T-cell interferon-γ release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings

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Purpose of review

The utility of T-cell interferon-γ (IFN-γ) responses to *Mycobacterium tuberculosis* specific antigens [interferon-γ release assays (IGRAs)] in high-burden settings remains unclear and there is growing evidence that IGRA performance varies across high tuberculosis (TB) burden vs. low TB burden settings. Here we review the evidence supporting the utility of IGRAs in specific subgroups and compare their performance in high-burden vs. low-burden settings.

Recent findings

Although the IGRA, compared with the tuberculin skin test (TST), has greater specificity in BCG-vaccinated individuals, treatment of latent tuberculosis infection is not a priority in high-burden setting. Nevertheless, in high-burden settings, the TST performs reasonably well and correlates as well, or better, with proxy measures of exposure.

Summary

IGRAs may still be useful in high-burden settings in specific subgroups at high risk of progression, including young children, HIV-infected individuals and healthcare workers, but this requires confirmation. Although the IGRAs cannot distinguish between latent and active TB, their utility as rule-out tests, when combined with smear microscopy or the TST, requires further study. Prospective studies are required in high-burden settings to confirm whether IFN-γ responses are predictive of high risk of progression to active TB, particularly in HIV-infected individuals.

Keywords

early secreted antigenic target 6 kDa, ELISPOT, enzyme-linked immunosorbent assay, immunodiagnosis, interferon-γ, tuberculosis

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Introduction

Tuberculosis (TB) is a public health catastrophe resulting in one death every 15 s [1]. It is estimated that a third of the world's population is infected with *Mycobacterium tuberculosis*. In high-burden countries, almost two-thirds of new cases are co-infected with HIV [2]. Until recently, the tuberculin skin test (TST) was the only available test to identify underlying *M. tuberculosis* infection. However, many factors may negatively impact upon the utility of the TST (Table 1).

More recently, peripheral blood-derived T-cell interferon-γ (IFN-γ) responses to *M. tuberculosis*-specific antigens [early secreted antigenic target 6 kDa (ESAT-6) and culture filtrate protein 10 (CFP-10)] have been investigated as a proxy biomarker of latent tuberculosis infection (LTBI). This review outlines factors that may modulate test results, and in particular, focuses on performance outcomes and applicability in high-burden settings. Peer-reviewed data for this manuscript were identified by searches of the PubMed database, up to and including December 2008, in all languages, using the search terms 'tuberculosis' and 'ESAT-6', 'CFP-10', 'RD-1', 'IFN-γ', 'T-cell epitopes' and 'immunodiagnosis'. Other sources were experts in the field, manufacturers, the references of retrieved articles including previous systematic reviews, textbooks and the files of the authors. This review focuses on assays that use standardized protocols incorporating both ESAT-6 and CFP-10 antigens, and that use incubation times of 24 h or less.

The interferon-γ release assays: principle and test formats

This has been outlined in detail [3–6] but will be reviewed here briefly. LTBI is typically diagnosed by performing a TST that measures cell-mediated immunity as a delayed type hypersensitivity reaction to purified

Table 1 Comparison of factors impacting upon utility of the tuberculin skin test and interferon-γ release assays

Parameter	Tuberculin skin test	RD-1 IFN-γ release assay
Specificity	Cross-reactivity with BCG and environmental bacteria	Relatively *Mycobacterium tuberculosis* specific (may be positive with *M. kansasii* and *M. marinum* exposure/infection)
Workload	Requires return visit for which attendance is poor	Single visit; however, in most settings a second visit may be required for information and advice purposes
Chemoprophylaxis	Casts a wide net – may result in 'overtreatment' due to BCG effect	May avoid unnecessary treatment and toxicity but may also potentially undertreat
Subjectivity	Results dependent on observer and technique	Provides 'yes'/'no' answer (however within-patient variability is significant therefore values close to the cut-point must be interpreted with caution)
Booster phenomenon	Yes (the TST may boost subsequent TST reactions)	Yes (the TST may boost down-stream IGRA responses)
Cost	High in developed countries; low in developing countries	Affordable in developed countries
Longitudinal efficacy data	Plentiful	Limited
Other factors	Prone to breakage of cold chain and syringe re-use in resource poor setting	Requires laboratory expertise and equipment, phlebotomy facilities, and laboratory closing times may impact on test availability. Phlebotomy may be unsuccessful in children and needle-phobic adults

IFN-γ, interferon-γ; IGRA, interferon-γ release assays; TST, tuberculin skin test.

protein derivative (PPD), a culture filtrate of *M. tuberculosis*. By contrast, the *M. tuberculosis* specific antigens [interferon-γ release assays (IGRAs)] detect TB antigen-specific (ESAT-6, CFP-10 and also TB 7.7) circulating effector memory T cells through IFN-γ secretion measured by an ELISPOT or enzyme-linked immunosorbent assay (ELISA). Both the proteins are encoded on the region of difference (RD-1) domain of the genome, although there are regions where ESAT-6 and CFP-10 paralogs are duplicated along with the PE and PPE gene families, which encode relatively TB-specific proteins that also form a heterodimeric complex consisting of an extended alpha helical structure [7]. Their expression is known to be absent in BCG substrains and many environmental mycobacteria except *M. marinum*, *M. szulgai* and *M. kansasii*; homologues of these proteins are also found in the genome of *M. leprae* [8]. There are two commercial IGRAs and both measure overnight IFN-γ responses (<24 h) to overlapping ESAT-6 and CFP-10 peptides. The T-SPOT.TB assay (Oxford Immunotec, Oxford, England) is an ELISPOT assay that uses peripheral blood mononuclear cells and QuantiFERON-TB Gold (QFT-G) and QuantiFERON-TB Gold In Tube (QFT-GIT Cellestis, Victoria, Australia) are ELISA assays utilizing whole blood. Both assays have European CE Mark and US FDA approval. The QFT-GIT assay has replaced the QFT-G test and also contains the TB 7.7 peptides.

Diagnosis of latent tuberculosis infection

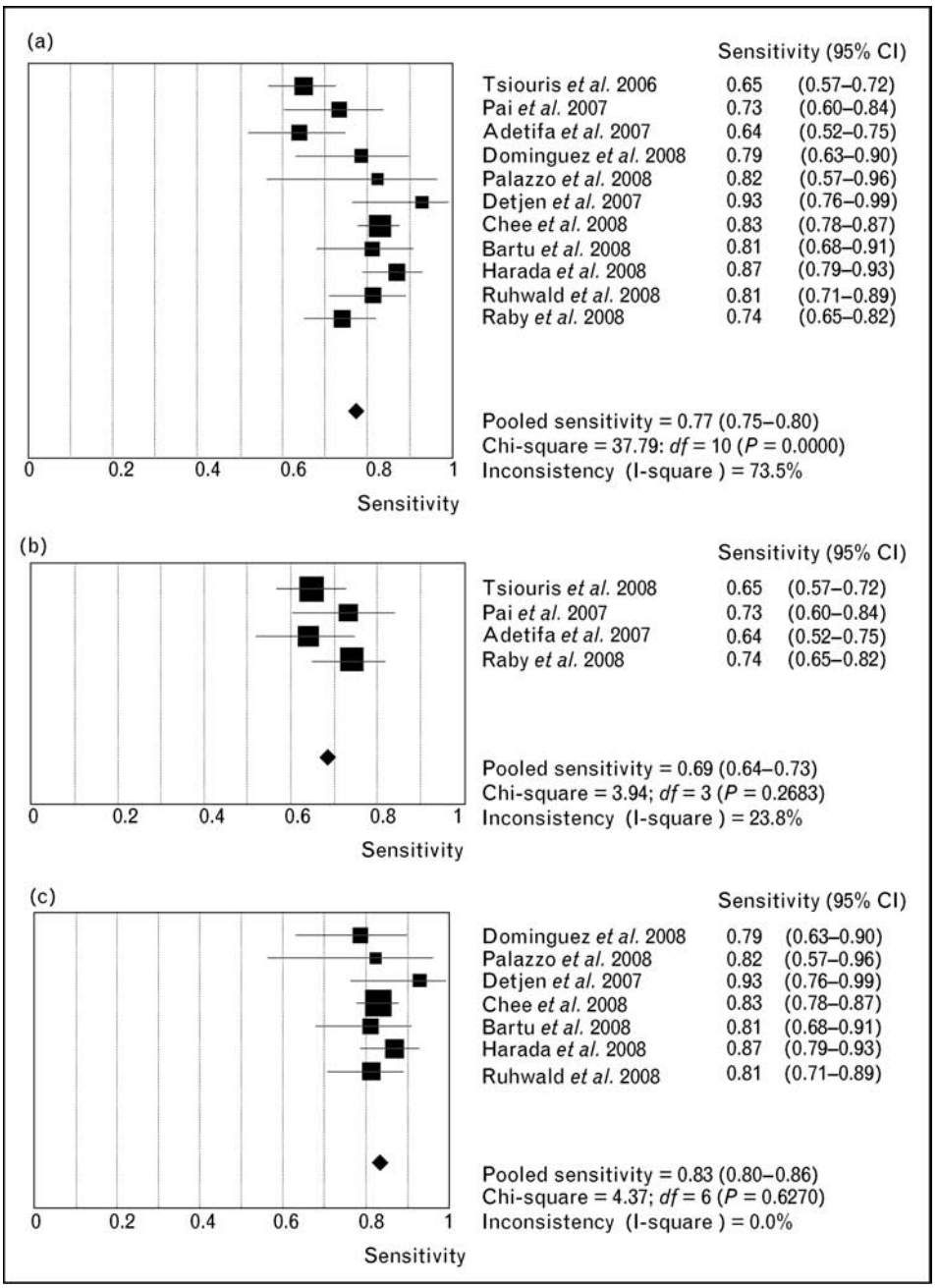
The lack of a gold standard for diagnosing LTBI is a problem that investigators face when trying to determine performance outcomes of the IGRA.

Comparison of interferon-γ release assays performance outcomes in high-burden vs. low-burden settings

In view of the lack of a gold standard, it is assumed that sensitivity should be at least as good as, or better than, in active TB, which tends to attenuate IFN-γ levels due to the immunosuppressive effect of disease itself and trafficking of cells out of the peripheral blood compartment [9]. In a recent meta-analysis, the pooled sensitivity of QFT was between 70 and 78% (depending on whether QFT GIT or QFT-G was used), compared with 77% for the TST and 90% for T-SPOT.TB [10••]. Notably, only five studies were carried out in high-burden settings [11–15]. Here, the QFT sensitivity results mirrored those found in low-burden settings; however, only two studies evaluated the ELISPOT assay (79 and 71% sensitivity in The Gambia) [11,15]. In an updated meta-analysis presented here (Fig. 1), there is a clear trend towards lower QFT sensitivity in high-burden settings compared with low-burden countries. The overall pooled QFT sensitivity was 77%, but in high-burden ($n = 4$ studies) vs. low-burden countries ($n = 7$ studies), the sensitivity was 69 vs. 83%, respectively. The lower sensitivity in high-burden countries may be due to several factors including HIV co-infection, advanced disease, malnutrition, immunological host phenotype, effect of strain variation, and so on. In the only two studies from high-burden settings that evaluated comparative IGRA vs. TST responses, the TST sensitivities were 90 [14] and 100% [15] at a 10 mm cut-point.

Specificity was determined by studying T-cell IFN-γ responses, usually in low TB incidence country persons, who were asymptomatic and at low risk for LTBI, and by comparing test performance in BCG-vaccinated and

Figure 1 Meta-analysis of QuantiFERON-TB Gold sensitivity in active tuberculosis disease



Sensitivity of QuantiFERON-TB Gold In Tube (QFT-GIT) in high-incidence (b) vs. low-incidence (c) settings (both high-burden and low-burden settings are combined in (a). Sensitivity was evaluated in culture-confirmed tuberculosis (TB) patients (updated with permission from [10••]).

nonvaccinated individuals. These studies showed a pooled specificity of between 93 and 99% for IGRAs [10••]. In particular, QFT specificity was found to be consistently high in many studies, whereas few studies were available on T-SPOT.TB specificity. IGRAs showed no difference in specificity in BCG-vaccinated and unvaccinated individuals. By contrast, the pooled specificity of TST was 97% in non-BCG-vaccinated individuals and 59% in BCG-vaccinated individuals [10••]. An alternative way to evaluate sensitivity and specificity is through comparison along a gradient of exposure. Owing to the fact that the risk of LTBI is closely related to proximity and duration of exposure to infectious pulmonary TB cases, investigators have used quantified TB exposure as a surrogate gold standard for LTBI. Studies from low-burden countries indicate that the IGRAs correlated better, along a gradient of exposure, than the TST [16–24]. By contrast, studies from Uganda,

India and The Gambia showed that the TST correlated well with proximity to an index case [11,25–29,30•] and performed better than the IGRA along a *M. tuberculosis* gradient of exposure (summarized in Table 2 [11,26,27,31–33]).

Notably, most studies were performed in The Gambia. Most developing countries have a policy of giving BCG at birth and not repeating the vaccine. In such situations, there is evidence that TST specificity is not seriously compromised [34].

Test specificity may also be extrapolated from studies evaluating response to treatment of LTBI and active TB. Theoretically, a highly specific test should be positive during the disease and revert to negative after successful treatment of the disease. Study findings have been variable with some [35,36•,37], but not other studies [38] from low-burden settings showing rapidly declining IGRA responses during or after anti-TB treatment. By contrast, three studies from high-burden settings (The Gambia, India and Uganda) showed a persistently high frequency of antigen-specific T cells 3–12 months after the commencement of anti-TB treatment [13,39,40]. There is also significant discordance between the IGRA and the TST. In high-burden countries, there is generally good agreement between the IGRA and TST in close contacts [11,15,25]. By contrast, in low-burden settings, agreement between IGRA and TST depends on the BCG vaccination status of participants (good [16,23,41] and poor [17–19,23,42] agreement in BCG nonvaccinated and vaccinated participants, respectively). Some low-burden countries offer BCG vaccination after infancy and/or provide several booster BCG shots. This has been shown to compromise TST specificity [34] and might explain some of the IGRA/TST discordance in low-burden settings. To facilitate the interpretation of TST, a World Atlas of BCG Policies and Practices has been put together (available at www.bcgatlas.org), along with a Web-based algorithm for a three-dimensional interpretation of TST [43•].

In summary, these data indicate that in low-burden countries, the IGRA is as sensitive as the TST, is more specific in BCG-vaccinated individuals, correlates better with a gradient of exposure than the TST, may revert to negative during successful anti-TB treatment, and concordance with the TST is variable but correlates well with the BCG status of the individual. By contrast, in high-burden settings, the picture is less clear. Here, there is some evidence that IGRA sensitivity (especially QFT) might be lower in high-incidence settings. In addition, although the TST is also less specific in BCG-vaccinated individuals, it is at least as sensitive or more sensitive than the IGRAs in active TB, correlates as well or better with exposure status than the IGRAs, and concordance with TST is modest to good (predominantly TST-positive/IGRA-negative discordance). How do we explain these results?

Interpretation, diagnosis of latent tuberculosis infection and factors that may modulate test results

IGRAs measure the frequency of effector memory T cells. It is unclear whether, because of ongoing *M. tuberculosis* exposure, such memory cells are detectable in the absence of LTBI. This may account for the poorer specificity of IGRAs in high-burden settings and lack of reversion even after treatment. There are a multitude of other factors that may modulate the sensitivity of IGRAs including HIV infection, malnutrition, high ambient exposure to *M. tuberculosis*, transmission dynamics and repeat exposures, helminth infection, high exposure to RD-1 homologue-producing environmental mycobacterial, disease phenotype and severity and related to this, underlying host immunity including T helper and regulatory T-cell profiles [44], and levels of immunosuppressive cytokines (IL-4, IL-10, TGF-β, and IL-9) [45,46]. There is evidence from The Gambia that strain differences impact on IGRA results [47]. Furthermore, it is possible that intra-pulmonary clearance of the organism at initial contact may still evoke a transient T-cell response, and that established LTBI may be associated with an undetectable T-cell response when organisms enter a state of dormancy; it is unclear whether ESAT-6 secretion intermittently ceases at this point. Thus, it remains unclear whether IGRAs may be detecting more recent infection. Many of these factors, including regulatory T-cell pathways, may also explain the higher rates of discordance in high-burden settings. Furthermore, the TST is composite 48–72 h readout of the pro-inflammatory effects of antigen presenting cells, chemokines and lymphocytes in response to several antigens, whereas the IGRAs represent a single cytokine readout after overnight T-cell stimulation to two specific antigens. It is worth noting that correlation with exposure does not necessarily mean correlation with LTBI. Thus, in the absence of a gold standard, the absolute sensitivity of the IGRA and the TST for that matter, for diagnosing LTBI still remains unclear.

In contrast to the TST [48,49], the IGRAs remain negative in the face of proven *M. avium* disease [50] but not active *M. marinum* and *M. kansasii* disease [51,52]. Positive responses are also found in individuals with high environmental mycobacterial exposure, such as flower sellers and tropical fish tank owners [52]. *M. leprae* homologues of *M. tuberculosis* CFP-10 [53] and ESAT-6 [54], designated L-CFP-10 and L-ESAT-6, respectively, induce IFN-γ from T cells of patients with leprosy, active TB [53–55] and also in healthy volunteers from Brazil where leprosy is endemic [55]. Consequently, the standardized IGRAs will need validation in populations who come from or reside in countries where both TB and

Table 2 Summary of studies performed in high burden countries where test results were correlated to exposure status

Study	Country and assay type	No. of individuals	No. of IGRA-positive/total tested[a]	IGRA positivity OR (95% CI)	No. TST-positive/total tested	TST OR (95% CI)	ROR (TST/IGRA)	Comments
Hill *et al.* [26]	The Gambia; ELISPOT	593	58/135	2.2 (1.1–4.3)	78/126	15.7 (7.0–35.3)	7.16 (4.55–11.71)	TST 10 mm cut-point; similar result with 15 mm cut-point[b]
Hill *et al.* [25]	The Gambia; ELISPOT	735	57/150	1.96 (1.13; 3.38)	93/150	5.02 (2.87–8.78)	2.56 (2.17–3.04)	TST 10 mm cut-point[b]
Adetifa *et al.* [11]	The Gambia; ELISPOT	174	28/40 24/38	3.8 (1.2–12.5) for QFT 4.2 (1.0–18.0) for ELISPOT	25/41	4.8 (1.3–17.1)	1.26 (0.81–1.99) 1.14 (0.74–1.77)	TST 10 mm cut-point[b]
Mahomed *et al.* [31]	South Africa; QFT-GIT	358	12/32	0.87 (0.36–2.13)	20/22	2.25 (0.51–9.93)	2.59 (2.01–3.35)	TST 10 mm cut-point, healthy adults who reported exposure status
Pai *et al.* [32]	India; QFT-G	726	109/169	3.34 (1.13–9.81)	117/165	3.20 (1.08–9.45)	0.96 (0.82–1.12)	TST 10 mm cut-point, HCWs >10 years exposure vs. less than 1 year
Pai *et al.* [30•]	India; QFT-GIT	250	115/201	2.03 (0.92, 4.50)	103/201	3.61 (1.50–8.63)	1.78 (1.49–2.12)	TST 10 mm cut-point[b]
Nakaoka *et al.* [33]	Nigeria; QFT-GIT	207	155/192	7.4 (53/72 + in smear positive vs. 4/39 in control)	57/193	3.5 (38/78 in smear positive vs. 6/48 in control)	0.47	TST 10 mm cut-point; exposure defined as contact with smear positive vs. smear negative vs. control case

ROR, relative odds ratio [a ROR of greater than one suggests that the tuberculin skin test (TST) correlates significantly better along a gradient of exposure than the interferon-γ release assay (IGRA), provided that the 95% confidence interval (CI) does not include the value 1.0; a ROR less than one suggests the reverse provided that the 95% CI does not include the value 1.0]. HCWs, healthcare workers; QFT-G, QuantiFERON-TB Gold; QFT-GIT, QuantiFERON-TB Gold In Tube.

[a] For the highest exposure category (e.g. same room as the index, or smear positive case, etc.).

[b] All assays were evaluated in contacts unless otherwise stated; exposure was defined through proximity to the index case (slept in the same room vs. same house vs. different house as the index case) unless otherwise stated in the text.

leprosy are endemic, and where there is a high environmental mycobacterial load [56].

The optimal strategy that should be employed to detect LTBI using the IGRAs is controversial. One approach, despite the lack of sufficient prospective data, is to define LTBI by using the IGRA alone ([57]; CDC guidelines). However, significant rates of reversion [30•,58] and discordance between the two IGRA formats make this strategy questionable. An alternative approach, as suggested by the UK National Institute for Health and Clinical Excellence (NICE) and Canadian guidelines, is to first perform a screening TST, and if positive to then perform an IGRA [59,60]. In the appropriate TST-negative individuals, an IGRA should be performed up to 6 weeks after the screening TST [59]. This rationale is based on cost-containment analysis and that a sensitive and cheaper screening test should logically precede a more complex and less widely available, but more specific assay. A similar approach could be used is developing countries in specific situations such as healthcare worker screening, children and other individuals at high risk of progression to active TB. However, this approach relies on the assumption that the TST does not evoke a subsequent 'false-positive' downstream IGRA response. Our data indicate that definite boosting of IFN-γ responses occurs by day 7 after TST administration and with other antigens by day 3 [61]. Therefore, if the two-step strategy is used for IGRA testing, it should be performed by day 3 (i.e. at the time of reading of the TST). This approach is consistent with the updated Canadian guidelines on IGRA [60]. Larger studies are required to validate this recommendation as PPD and HBHA-driven responses occur by day 3 [61].

A recent meta-analysis indicated that the QFT-GIT sensitivity is nearly 10% lower than the T-SPOT.TB [10••]. Our preliminary work has shown that this is unlikely to be explained by the nonstandardized and variable mononuclear cell count in the QFT tube (van Zyl Smit R and Dheda K, submitted). Rather, lymphocyte-independent factors including the inherent nature of the technique and setting of the assay cut-point may explain this finding [62]. The QFT assay cut-off appears to be designed for maximizing specificity (which is consistently high in all studies), whereas the T-SPOT.TB cut-off appears to maximize sensitivity (with a potentially slight impact on specificity). Further work is necessary to firmly establish the specificity of the commercial T-SPOT.TB assay.

Finally, interpretation of test results, particularly conversions and reversions, and values near the cut-point require an understanding about the day-to-day test variability in high-burden and low-burden settings. However, published data are limited [63•]. Our studies in Cape Town, South Africa, indicate high within-person variability of IGRA results [61]. Ninety-five percent of this variability falls within 80% on either side of a given QFT-GIT IFN-γ response, and three spots on either side of a given T-SPOT.TB value [actual spot-forming cells (SFCs)]. Thus, results close to the cut-point (3–9 SFCs with T-SPOT.TB and between 0.2 and 0.7 IU/ml with QFT-GIT) should be repeated and evaluated in the clinical context. Recently, a borderline (uncertainty) zone analysis has been proposed to take into account the variability around the cut-off for interpretation of serial testing data [30•]. Similarly a change from the baseline IFN-γ value below 0.35 IU/ml and crossing the cut-point to above 0.7 IU/ml might suggest a true QFT conversion, although further data are required to confirm these findings [58].

Clinical utility for the diagnosis of latent tuberculosis infection in high-burden vs. low-burden settings

Diagnosing and treating LTBI is a less important strategy in the developing world because the priority is treatment of large numbers of active cases that promote ongoing transmission. Thus, current priorities of TB programs, limited infrastructure, consideration of isoniazid (INH) mono-resistance patterns [64], and lack of evidence on long-term efficacy of preventive therapy in populations with repeated exposures dictate that treatment of LTBI is not currently feasible in developing countries. Although concerns have been raised about laboratory expertise and infrastructure, IGRAs have been performed in resource-poor settings by personnel with no laboratory experience and only a week's training using microscope, centrifuge and incubator [65–67].

Children

LTBI has a high risk of progression to active disease in children (40% in <2-year-old infants and 24% in <5-year-old children), often within 12 months of infection [68]. Studies evaluating the prevalence of presumed childhood LTBI in low-burden or intermediate-burden settings showed that, although both the IGRA and TST correlated well with proximity and exposure to *M. tuberculosis*, the IGRA correlated better [20,69]. Several studies in low-burden settings showed modest-to-poor agreement between the IGRA and TST with the majority of discordance being TST-positive/IGRA-negative [70–75]. Higuchi *et al.* [73], in a low-burden setting, found that none of the TST-positive/IGRA-negative test individuals developed active disease over a 3.5-year follow-up period. Further prospective studies are required to clarify the significance of TST-positive/IGRA-negative results in this setting. By contrast, in high-burden settings, with some exception [33], the TST and IGRA correlated remarkably well, and the TST was as sensitive or more sensitive than the IGRA [76–79]. Two studies from the The Gambia and Cambodia showed that both the TST and IGRA correlated with exposure and, where relevant, proximity [77,78].

Healthcare worker screening

Healthcare workers (HCWs) in high-burden countries, particularly in medical wards, emergency departments, primary care clinics and TB hospitals are at high risk of acquiring TB [80]. A recent study suggests TB incidence rates of more than 1000 per 100 000 per year in South African HCWs [81]. In these settings, there are also high rates of HIV infection and undetected TB has a substantial impact on an already depleted workforce. Because the TST is prone to boosting, surveillance of HCWs could be facilitated by a simple blood test like the IGRA. Several studies have evaluated the feasibility and utility of the IGRA as a potential tool for screening HCWs [32,58,82–87]. In these studies, the prevalence of LTBI in India and territories within the former Soviet Union varied between approximately 30 and 60% [32,82,85]. Agreement between the IGRA and TST was variable; discordance was higher in populations receiving BCG after infancy [83]. In India, there was significant agreement between the TST and IGRA [32]. Only one study evaluated serial responses over 18 months and found a TST and QFT-GIT conversion rate of 9.5 and 11.6, respectively [58]. LTBI-treated HCWs in India, at 6-month follow-up, had persistently positive IGRA responses [86]. Prospective studies are now urgently needed, in HCWs from high-burden settings, to determine the predictive value of IGRAs for active disease and whether chemoprophylaxis in IGRA-positive individuals will be effective in reducing rates of active TB. To facilitate interpretation of serial test results in HCWs, it is also important to determine the best definitions for IGRA conversions and reversions. Available data, although limited, suggest that IGRA conversion rates may be much higher in high-burden settings compared with low-burden settings [88].

HIV-positive individuals with latent tuberculosis infection

The risk of active TB doubles in the first year of HIV co-infection [89], and the risk of developing active disease in those who have LTBI is approximately 10% per year [90]. HIV–TB co-infected individuals have reduced survival rate [91] and are at higher risk for subsequent opportunistic infections [92,93]. Treating LTBI in the HIV-positive patients substantially reduces the subsequent risk of TB [94]. Consequently, treatment of LTBI is recommended and is suggested by a TST exceeding 5 mm [95,96]. However, meaningful interpretation of the TST is hampered by anergy in HIV-positive and other immuno-compromised individuals [97], and this worsens with increasing immunosuppression [97]. The IGRA may circumvent this problem. In low-burden settings, two studies comparatively evaluated the TST and QFT-G test and reported low rates of presumed LTBI; sensitivity of both tests were similar and their positivity diminished with decreasing CD4 cell counts [98,99]. Other studies indicated that the QFT-GIT or T-SPOT.TB IFN-γ responses were attenuated at lower CD4 cell counts [98–101]. In high-burden countries, several studies have documented that rate of LTBI is high (more than 50%), the TST has a lower sensitivity than the IGRA in HIV-positive individuals [102–104,105•,106], and that IGRAs (especially ELISPOT) are less prone, but not unaffected by T-cell anergy at lower CD4 cell counts [103,104,105•,106]. By contrast, one study, using an in-house ELISPOT assay, reported increasing RD-1 responses with decreasing CD4 T cell counts [107]. In resource-poor settings, treatment of LTBI is one of the few interventions proven to reduce morbidity in the absence of antiretroviral therapy [108]. Collectively, these preliminary data suggest that the ELISPOT IFN-γ assay may be more sensitive than the TST in HIV-positive individuals with LTBI. However, whether this will confer benefit in HIV-positive individuals requires confirmation in well designed prospective studies, as those anergic by the TST appear not to be at high risk for developing active TB [109,110]. A crucial question, given the poor sensitivity of TST, is whether IGRAs can accurately target preventive therapy in this group. Prospective studies are currently underway.

Epidemiological surveillance studies of latent tuberculosis infection

Accurate determination of the prevalence of latent infection in a community is essential for an improved understanding of the epidemiology of TB and to guide TB-control strategies. One approach to improving the estimation of LTBI prevalence is to use both TST and IGRAs and estimate the prevalence using Bayesian mixture models and latent class analyses [111].

Predictive value for active tuberculosis in those with latent tuberculosis infection

Despite the shortcomings of the TST, large studies have shown that treatment of LTBI, as defined by the TST, substantially reduces the risk of developing active disease [112–114]. Consequently, the important clinical question is whether treatment, of those identified by the IGRA as having LTBI, will actually reduce the incidence of subsequent clinical TB. Secondly, relevant to both industrialized and developing nations, it is important to determine whether IFN-γ responses are predictive of those who have a high risk of progression to active TB [115•]. Preliminary studies suggest that this approach may be promising. Studies from low-burden [73,116••], intermediate-burden [117], and high-burden [30•,118•,119] countries evaluating the predictive value of IGRA are summarized in Table 3. Diel *et al.* found that although progressors had significantly higher initial IFN-γ levels, there was wide overlap in IFN-γ values between progressors and nonprogressors. Kik *et al.* [120], who evaluated 433 close immigrant contacts in the

Table 3 Summary of studies published in peer-reviewed journals that evaluated the comparative predictive value of the interferon-γ release assays and tuberculin skin test

Study and assay type	Country and follow-up period	Number of participants	Number of progressors/IGRA-positive (%)	Number of progressors/TST-positive	Relative OR of IGRA vs. TST (95% CI)	Number (%) of progressors detected by TST or IGRA vs. IGRA alone vs. TST alone
Diel *et al.* [116••]; QFT-GIT	Low burden (Germany); 24 months	601 Close contacts	6/41 (14.6)	5/90 (5.6) 10 mm cut-point	2.63 (0.67–10.9)	6/6 (100) vs. 6/6 (100) vs. 5/5 (100)
Bakir *et al.* [117]; in-house ELISPOT	Intermediate burden (Turkey)[a]; 24 months	908 Contacts*	11/381 (2.9)	12/550 (2.2)	1.32) (0.53–3.28)	11/15 (73) vs. 11/15 (73) vs. 6/15 (40)
Hill *et al.* [118•]; in-house ELISPOT	High burden (The Gambia); 24 months	2348 Close contacts[b]	11/649 (1.7)	14/843 (1.7)	1.02 (0.42–2.43)	15/21 (71) vs. 11/21 (52) 14/25 (56)
Doherty *et al.* [119]; in-house 5 day ELISA	High burden (Ethiopia); 24 months	24 Close contacts	7/9 (77.8)	7/21 (33.3)	NA	NA

Preliminary data from Kik and Mahomed are not included in the table. ELISA, enzyme-linked immunosorbent assay; IGRA, interferon-γ release assay; NA, not applicable because of the small number of participants; OR, odds ratio; TST, tuberculin skin test. In all the studies a clinical definition of tuberculosis was used.
[a]Isoniazid preventive therapy (IPT) administered in 76% of contacts. TST and IGRA repeated at 6 months (analysis of incident cases in those who did not receive IPT is presented in the manuscript) test-specific incidence rate (1000 person-years) calculated.
[b]Strain-typing of isolates was performed. Of the six contacts who had concordant isolates with their respective index case, four (67%) were mantoux positive at recruitment, three (50%) were ELISPOT positive, and five (83%) were positive by one or other of the two tests.
*Study participants less than 16 years of age.

Netherlands, found that the PPV value for developing active TB was 3.8% [95% confidence interval (CI) 1.0–6.6%] for TST (cut-off ≥15 mm), 2.8% (95% CI 0.38–5.2) for QFT-GIT and 3.3% (95% CI 0.70–5.9) for T-SPOT.TB. Notably, three (culture confirmed) of the eight cases that developed TB had a negative IGRA result. In the largest study, Hill *et al.* [118•] evaluated the comparative predictive value of the TST and RD-1 ELISPOT assay in 2348 The Gambian household contacts. Each test was initially positive in just over half the contacts that developed TB and a combination of the TST and IGRA were positive in 71% of cases. More recently, Bakir *et al.* [117] evaluated the predictive value of ELISPOT among contacts in Turkey, an intermediate prevalence setting. Both ELISPOT and TST had similar rates of progression to disease, but prevalence of LTBI was lower with ELISPOT. Preliminary data from a healthy adolescent cohort study (6363 participants enrolled in Cape Town so far) suggest that a baseline positive QFT-GIT and positive TST (10 mm cut-off) are similar in predicting the onset of TB disease during a 2-year follow-up period (H. Mahomed, SATVI, personal communication).

Collectively, these data suggest that IGRAs may be a promising predictive tool, but a combination of tests (IGRA and TST), including yet unidentified new biomarkers, may be required to determine which individuals are at highest risk of progression to active disease. It is likely that these tools will need to be interpreted in the relevant clinical and geographical context. It is possible that IGRAs and TST may have similar rates of progression and predictive values in high-burden countries, but different predictive values in low-incidence settings. Several large studies are currently underway to address these questions.

Utility for the diagnosis and management of active tuberculosis

In patients with active disease, even when HIV seroprevalence rates are low and there is good laboratory infrastructure, only approximately 50% of treated patients have microbiologically confirmed disease [121]. Moreover in children, immuno-compromised patients and those with extra-pulmonary TB, bacterial load may be low and sample acquisition difficult; consequently, diagnosis is problematic for the clinician.

Diagnosis of active tuberculosis in adults using peripheral blood

A negative IGRA may be a convenient 'rule-out' test for TB if the diagnostic sensitivity of the assay is sufficiently high, for example nearly 95%. However, in contrast to several case–control studies (outlined in [10••]), there are few studies that have evaluated IGRA outcomes in

consecutive TB suspects in a realistic clinical setting. Seven clinical studies from intermediate-burden to low-burden settings, evaluating IGRAs in TB suspects, have shown variable accuracy as rule-in or rule-out tests [122–127]. Sensitivities in active TB ranged from 64 to 92%. Thus, IGRAs can potentially miss 10–30% of active TB cases and are hence unsuitable for use as 'rule-in' tests. Interestingly, two studies showed a very high negative predictive value (NPV) when IGRAs where combined with smear [127] or TST results [123], allowing rapid exclusion of TB suspects from further investigation. Notably, no studies have been undertaken to evaluate the utility of such an approach in a high-burden country. Thus, as IGRAs can yield rapid results, prospective studies are urgently required to evaluate the utility of IGRAs as rule-out tests when combined with smear and TST results. In a cohort of TB suspects in Cape Town, South Africa, preliminary data indicate that combining IGRA with smear results has a good NPV in TB suspects (K. Dheda *et al.*, European Respiratory Society, Berlin, 2008, p. 2529).

Diagnosis of active tuberculosis in HIV-positive adults

TB is the commonest opportunistic infection in HIV-positive individuals in high-burden countries, associated with considerable mortality and morbidity, and diagnostically challenging [92]. Thus, a simple blood test, if proven useful, is an attractive diagnostic option in this group. In three small studies from Italy and the UK, each with less than 40 active HIV–TB co-infected cases, the sensitivity of the T-SPOT.TB test varied from 79 to 95% and specificity between 64 and 100% [128–130]. The rate of indeterminate results was up to 19% [130]. In three African studies, none of which recruited consecutive TB suspects but used the QFT-GIT or an in-house ELISPOT assay, the IGRA sensitivity varied from 74 to 100% (between 39 to 74 individuals in each study) [65,131,132]. ELISPOT performed better than the TST, but IGRA sensitivity dropped with advancing immunosuppression. Interestingly, the ratio of the IGRA response to the CD4 cell count was useful to distinguish latent from active TB [128,132]. In summary, the IGRAs appear promising for the diagnosis of active TB in HIV co-infected patients. Prospective studies enrolling consecutive TB suspects are now required in settings with high rates of LTBI to evaluate the value of this assay in co-infected patients. The incremental value of these tests over smear for rapid rule-in or rule-out also deserves further study. One drawback of the IGRAs is the increasing rate of indeterminate results at lower CD4 cell counts. The CD4 cell count cut-point at which antigen-specific responses are attenuated requires further study.

Diagnosis of active tuberculosis in children using peripheral blood

In the developing world, children carry a large proportion of the TB burden and the rates of TB–HIV co-infection are increasing. Acquisition of sputum or other biological samples is challenging, treatment is often empiric and better diagnostic tools are urgently needed. However, studies on the utility of IGRAs in active TB are limited. In Spain and Italy, the TST was as or more sensitive than the IGRA [72,133]. In two South African studies, the sensitivity of an in-house RD-1 ELISPOT assay varied between 72 and 83% depending on TB case definition [67,134]. Significantly, in the only study that recruited consecutive TB suspects up to 14 years of age, the ELISPOT sensitivity was 83% and although it would have allowed earlier diagnosis and treatment in the 52% of children who were smear negative but culture positive, almost a third of the non-TB group were also ELISPOT positive [67]. Considerably more malnourished children had a positive IFN-γ ELISPOT assay compared with the TST (78 vs. 44%) [67]. More recently, Nicol *et al.* [135] from Cape Town, using the T-SPOT.TB assay in 243 young children, showed that in the combined group of culture-confirmed and clinically probable tuberculosis, the T-SPOT.TB assay was significantly less sensitive than the TST (40 and 52%, respectively). Collectively, these data suggest that the IGRA cannot be used as a rule-out test. Whether treatment can be initiated in different age groups on the basis of a positive result is less clear. The Canadian guideline states that, in addition to routine TB-related tests, IGRAs may be used as a supplementary diagnostic aid in combination with the TST and other investigations to help support a diagnosis of TB. However, IGRA should not be a substitute for, or obviate the need for, appropriate specimen collection [60].

Diagnosis of active tuberculosis using pleural, alveolar lavage and cerebrospinal fluid mononuclear cells

At the site of disease (pleural space or lung), the frequency of antigen-specific T cells is almost 10 times higher than in peripheral blood. It is therefore reasonable to hypothesize that, in contrast to non-TB disorder, at the site of active TB disease, there will be a high frequency of antigen-specific T cells [136]. Indeed, IGRA responses of alveolar lavage lymphocytes [137] and pleural mononuclear cells [138] have been shown to be useful for diagnosis in preliminary studies. Other studies evaluating IGRAs in pleural fluid have shown promise [139–141], although unstimulated IFN-γ levels are more accurate in diagnosing pleural from nonpleural effusions [142]. We have recently completed a study in almost 80 South African pleural TB suspects and showed that both IGRA formats performed sub-optimally compared with unstimulated IFN-γ [143] and other biomarkers [144]. A key finding was the detection of antigen-specific T cells in the pleural space of individuals with other biopsy-proven pathologies but who also had LTBI, and hence high circulating frequencies of antigen-specific T cells. We have observed a similar pattern in a cohort of almost 100

South African TB meningitis suspects (unpublished data). We have also evaluated the IGRA using alveolar lavage cells in a cohort of almost 100 South African TB suspects who underwent bronchoscopy. Although the ELISPOT assay had good predictive value, as previously shown [137], almost a third of patients had inconclusive results, thus limiting the clinical utility of these assays (K. Dheda, submitted).

Monitoring of disease activity and efficacy of anti-tuberculosis treatment

In contrast to studies from low-burden countries, which generally showed rapidly declining responses [35,36•, 37,145], those from high-burden countries showed highly inconsistent and modest-to-minimal changes [13,39,146]. These observations are more likely to be due to biological and other factors, including re-infection, residual post-treatment persistent infection, persistent exposure to environmental mycobacteria, and possible maintenance of circulating pool of effector memory T cells, rather than due to technical factors [36•]. If the IGRAs can be shown to be proxy markers of disease activity, they may have several useful applications. The current benchmark for assessing the efficacy of new immunotherapeutic agents is clinical cure and failure to relapse at 2 years. The IGRA or a modified assay may thus serve as a useful marker of disease activity that will expedite the selection and evaluation of new immunotherapeutic agents. It may also facilitate the search for correlates of protective anti-tuberculous immunity and monitoring treatment efficacy in extra-pulmonary or drug-resistant TB. Large prospective studies evaluating how responses change in relation to anti-TB treatment are now required.

Conclusion

IGRAs have revolutionized the diagnosis of LTBI in low-burden countries. In high-burden settings, however, the performance of IGRAs may be modulated by several factors. In all settings, IGRAs retain specificity in those who are BCG vaccinated or have a false positive TST due to environmental mycobacteria. Sensitivity and correlation with exposure are not consistent between low-incidence and high-incidence settings. It is possible that even predictive value might vary between high-incidence and low-incidence settings. Therefore, prospective studies in high-burden and low-burden countries will need to confirm a reduction in active TB when IFN-γ defined LTBI is treated, and whether the IGRAs will identify those that have a high likelihood of progression to active disease (confirmation that it is a marker of LTBI and not exposure). Future work in high-burden and low-burden countries will also have to address the utility of this test in children, HIV-positive individuals and other immunosuppressed individuals, as a 'rule-out' test for active TB in unselected cohorts of TB suspects, and as a marker of disease activity [147•]. Finally, new antigens [7,148•] and/or cytokines/biomarkers [149] may be necessary to improve the utility of the current IGRAs.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 284–285).

1 Dolin PJ, Raviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990–2000. Bull World Health Organ 1994; 72:213–220.

2 World Health Organization. Global tuberculosis control-surveillance, planning, financing. Geneva: World Health Organisation; 2007.

3 Dheda K, Udwadia ZF, Huggett JF, *et al.* Utility of the antigen-specific interferon-gamma assay for the management of tuberculosis. Curr Opin Pulm Med 2005; 11:195–202.

4 Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis. Part I: Latent tuberculosis. Expert Rev Mol Diagn 2006; 6:413–422.

5 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.

6 Richeldi L. An update on the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2006; 174:736–742.

7 Gey van Pittius NC, Sampson SL, Lee H, *et al.* Evolution and expansion of the *Mycobacterium tuberculosis* PE and PPE multigene families and their association with the duplication of the ESAT-6 (esx) gene cluster regions. BMC Evol Biol 2006; 6:95.

8 Gey van Pittius NC, Warren RM, van Helden PD. ESAT-6 and CFP-10: what is the diagnosis? Infect Immun 2002; 70:6509–6510; author reply 6511.

9 Vekemans J, Lienhardt C, Sillah JS, *et al.* Tuberculosis contacts but not patients have higher gamma interferon responses to ESAT-6 than do community controls in The Gambia. Infect Immun 2001; 69:6554–6557.

10 •• Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection – an update. Ann Intern Med 2008; 149:177–184.

A systematic review and meta-analysis of the IFN-γ release assays and their performance characteristics in the diagnosis of tuberculosis infection.

11 Adetifa IM, Lugos MD, Hammond A, *et al.* Comparison of two interferon gamma release assays in the diagnosis of *Mycobacterium tuberculosis* infection and disease in The Gambia. BMC Infect Dis 2007; 7:122.

12 Kanunfre KA, Leite OH, Lopes MI, *et al.* Enhancement of diagnostic efficiency by a gamma interferon release assay for pulmonary tuberculosis. Clin Vaccine Immunol 2008; 15:1028–1030.

13 Pai M, Joshi R, Bandyopadhyay M, *et al.* Sensitivity of a whole-blood interferon-gamma assay among patients with pulmonary tuberculosis and variations in T-cell responses during antituberculosis treatment. Infection 2007; 35:98–103.

14 Tsiouris SJ, Coetzee D, Toro PL, *et al.* Sensitivity analysis and potential uses of a novel gamma interferon release assay for diagnosis of tuberculosis. J Clin Microbiol 2006; 44:2844–2850.

15 Jackson-Sillah D, Hill PC, Fox A, *et al.* Screening for tuberculosis among 2381 household contacts of sputum-smear-positive cases in The Gambia. Trans R Soc Trop Med Hyg 2007; 101:594–601.

16 Arend SM, Thijsen SF, Leyten EM, *et al.* Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. Am J Respir Crit Care Med 2007; 175:618–627.

17 Brodie D, Lederer DJ, Gallardo JS, *et al.* Use of an interferon-gamma release assay to diagnose latent tuberculosis infection in foreign-born patients. Chest 2008; 133:869–874.

18 Diel R, Ernst M, Doscher G, *et al.* Avoiding the effect of BCG vaccination in detecting *Mycobacterium tuberculosis* infection with a blood test. Eur Respir J 2006; 28:16–23.

19 Diel R, Nienhaus A, Lange C, *et al.* Tuberculosis contact investigation with a new, specific blood test in a low-incidence population containing a high proportion of BCG-vaccinated persons. Respir Res 2006; 7:77.

20 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.

21 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.

22 Richeldi L, Ewer K, Losi M, *et al.* T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med 2004; 170:288–295.

23 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.

24 Zellweger JP, Zellweger A, Ansermet S, *et al.* Contact tracing using a new T-cell-based test: better correlation with tuberculosis exposure than the tuberculin skin test. Int J Tuberc Lung Dis 2005; 9:1242–1247.

25 Hill PC, Brookes RH, Fox A, *et al.* Large-scale evaluation of enzyme-linked immunospot assay and skin test for diagnosis of *Mycobacterium tuberculosis* infection against a gradient of exposure in The Gambia. Clin Infect Dis 2004; 38:966–973.

26 Hill PC, Brookes RH, Fox A, *et al.* Surprisingly high specificity of the PPD skin test for *M. tuberculosis* infection from recent exposure in The Gambia. PLoS ONE 2006; 1:e68.

27 Hill PC, Fox A, Jeffries DJ, *et al.* Quantitative T cell assay reflects infectious load of *Mycobacterium tuberculosis* in an endemic case contact model. Clin Infect Dis 2005; 40:273–278.

28 Hill PC, Jeffries DJ, Brookes RH, *et al.* Using ELISPOT to expose false positive skin test conversion in tuberculosis contacts. PLoS ONE 2007; 2:e183.

29 Whalen CC, Chiunda A, Zalwango S, *et al.* Immune correlates of acute *Mycobacterium tuberculosis* infection in household contacts in Kampala, Uganda. Am J Trop Med Hyg 2006; 75:55–61.

30 • Pai M, Joshi R, Dogra S, *et al.* T-cell assay conversions and reversions among household contacts of tuberculosis patients in rural India. Int J Tuberc Lung Dis 2009; 13:84–92.

A study of 250 tuberculosis contacts and the changes in longitudinal T-cell responses on repeat testing. Reversions in test results were more common in those with low positive QFT results and a negative TST.

31 Mahomed H, Hughes EJ, Hawkridge T, *et al.* Comparison of mantoux skin test with three generations of a whole blood IFN-gamma assay for tuberculosis infection. Int J Tuberc Lung Dis 2006; 10:310–316.

32 Pai M, Gokhale K, Joshi R, *et al.* Mycobacterium tuberculosis infection in healthcare workers in rural India: comparison of a whole-blood, interferon-g assay with tuberculin skin testing. JAMA 2005; 293:2746–2755.

33 Nakaoka H, Lawson L, Squire B, *et al.* Risk for tuberculosis among children. Emerg Infect Dis 2006; 12:1383–1388.

34 Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and nontuberculous mycobacteria? Int J Tuberc Lung Dis 2006; 10:1192–1204.

35 Carrara S, Vincenti D, Petrosillo N, *et al.* Use of a T cell-based assay for monitoring efficacy of antituberculosis therapy. Clin Infect Dis 2004; 38:754–756.

36 • Dheda K, Pooran A, Pai M, *et al.* Interpretation of Mycobacterium tuberculosis antigen-specific IFN-gamma release assays (T-SPOT.TB) and factors that may modulate test results. J Infect 2007; 55:169–173.

A study exploring how technical factors may modulate IFN-γ release assay results.

37 Pathan AA, Wilkinson KA, Klenerman P, *et al.* Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in *Mycobacterium tuberculosis*-infected individuals: associations with clinical disease state and effect of treatment. J Immunol 2001; 167:5217–5225.

38 Millington KA, Innes JA, Hackforth S, *et al.* Dynamic relationship between IFN-gamma and IL-2 profile of *Mycobacterium tuberculosis*-specific T cells and antigen load. J Immunol 2007; 178:5217–5226.

39 Aiken AM, Hill PC, Fox A, *et al.* Reversion of the ELISPOT test after treatment in Gambian tuberculosis cases. BMC Infect Dis 2006; 6:66.

40 Goletti D, Carrara S, Vincenti D, *et al.* Accuracy of an immune diagnostic assay based on RD1 selected epitopes for active tuberculosis in a clinical setting: a pilot study. Clin Microbiol Infect 2006; 12:544–550.

41 Brock I, Weldingh K, Lillebaek T, *et al.* Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. Am J Respir Crit Care Med 2004; 170:65–69.

42 Anderson ST, Williams AJ, Brown JR, *et al.* Transmission of Mycobacterium tuberculosis undetected by tuberculin skin testing. Am J Respir Crit Care Med 2006; 173:1038–1042.

43 • Menzies D, Gardiner G, Farhat M, *et al.* Thinking in three dimensions: a web-based algorithm to aid the interpretation of tuberculin skin test results. Int J Tuberc Lung Dis 2008; 12:498–505.

A mathematically generated prediction algorithm developed to aid in assessing the risk of active tuberculosis in individuals who are tuberculin skin test positive.

44 Guyot-Revol V, Innes JA, Hackforth S, *et al.* Regulatory T cells are expanded in blood and disease sites in tuberculosis patients. Am J Respir Crit Care Med 2006; 173:803–810.

45 Dheda K, Chang JS, Breen RA, *et al.* Expression of a novel cytokine, IL-4delta2, in HIV and HIV-tuberculosis co-infection. AIDS 2005; 19:1601–1606.

46 Rook GA, Dheda K, Zumla A. Immune responses to tuberculosis in developing countries: implications for new vaccines. Nat Rev Immunol 2005; 5:661–667.

47 de Jong BC, Hill PC, Brookes RH, *et al. Mycobacterium africanum* elicits an attenuated T cell response to early secreted antigenic target, 6 kDa, in patients with tuberculosis and their household contacts. J Infect Dis 2006; 193:1279–1286.

48 Hersh AL, Tosteson AN, von Reyn CF. Dual skin testing for latent tuberculosis infection: a decision analysis. Am J Prev Med 2003; 24:254–259.

49 von Reyn CF, Horsburgh CR, Olivier KN, *et al.* Skin test reactions to *Mycobacterium tuberculosis* purified protein derivative and *Mycobacterium avium* sensitin among healthcare workers and medical students in the United States. Int J Tuberc Lung Dis 2001; 5:1122–1128.

50 Lein AD, von Reyn CF, Ravn P, *et al.* Cellular immune responses to ESAT-6 discriminate between patients with pulmonary disease due to *Mycobacterium avium* complex and those with pulmonary disease due to *Mycobacterium tuberculosis*. Clin Diagn Lab Immunol 1999; 6:606–609.

51 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.

52 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.

53 Geluk A, van Meijgaarden KE, Franken KL, *et al.* Immunological crossreactivity of the *Mycobacterium leprae* CFP-10 with its homologue in *Mycobacterium tuberculosis*. Scand J Immunol 2004; 59:66–70.

54 Geluk A, van Meijgaarden KE, Franken KL, *et al.* Identification and characterization of the ESAT-6 homologue of *Mycobacterium leprae* and T-cell cross-reactivity with *Mycobacterium tuberculosis*. Infect Immun 2002; 70:2544–2548.

55 Cardoso FL, Antas PR, Milagres AS, *et al.* T-cell responses to the *Mycobacterium tuberculosis*-specific antigen ESAT-6 in Brazilian tuberculosis patients. Infect Immun 2002; 70:6707–6714.

56 Dheda K, Rook G, Zumla A. Peripheral T cell interferon-gamma responses and latent tuberculosis. Am J Respir Crit Care Med 2004; 170:97–98; author reply 98.

57 Mazurek GH, Jereb J, Lobue P, *et al.* Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recomm Rep 2005; 54:49–55.

58 Pai M, Joshi R, Dogra S, *et al.* Serial testing of healthcare workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med 2006; 174:349–355.

59 Clinical Guideline 33. Tuberculosis: clinical diagnosis and management of tuberculosis, and measures for its prevention and control. http://www.nice.org.uk/page.aspx?o=CG033NICEgui on http://www.nice.org.uk/page.aspx?o=271310.

60 Pai M, Gardam M, Haldane D, *et al.* Canadian Tuberculosis Committee: Updated recommendations on interferon gamma release assays for latent tuberculosis infection. Can Commun Dis Rep 2008; 34:1–12.

61 van Zyl Smit RN, Pai M, Peprah K, *et al.* Within-subject variability and boosting of T cell interferon gamma responses following tuberculin skin testing. Am J Respir Crit Care Med (in press).

62 Ekerfelt C, Lidstrom C, Matthiesen L, *et al.* Spontaneous secretion of interleukin-4, interleukin-10 and interferon-gamma by first trimester decidual mononuclear cells. Am J Reprod Immunol 2002; 47:159–166.

63 • Veerapathran A, Joshi R, Goswami K, *et al.* T-cell assays for tuberculosis infection: deriving cut-offs for conversions using reproducibility data. PLoS ONE 2008; 3:e1850.

A study examining the results in serially tested individuals and proposing a cut-off value for conversions when using the QuantiFERON GIT assay.

64 Khan K, Muennig P, Behta M, Zivin JG. Global drug-resistance patterns and the management of latent tuberculosis infection in immigrants to the United States. N Engl J Med 2002; 347:1850–1859.

65 Chapman AL, Munkanta M, Wilkinson KA, *et al.* Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. AIDS 2002; 16:2285–2293.

66 Lalvani A, Nagvenkar P, Udwadia Z, *et al.* Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. J Infect Dis 2001; 183:469–477.

67 Liebeschuetz S, Bamber S, Ewer K, *et al.* Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 2004; 364:2196–2203.

68 Brailey M. Mortality in the children of tuberculous households. Am J Public Health Nations Health 1940; 30:816–823.

69 Soysal A, Millington KA, Bakir M, *et al.* Effect of BCG vaccination on risk of *Mycobacterium tuberculosis* infection in children with household tuberculosis contact: a prospective community-based study. Lancet 2005; 366:1443–1451.

70 Connell TG, Curtis N, Ranganathan SC, Buttery JP. Performance of a whole blood interferon gamma assay for detecting latent infection with *Mycobacterium tuberculosis* in children. Thorax 2006; 61:616–620.

71 Connell TG, Ritz N, Paxton GA, *et al.* A three-way comparison of tuberculin skin testing, QuantiFERON-TB gold and T-SPOT.TB in children. PLoS ONE 2008; 3:e2624.

72 Dominguez J, Ruiz-Manzano J, De Souza-Galvao M, *et al.* Comparison of two commercially available gamma interferon blood tests for immunodiagnosis of tuberculosis. Clin Vaccine Immunol 2008; 15:168–171.

73 Higuchi K, Harada N, Mori T, Sekiya Y. Use of QuantiFERON-TB Gold to investigate tuberculosis contacts in a high school. Respirology 2007; 12:88–92.

74 Soysal A, Turel O, Toprak D, Bakir M. Comparison of positive tuberculin skin test with an interferon-gamma-based assay in unexposed children. Jpn J Infect Dis 2008; 61:192–195.

75 Taylor RE, Cant AJ, Clark JE. Potential effect of NICE tuberculosis guidelines on paediatric tuberculosis screening. Arch Dis Child 2008; 93:200–203.

76 Dogra S, Narang P, Mendiratta DK, *et al.* Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. J Infect 2007; 54:267–276.

77 Hill PC, Brookes RH, Adetifa IM, *et al.* Comparison of enzyme-linked immunospot assay and tuberculin skin test in healthy children exposed to *Mycobacterium tuberculosis*. Pediatrics 2006; 117:1542–1548.

78 Okada K, Mao TE, Mori T, *et al.* Performance of an interferon-gamma release assay for diagnosing latent tuberculosis infection in children. Epidemiol Infect 2008; 136:1179–1187.

79 Tsiouris SJ, Austin J, Toro P, *et al.* Results of a tuberculosis-specific IFN-gamma assay in children at high risk for tuberculosis infection. Int J Tuberc Lung Dis 2006; 10:939–941.

80 Joshi R, Reingold AL, Menzies D, Pai M. Tuberculosis among health-care workers in low- and middle-income countries: a systematic review. PLoS Med 2006; 3:e494.

81 Naidoo S, Jinabhai CC. TB in healthcare workers in KwaZulu-Natal, South Africa. Int J Tuberc Lung Dis 2006; 10:676–682.

82 Drobniewski F, Balabanova Y, Zakamova E, *et al.* Rates of latent tuberculosis in healthcare staff in Russia. PLoS Med 2007; 4:e55.

83 Hotta K, Ogura T, Nishii K, *et al.* Whole blood interferon-gamma assay for baseline tuberculosis screening among Japanese healthcare students. PLoS ONE 2007; 2:e803.

84 Joshi R, Patil S, Kalantri S, *et al.* Prevalence of abnormal radiological findings in healthcare workers with latent tuberculosis infection and correlations with T cell immune response. PLoS ONE 2007; 2:e805.

85 Mirtskhulava V, Kempker R, Shields KL, *et al.* Prevalence and risk factors for latent tuberculosis infection among healthcare workers in Georgia. Int J Tuberc Lung Dis 2008; 12:513–519.

86 Pai M, Joshi R, Dogra S, *et al.* Persistently elevated T cell interferon-gamma responses after treatment for latent tuberculosis infection among healthcare workers in India: a preliminary report. J Occup Med Toxicol 2006; 1:7.

87 Barsegian V, Mathias KD, Wrighton-Smith P, *et al.* Prevalence of latent tuberculosis infection in German radiologists. J Hosp Infect 2008; 69:69–76.

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

89 Sonnenberg P, Glynn JR, Fielding K, *et al.* How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. J Infect Dis 2005; 191:150–158.

90 Selwyn PA, Hartel D, Lewis VA, *et al.* A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. N Engl J Med 1989; 320:545–550.

91 Whalen C, Horsburgh CR, Hom D, *et al.* Accelerated course of human immunodeficiency virus infection after tuberculosis. Am J Respir Crit Care Med 1995; 151:129–135.

92 Dheda K, Lampe FC, Johnson MA, Lipman MC. Outcome of HIV-associated tuberculosis in the era of highly active antiretroviral therapy. J Infect Dis 2004; 190:1670–1676.

93 Munsiff SS, Alpert PL, Gourevitch MN, *et al.* A prospective study of tuberculosis and HIV disease progression. J Acquir Immune Defic Syndr Hum Retrovirol 1998; 19:361–366.

94 Pape JW, Jean SS, Ho JL, *et al.* Effect of isoniazid prophylaxis on incidence of active tuberculosis and progression of HIV infection. Lancet 1993; 342:268–272.

95 Anergy skin testing and tuberculosis [corrected] preventive therapy for HIV-infected persons: revised recommendations. Centers for Disease Control and Prevention. MMWR Recomm Rep 1997; 46:1–10.

96 Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. Am J Respir Crit Care Med 2000; 161:S221–S247.

97 Duncan LE, Elliott AM, Hayes RJ, *et al.* Tuberculin sensitivity and HIV-1 status of patients attending a sexually transmitted diseases clinic in Lusaka, Zambia: a cross-sectional study. Trans R Soc Trop Med Hyg 1995; 89:37–40.

98 Brock I, Ruhwald M, Lundgren B, *et al.* Latent tuberculosis in HIV positive, diagnosed by the *M. tuberculosis* specific interferon-gamma test. Respir Res 2006; 7:56.

99 Luetkemeyer AF, Charlebois ED, Flores LL, *et al.* Comparison of an interferon-gamma release assay with tuberculin skin testing in HIV-infected individuals. Am J Respir Crit Care Med 2007; 175:737–742.

100 Dheda K, Lalvani A, Miller RF, *et al.* Performance of a T-cell-based diagnostic test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count. AIDS 2005; 19:2038–2041.

101 Hoffmann M, Reichmuth M, Fantelli K, *et al.* Conventional tuberculin skin testing versus T-cell-based assays in the diagnosis of latent tuberculosis infection in HIV-positive patients. AIDS 2007; 21:390–392.

102 Balcells ME, Perez CM, Chanqueo L, *et al.* A comparative study of two different methods for the detection of latent tuberculosis in HIV-positive individuals in Chile. Int J Infect Dis 2008; 12:645–652.

103 Karam F, Mbow F, Fletcher H, *et al.* Sensitivity of IFN-gamma release assay to detect latent tuberculosis infection is retained in HIV-infected patients but dependent on HIV/AIDS progression. PLoS ONE 2008; 3:e1441.

104 Lawn SD, Bangani N, Vogt M, *et al.* Utility of interferon-gamma ELISPOT assay responses in highly tuberculosis-exposed patients with advanced HIV infection in South Africa. BMC Infect Dis 2007; 7:99.

105 • Rangaka MX, Wilkinson KA, Seldon R, *et al.* Effect of HIV-1 infection on T-Cell-based and skin test detection of tuberculosis infection. Am J Respir Crit Care Med 2007; 175:514–520.

A study investigating the effect of HIV infection and increasing immunosuppression on the performance of the tuberculin skin test and the IFN-γ release assay in a high-burden setting. The performance of the QuantiFERON assay, in contrast to the TST, was unaffected by moderate immunosupression.

106 Mandalakas AM, Kirchner HL, Zhu X, *et al.* Interpretation of repeat tuberculin skin testing in international adoptees: conversions or boosting. Pediatr Infect Dis J 2008; 27:913–919.

107 Hammond AS, McConkey SJ, Hill PC, *et al.* Mycobacterial T cell responses in HIV-infected patients with advanced immunosuppression. J Infect Dis 2008; 197:295–299.

108 Godfrey-Faussett P, Ayles H. The impact of HIV on tuberculosis control: towards concerted action. Lepr Rev 2002; 73:376–385.

109 Daley CL, Hahn JA, Moss AR, *et al.* Incidence of tuberculosis in injection drug users in San Francisco: impact of anergy. Am J Respir Crit Care Med 1998; 157:19–22.

110 Gordin FM, Matts JP, Miller C, *et al.* A controlled trial of isoniazid in persons with anergy and human immunodeficiency virus infection who are at high risk for tuberculosis. Terry Beirn Community Programs for Clinical Research on AIDS. N Engl J Med 1997; 337:315–320.

111 Pai M, Dendukuri N, Wang L, *et al.* Improving the estimation of tuberculosis infection prevalence using T-cell-based assay and mixture models. Int J Tuberc Lung Dis 2008; 12:895–902.

112 Efficacy of various durations of isoniazid preventive therapy for tuberculosis: five years of follow-up in the IUAT trial. International Union Against Tuberculosis Committee on Prophylaxis. Bull World Health Organ 1982; 60:555–564.

113 Hsu KH. Thirty years after isoniazid. Its impact on tuberculosis in children and adolescents. JAMA 1984; 251:1283–1285.

114 Stead WW, To T, Harrison RW, Abraham JH 3rd. Benefit–risk considerations in preventive treatment for tuberculosis in elderly persons. Ann Intern Med 1987; 107:843–845.

115 • Andersen P, Doherty TM, Pai M, Weldingh K. The prognosis of latent tuberculosis: can disease be predicted? Trends Mol Med 2007; 13:175–182.

A review exploring the role of the IFN-γ release assays as predictive tools for active disease in those with LTBI.

116 •• Diel R, Loddenkemper R, Meywald-Walter K, *et al.* Predictive value of a whole-blood IFN-{gamma} assay for the development of active TB disease. Am J Respir Crit Care Med 2008; 177:1164–1170.

A prospective 2-year follow-up study comparing the positive predictive value of the tuberculin skin test and the IGRA (QuantiFERON TB GIT) in latently infected contacts from Germany (see Table 3 for interpretation). Individuals who were QFT-GIT positive had higher rates of progression to active TB.

117 Bakir M, Millington KA, Soysal A, *et al.* Prognostic value of a T-cell-based, interferon-gamma biomarker in children with tuberculosis contact. Ann Intern Med 2008; 149:777–787.

118 • Hill PC, Jackson-Sillah DJ, Fox A, *et al.* Incidence of tuberculosis and the predictive value of ELISPOT and mantoux tests in Gambian case contacts. PLoS ONE 2008; 3:e1379.

A prospective 2-year follow-up study comparing the positive predictive value of the tuberculin skin test and the IGRA (QuantiFERON TB GIT) in latently infected contacts from The Gambia. The predictive value of ELISPOT was no better than the TST, but a combination of tests had the best predictive value (see Table 3).

119 Doherty TM, Demissie A, Olobo J, *et al.* Immune responses to the *Mycobacterium tuberculosis*-specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. J Clin Microbiol 2002; 40:704–706.

120 Kik SV, Franken PJ, Mensen M, *et al.* Predicting tuberculosis by IGRA among foreign-born contacts. In: Proceedings of the 2008 IUTALD Meeting in Paris, France; 2008.

121 Ormerod LP, Bentley C. The management of pulmonary tuberculosis notified in England and Wales in 1993. J R Coll Phys Lond 1997; 31:662–665.

122 Dewan PK, Grinsdale J, Kawamura LM. Low sensitivity of a whole-blood interferon-gamma release assay for detection of active tuberculosis. Clin Infect Dis 2007; 44:69–73.

123 Dosanjh DP, Hinks TS, Innes JA, *et al.* Improved diagnostic evaluation of suspected tuberculosis. Ann Intern Med 2008; 148:325–336.

124 Kang YA, Lee HW, Hwang SS, *et al.* Usefulness of whole-blood interferon-γ assay and interferon-γ enzyme-linked immunospot assay in the diagnosis of active pulmonary tuberculosis. Chest 2007; 132:959–965.

125 Kobashi Y, Mouri K, Yagi S, *et al.* Usefulness of the QuantiFERON TB-2G test for the differential diagnosis of pulmonary tuberculosis. Intern Med 2008; 47:237–243.

126 Nishimura T, Hasegawa N, Mori M, *et al.* Accuracy of an interferon-gamma release assay to detect active pulmonary and extra-pulmonary tuberculosis. Int J Tuberc Lung Dis 2008; 12:269–274.

127 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.

128 Clark SA, Martin SL, Pozniak A, *et al.* Tuberculosis antigen-specific immune responses can be detected using enzyme-linked immunospot technology in human immunodeficiency virus (HIV)-1 patients with advanced disease. Clin Exp Immunol 2007; 150:238–244.

129 Goletti D, Carrara S, Vincenti D, Girardi E. T cell responses to commercial mycobacterium tuberculosis-specific antigens in HIV-infected patients. Clin Infect Dis 2007; 45:1652–1654.

130 Vincenti D, Carrara S, Butera O, *et al.* Response to region of difference 1 (RD1) epitopes in human immunodeficiency virus (HIV)-infected individuals enrolled with suspected active tuberculosis: a pilot study. Clin Exp Immunol 2007; 150:91–98.

131 Raby E, Moyo M, Devendra A, *et al.* The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay in Zambian adults with active tuberculosis. PLoS ONE 2008; 3:e2489.

132 Rangaka MX, Diwakar L, Seldon R, *et al.* Clinical, immunological, and epidemiological importance of antituberculosis T cell responses in HIV-infected Africans. Clin Infect Dis 2007; 44:1639–1646.

133 Molicotti P, Bua A, Mela G, *et al.* Performance of QuantiFERON-TB testing in a tuberculosis outbreak at a primary school. J Pediatr 2008; 152:585–586.

134 Nicol MP, Pienaar D, Wood K, *et al.* Enzyme-linked immunospot assay responses to early secretory antigenic target 6, culture filtrate protein 10, and purified protein derivative among children with tuberculosis: implications for diagnosis and monitoring of therapy. Clin Infect Dis 2005; 40:1301–1308.

135 Nicol MP, Davies MA, Wood K, *et al.* Comparison of T-SPOT.TB assay and tuberculin skin test for the evaluation of young children at high risk for tuberculosis in a community setting. Pediatrics 2009; 123:38–43.

136 Jafari C, Ernst M, Strassburg A, *et al.* Local immunodiagnosis of pulmonary tuberculosis by enzyme-linked immunospot. Eur Respir J 2008; 31:261–265.

137 Jafari C, Ernst M, Diel R, *et al.* Rapid diagnosis of smear-negative tuberculosis by bronchoalveolar lavage enzyme-linked immunospot. Am J Respir Crit Care Med 2006; 174:1048–1054.

138 Wilkinson KA, Wilkinson RJ, Pathan A, *et al.* Ex vivo characterization of early secretory antigenic target 6-specific T cells at sites of active disease in pleural tuberculosis. Clin Infect Dis 2005; 40:184–187.

139 Ariga H, Kawabe Y, Nagai H, *et al.* Diagnosis of active tuberculous serositis by antigen-specific interferon-g response of cavity fluid cells. Clin Infect Dis 2007; 45:1559–1567.

140 Baba K, Sornes S, Hoosen AA, *et al.* Evaluation of immune responses in HIV infected patients with pleural tuberculosis by the QuantiFERON TB-Gold interferon-gamma assay. BMC Infect Dis 2008; 8:35.

141 Chegou NN, Walzl G, Bolliger CT, *et al.* Evaluation of adapted whole-blood interferon-gamma release assays for the diagnosis of pleural tuberculosis. Respiration 2008; 76:131–138.

142 Jiang J, Shi HZ, Liang QL, *et al.* Diagnostic value of interferon-gamma in tuberculous pleurisy: a metaanalysis. Chest 2007; 131:1133–1141.

143 Dheda K, van Zyl Smit RN, Sechi LA, *et al.* T cell responses versus unstimulated interferon gamma for the diagnosis of pleural tuberculosis. Eur Respir J (in press).

144 Dheda K, van-Zyl Smit RN, Sechi LA, *et al.* Clinical diagnostic utility of IP-10 and LAM antigen levels for the diagnosis of tuberculous pleural effusions in a high burden setting. PLoS ONE 2009; 4:e4689. [Epub Mar 11].

145 Lalvani A, Pathan AA, McShane H, *et al.* Rapid detection of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. Am J Respir Crit Care Med 2001; 163:824–828.

146 Goletti D, Carrara S, Mayanja-Kizza H, *et al.* Response to *M. tuberculosis* selected RD1 peptides in Ugandan HIV-infected patients with smear positive pulmonary tuberculosis: a pilot study. BMC Infect Dis 2008; 8:11.

147 • Pai M, Dheda K, Cunningham J, *et al.* T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. Lancet Infect Dis 2007; 7:428–438.

An overview of recommendations for future research priorities to improve the diagnosis of tuberculosis when using IGRAs.

148 • Hougardy JM, Schepers K, Place S, *et al.* Heparin-binding-hemagglutinin-induced IFN-gamma release as a diagnostic tool for latent tuberculosis. PLoS ONE 2007; 2:e926.

A study suggesting that HBHA is a sensitive and specific test for LTBI and has potential to discriminate latent and active TB.

149 Ruhwald M, Bodmer T, Maier C, *et al.* Evaluating the potential of IP-10 and MCP-2 as biomarkers for the diagnosis of tuberculosis. Eur Respir J 2008; 32:1607–1615.