T-cell interferon-γ release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings
Keertan Dheda, Richard van Zyl Smit, Motasim Badri and Madhukar Pai

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 Mycobacterium 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 Mycobacterium 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 vs. low-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 protein derivatives (PPD) of Mycobacterium tuberculosis. The interferon-γ release assays (IGRAs) measure T-cell responses to ESAT-6 and CFP-10 antigens. These antigens are highly conserved, and therefore, likely to be present in all strains of M. tuberculosis. The IGRA is a more specific test than the TST, as it does not react with BCG-vaccinated individuals. However, the IGRA is a more expensive test, as compared with the TST. The IGRA also requires more technical expertise to perform and interpret.

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

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

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

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.

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.

IFN-γ assays and tuberculosis Dheda et al. 189

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.
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 IGRAs 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 \([11,15,25]\). A study performed in The Gambia showed that the TST is more specific in BCG-vaccinated individuals, correlates better with a gradient of exposure than the IGRAs, and concordance with 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-\(\beta\), 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-\(\gamma\) 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

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

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.

*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 IGRA 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 in 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 [106]. 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, IGRA 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 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 100000 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 IGRA 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 IGRA (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 IGRA 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 IGRA 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>
<thead>
<tr>
<th>Study and assay type</th>
<th>Country and follow-up period</th>
<th>Number of participants</th>
<th>Number (%) of progressors/IGRA-positive (%)</th>
<th>Number (%) of progressors/TST-positive (%)</th>
<th>Relative OR of IGRA vs. TST (95% CI)</th>
<th>Number (%) of progressors detected by TST or IGRA vs. IGRAlone vs. TST alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diel et al. [116**]; QFT-GIT</td>
<td>Low burden (Germany); 24 months</td>
<td>601 Close contacts</td>
<td>6/41 (14.6)</td>
<td>5/90 (5.6)</td>
<td>2.63 (0.67–10.9)</td>
<td>6/6 (100) vs. 6/6 (100) vs. 5/5 (100)</td>
</tr>
<tr>
<td>Bakir et al. [117]; in-house ELISPOT</td>
<td>Intermediate burden (Turkey)b; 24 months</td>
<td>908 Contacts</td>
<td>11/381 (2.9)</td>
<td>12/550 (2.2)</td>
<td>1.32 (0.53–3.28)</td>
<td>11/15 (73) vs. 11/15 (73) vs. 6/15 (40)</td>
</tr>
<tr>
<td>Hill et al. [118]; in-house ELISPOT</td>
<td>High burden (The Gambia); 24 months</td>
<td>2348 Close contactsb</td>
<td>11/649 (1.7)</td>
<td>14/843 (1.7)</td>
<td>1.02 (0.42–2.43)</td>
<td>15/21 (71) vs. 11/21 (52) 14/25 (56)</td>
</tr>
<tr>
<td>Doherty et al. [119]; in-house 5 day ELISA</td>
<td>High burden (Ethiopia); 24 months</td>
<td>24 Close contacts</td>
<td>7/9 (77.8)</td>
<td>7/21 (33.3)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Preliminary data from Kk 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.

**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 T-SPOT positive, and five (83%) were positive by one or other of the two tests.

Study participants less than 16 years of age.

Collectively, these data suggest that IGRA may be a promising diagnostic tool, but a combination of tests (IGRA and TST), including yet unidentified new biomarkers, may be required to determine which individuals have active TB if the diagnostic sensitivity of the assay is sufficiently high, for example nearly 95%. However, in contrast to peripheral blood markers, there is some evidence that IGRA and TST have similar rates of predictive values in low-incidence settings, but different predictive values in high-incidence settings. Several large studies are currently underway to address these questions.
consecutive TB suspects in a realistic clinical setting. Seven clinical studies from intermediate-burden to low-burden settings, evaluating IGRA s 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, IGRA s 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 IGRA s 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 IGRA s can yield rapid results, prospective studies are urgently required to evaluate the utility of IGRA s 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 s 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 IGRA s 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 IGRA s 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 IGRA s 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, IGRA s 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 IGRA s 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 IGRA 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.

**Acknowledgement**

K.D. is supported by a South African MRC Career Development Award and the South African Research Chair Initiative (SARChI). R.V.Z.S. is supported by a Discovery Foundation Fellowship and MRC Development award. M.P. is supported by a career award by the Canadian Institutes of Health Research (CIHR).

**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. 284–295).


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.


Infectious diseases


31 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.


48 A methodologically generated prediction algorithm developed to aid in assessing the risk of active tuberculosis in individuals who are tuberculin skin test positive. Am J Respir Crit Care Med (in press).


IFN-γ assays and tuberculosis Dheda et al. 199


96 Targeted tuberculin testing and treatment of latent tuberculosis infection. This official 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.


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


