tuberculosis, high BCG coverage, and widespread NTM exposure. If interferon-γ assays can be shown to perform well in such settings, their applicability will be greatly enhanced. For example, a recent study from South Africa showed that a RD1-based ELISPOT assay had higher sensitivity than the TST in children with active tuberculosis.[101] Other studies are currently in progress in several countries across the world,[14] and the results of these studies will improve our understanding of the role of interferon-γ assays in less-developed countries. In addition, research among the following populations is required to clearly define the value and limitations of interferon-γ assays: (1) HIV-positive and other immunocompromised individuals (eg, dialysis and transplantation patients, and those on immunosuppressive drugs), (2) children, (3) patients with NTM exposure or infection, (4) patients with extrapulmonary and multidrug-resistant tuberculosis, and (5) high-risk populations such as health-care workers, contacts of infectious cases, and immigrants. There is also a need for research on the effect of treatment for latent and active tuberculosis on the performance of interferon-γ assays and their role in monitoring treatment response.[19]

With respect to assay methods, future research must attempt to enhance the sensitivity of RD1-based interferon-γ assays, without compromising the specificity; current evidence suggests that the addition of more specific antigens and use of them in combinations may be effective. There is a need for comparative studies to determine whether specific assay formats are associated with higher accuracy. For example, is the ELISPOT more sensitive than the ELISA format? Are short (24–48 h) incubation assays more sensitive and specific than assays that use longer (5–6 days) incubation periods?

Long-term cohort studies are needed to determine whether a positive interferon-γ result is associated with a higher incidence of active disease among those with latent infection.[12,39] Such studies are in progress and the results should help to settle the debate on whether interferon-γ assays can replace the TST.[21,39,66,87] It is important to determine whether treatment of individuals diagnosed to have latent tuberculosis by the interferon-γ assay will result in protection against active tuberculosis;[19,64] such experimental proof will greatly enable targeted interventions. Studies are needed to determine the cost-effectiveness of the interferon-γ assay compared with the TST. Studies are also required to explore the possibility of using both TST and interferon-γ assays in combination, to better exploit the higher sensitivity of PPD, and higher specificity of RD1 antigens.

Search strategy and selection criteria

These are described in the Methods section on page 762.

Conclusions

Current evidence suggests that interferon-γ assays, particularly those based on cocktails of RD1 antigens, have the potential to become useful diagnostic tools in clinical and public-health settings. Whether this potential can be realised in practice remains to be confirmed in large, well designed trials and long-term follow-up studies. Because interferon-γ assays might cost much more than the TST, cost will be a critical factor in determining the global applicability of this new assay. It will then be important to ensure that the benefits of this new technology, if shown to be valuable, reach the populations that need it most.

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Conflicts of interest

We declare that we have no conflicts of interest.

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