Effect of HIV-1 Infection on T-Cell–based and Skin Test Detection of Tuberculosis Infection

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Rationale: **Two forms of the IFN-γ release assay (IFNGRA) to detect tuberculosis infection are available, but neither has been evaluated in comparable HIV-infected and uninfected persons in a high tuberculosis incidence environment.**

Objective: **To compare the ability of the T-SPOT.*TB* (Oxford Immunotec, Abingdon, UK), QuantiFERON-TB Gold (Cellestis, Melbourne, Australia), and Mantoux tests to identify latent tuberculosis in HIV-infected and uninfected persons.**

Methods: **A cross-sectional study of 160 healthy adults without active tuberculosis attending a voluntary counseling and testing center for HIV infection in Khayelitsha, a deprived urban South African community with an HIV antenatal seroprevalence of 33% and a tuberculosis incidence of 1,612 per 100,000.**

Measurements and Main Results: **One hundred and sixty (74 HIV^+ and 86 HIV^-) persons were enrolled. A lower proportion of Mantoux results was positive in HIV-infected subjects compared with HIV-uninfected subjects ($p < 0.01$). By contrast, the proportion of positive IFNGRAs was not significantly different in HIV-infected persons for the T-SPOT.*TB* test (52 vs. 59%; $p = 0.41$) or the QuantiFERON-TB Gold test (43 and 46%; $p = 0.89$). Fair agreement between the Mantoux test (5- and 10-mm cutoffs) and the IFNGRA was seen in HIV-infected people ($\kappa = 0.52$–0.6). By contrast, poor agreement between the Mantoux and QuantiFERON-TB Gold tests was observed in the HIV-uninfected group ($\kappa = 0.07$–0.30, depending on the Mantoux cutoff). The pattern was similar for T-SPOT.*TB* ($\kappa = 0.18$–0.24).**

Interpretation: **IFNGRA sensitivity appears relatively unimpaired by moderately advanced HIV infection. However, agreement between the tests and with the Mantoux test varied from poor to fair. This highlights the need for prospective studies to determine which test may predict the subsequent risk of tuberculosis.**

Keywords: diagnostic techniques and procedures; antigens; interferons; ESAT-6 protein; CFP-10 protein

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Tuberculosis infection may be accurately diagnosed by IFN-γ release assay. However, these tests have not been rigorously evaluated in settings where there is a high prevalence of HIV infection and high incidence of tuberculosis.

What This Study Adds to the Field

IFN-γ release assay sensitivity appears relatively unaffected by moderately advanced HIV infection. However, agreement between the tests and with the Mantoux test varied from poor to fair.

The World Health Organization has called for "urgent and extraordinary actions" to control tuberculosis in Africa (1). Africa contains 9 of the 22 countries with the highest tuberculosis burden in the world, and the predominant factor driving the increased incidence of tuberculosis in these areas is the high prevalence of HIV (2).

Detection of latent or recent infection, which represents the reservoir of future tuberculosis cases, is an opportunity for intensified control (3). In HIV-infected people with latent tuberculosis infection (LTBI), isoniazid preventive therapy (IPT) is an underutilized public health strategy (4). However, the skin reaction to tuberculin PPD is impaired by HIV infection (5, 6). Furthermore, the tuberculin skin test (TST) has poor specificity in areas of high bacillus Calmette-Guérin (BCG) coverage and where there is high prevalence of environmental mycobacteria (7). The introduction of IFN-γ release assays (IFNGRAs) using immunogenic and specific *Mycobacterium tuberculosis* antigens (early secretory antigenic target [ESAT]-6 and culture filtrate protein 10 kD [CFP-10]) for immunodiagnosis is therefore a potential advance. Compared with the TST, research using IFNGRA indicates a high sensitivity for active tuberculosis (8–10). Positive scores in these assays have also been shown in contact studies to equate well with a history of exposure to tuberculosis (11–13).

Two commercial forms of the IFNGRA are now variously licensed for use in the developed world: the T-SPOT.*TB* (Oxford Immunotec, Abingdon, UK), which is based on the enzyme-linked immunospot (ELISpot) assay (14); and the whole blood–based QuantiFERON-TB Gold (QFT-G; Cellestis, Melbourne, Australia), which uses an ELISA to detect IFN-γ released into culture supernatant (15). Most data that supported licensing and introduction of these tests were obtained from studies in regions

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of the world where tuberculosis is uncommon and with emphasis on HIV-uninfected persons. Preliminary doubt that these assays can be applied to HIV-infected people has been raised (16, 17) although ELISpot-based analyses report encouraging sensitivity (73–90%) for active HIV-associated tuberculosis in both children and adults (18, 19). There have been relatively few published three-way comparisons of these tests (20–22), and no study has systematically assessed the decrement in sensitivity that might be conferred by HIV infection in comparable HIV-infected and uninfected persons. Further, studies in developing country environments are few. Some of the results of this study have been previously reported in the form of an abstract (23).

METHODS

For additional detail on methods, *see* the online supplement.

Study Setting

The study was conducted in Khayelitsha, South Africa, at an integrated tuberculosis/HIV clinic run by provincial and city health administrations together with Médecins sans Frontières (Geneva, Switzerland) (24). Khayelitsha has an incidence of tuberculosis of 1,612 per 100,000 and HIV infection is associated with a 36% per annum risk of developing active tuberculosis. Seventy-six percent of tuberculosis is HIV associated and the prevalence of HIV infection in women attending antenatal care is 33% (provincial and city data, 2005).

Recruitment and Selection

This study was approved by the University of Cape Town Research Ethics Committee (REC 443/2004). Adults resident in Khayelitsha and attending voluntary testing and counseling for HIV infection were invited to participate. Persons testing seronegative for HIV-1 were invited to participate after voluntary testing and counseling, on the day of their negative test. Recruitment of persons found to be HIV seropositive was deferred until their first visit to the HIV/AIDS clinic, because we did not wish to recruit them before they had time to accept their HIV diagnosis. Prior tuberculosis, IPT, steroid therapy, pregnancy, and (in HIV-infected people) a Karnofsky score not exceeding 60 or current opportunistic infection were exclusions.

Clinical Assessment

Assessment of eligibility included a symptom-screening questionnaire and physical examination for active tuberculosis based on validated approaches (25, 26). The presence of any one of the following—cough, chest pain, recent weight loss, night sweats, fever, loss of appetite, swelling of lymph nodes, generalized tiredness—formed an exclusion criterion and triggered referral. At the first study visit blood was taken and the intradermal (Mantoux) skin test was performed by placing tuberculin PPD RT23 2TU on the volar aspect of the forearm. On the second study visit, the transverse diameter of the TST induration was determined by the ballpoint pen and ruler method. HIV-infected persons with Mantoux reactions of or exceeding 5 mm were offered IPT according to South African national guidelines.

Laboratory Assays

Blood samples were processed within 4 hours of drawing. The T-SPOT.*TB* and QFT-G assays were performed and interpreted according to the manufacturer's insert guidelines (T-SPOT.*TB*) and, in the case of QFT-G, the latest update at the manufacturer's website. Laboratory staff were blind to the clinical status of samples. Scoring was carried out by a third investigator who had no role in clinical recruitment or in performing the laboratory assays. In HIV-infected persons, the $CD4^+$ cell count was simultaneously determined: persons qualifying for antiretroviral therapy ($CD4^+$ cell count less than 200/mm^3 or World Health Organization clinical stage 4) were referred to the antiretroviral clinic.

Data Analysis

Contingency analysis was by χ^2 test or Fisher exact test. McNemar paired analysis was used to compare proportions between the three tests. Mantel-Haentzel methods were used to explore the data before regression methods were employed. Adjustments were made for baseline factor(s) shown by univariate analysis to be significantly associated with either HIV status or any test. Logistic regression was used to obtain adjusted odds ratios and the likelihood ratio test was used to determine overall significance and test for trend for categorical variables with more than two categories. Diagnostic test comparability was assessed by computing κ statistics. Significance was inferred for $p < 0.05$.

RESULTS

Baseline Characteristics of Participants by HIV Status and Relationship to Test Positivity

One hundred and sixty persons were recruited, of whom 74 were HIV infected and 86 were HIV uninfected (Figure 1). Baseline characteristics were similarly distributed in the two groups except for the proportion with deltoid scarring (Table 1). This was present in 51% (36 of 71) of the HIV-infected group compared with 71% (56 of 79) in the HIV-uninfected group ($p = 0.01$).

Sixteen (10%) persons did not return for the TST reading, seven in the HIV-infected group and nine in the HIV-uninfected group. Three of 320 (0.9%, 2 QFT-G and 1 T-SPOT.*TB*) IFNGRAs

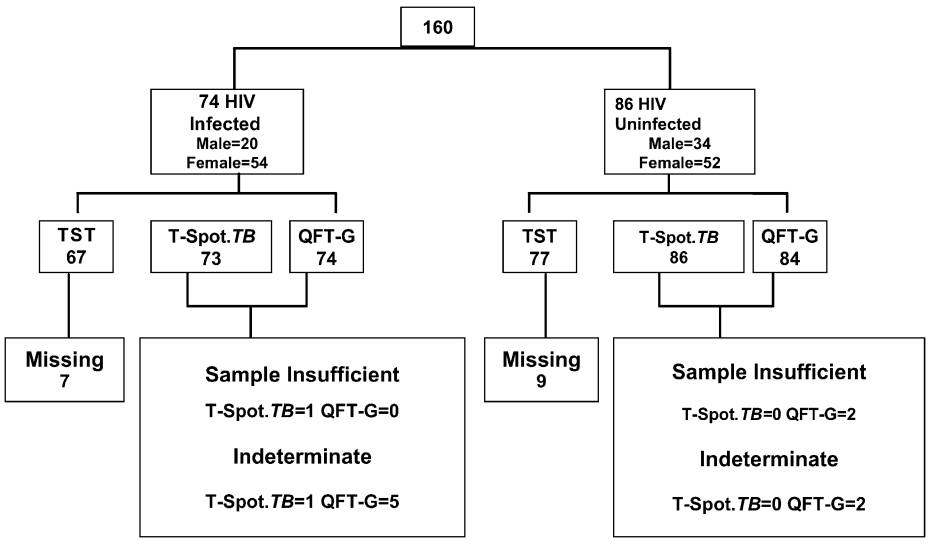

Figure 1. Flow chart showing numbers recruited in both HIV status groups and numbers obtained for final analysis. QFT-G = QuantiFERON-TB Gold (commercial form of IFN-γ release assay [IFNGRA] from Cellestis, Melbourne, Australia); T-SPOT.*TB* (commercial form of IFNGRA from Oxford Immunotec, Abingdon, UK); TST = tuberculin skin test.

TABLE 1. BASELINE CHARACTERISTICS OF YOUNG ADULTS ATTENDING VOLUNTARY COUNSELING AND TESTING FOR HIV-1 INFECTION IN KHAYELITSHA TOWNSHIP, SOUTH AFRICA

	HIV Infected (*n = 74*)	HIV Uninfected (*n = 86*)	p Value
Sex, % (n)			
Female	73 (54)	60 (52)	0.10
Age, yr			
Mean (SD)	30 (6.37)	30 (9.08)	0.68
TB contact in last 6 mo, % (n)			
Yes	12 (9/73)	21 (18/85)	0.14
Deltoid/BCG scarring seen, % (n)			
Yes	51 (36/71)	71 (56/79)	0.01
$CD4^+$ cell count			
Median (IQR)	392 (263–520)		
$< 200/mm^3$, % (n)	16 (10)		
$200–349/mm^3$, % (n)	27 (17)		
$350–499/mm^3$, % (n)	30 (19)		
$> 500/mm^3$, % (n)	28 (18)		

Definition of abbreviations: BCG = bacillus Calmette-Guérin; IQR = interquartile range; TB = tuberculosis.

A total of 160 adults were studied. *Note*: Numbers in parentheses indicate absolute number in group, and for factors where some information was missing, denominators are given.

could not be performed because of insufficient sample to perform both assays. Seven QFT-G results were scored indeterminate: five (7%) HIV infected and two HIV uninfected (2%) (Table 2). These persons were not obviously different in terms of baseline characteristics from the groups to which positive or negative scores could be assigned. Overall, therefore, the T-SPOT.*TB* (158 of 159) yielded a significantly higher proportion of interpretable results than did the QFT-G (151 of 158; $p = 0.04$) and the TST (144 of 160; $p < 0.001$).

We next analyzed potential risk factors for tuberculosis infection (Table 1) and related these to positivity in any test. No factor was consistently associated with positivity for all the tests in either the HIV-infected group or the uninfected group. Deltoid scarring, in particular, was not associated with either IFNGRA or TST positivity at any cutoff level.

Effect of HIV Infection on Proportion of Positive Results for Each Test

The median TST diameter was 8 mm (range, 0–30 mm) in HIV-infected subjects and 15 mm (range, 0–50 mm) in HIV-uninfected subjects ($p < 0.0001$). The distribution of TST reactions in both groups was bimodal, persons tending to be either TST negative or strongly positive (Figure 2). A significantly lower proportion of TST results was positive in the HIV-infected group compared with the HIV-uninfected group regardless of TST cutoff ($p \leq 0.01$; Table 2). This was evident even after controlling for the absence of deltoid scarring (likelihood ratio test, $p < 0.0001$; Table 3). By contrast, the proportion of IFNGRA tests positive in the HIV-infected group was not significantly lower for T-SPOT.*TB* (52 vs. 59%; $p = 0.41$), or for QFT-G (43 and 46%; $p = 0.89$).

McNemar's paired comparisons of the proportion positive by either IFNGRA or TST in both groups were also conducted. In HIV-uninfected persons, the proportion positive by T-SPOT.*TB* was significantly lower than by TST at both 5- and 10-mm cutoffs ($p < 0.001$; Figure 3B), a difference that disappeared at the 15-mm TST cutoff ($p = 0.56$). Similar results applied to QFT-G, with a significantly lower proportion of QFT-G results positive when compared with the TST at cutoff levels of 5 and 10 mm ($p < 0.001$; Figure 3C) but not at 15 mm ($p = 0.24$; Figure 3E).

In HIV-infected people, the proportion positive by T-SPOT.*TB* and TST at cutoffs of 5 and 10 mm did not differ (exact $p = 1.00$ and 0.58, respectively). However, a significantly greater proportion of T-SPOT.*TB* results was positive when compared with the TST at the 15-mm cutoff ($p = 0.01$). For the QFT-G, there was no statistically significant difference in proportions detected by the assay and TST at any cutoff point. When comparing the two forms of the IFNGRA within the HIV exposure groups, there was a trend toward greater positivity in the T-SPOT.*TB* assay in the HIV-uninfected group (59 vs. 46%, $p = 0.07$).

Stratification of Results in HIV-infected Persons by $CD4^+$ Cell Count

As both IFNGRAs depend predominantly on the CD4 recognition of *M. tuberculosis* antigens (27), a variable that will affect test performance in the HIV-infected group is the absolute $CD4^+$ cell count. Data were therefore explored by stratification by

TABLE 2. PROPORTION OF TESTS POSITIVE BY HIV STATUS

	HIV Infected (*n = 74*)	HIV Uninfected (*n = 86*)	p Value (χ^2)
TST, % (n)			
5-mm cutoff			
Negative	48 (32)	14 (11)	
Positive	52 (35)	86 (66)	< 0.0001
10-mm cutoff			
Negative	51 (34)	17 (13)	
Positive	49 (33)	83 (64)	< 0.0001
15-mm cutoff			
Negative	63 (42)	42 (32)	
Positive	37 (25)	58 (45)	0.01
T-SPOT.*TB*, % (n)			
Negative	47 (34)	41 (35)	
Positive	52 (38)	59 (51)	0.41
Indeterminate	1 (1)	0	
QuantiFERON-TB Gold, % (n)			
Negative	50 (37)	51 (43)	
Positive	43 (32)	46 (39)	0.89
Indeterminate	7 (5)	2 (2)	

Definition of abbreviation: TST = tuberculin skin test.

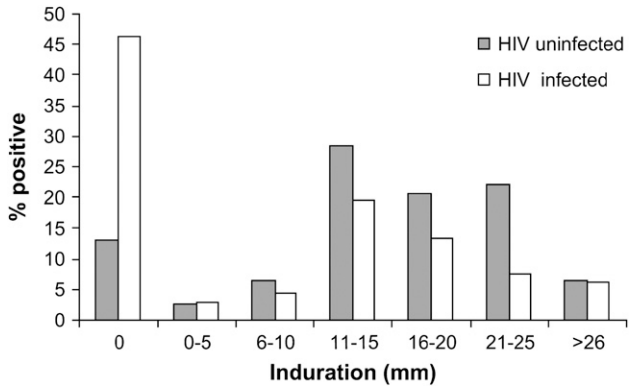

Figure 2. Distribution of TST results in HIV-infected and uninfected persons. The distribution of skin test reactions in both groups was bimodal, persons tending to be either negative or strongly positive.

$CD4^+$ cell count: < 350, < 250, and $< 200/mm^3$. The proportion identified as tuberculosis infected in the lower $CD4^+$ cell count group was higher for T-SPOT.*TB* compared with QFT-G (stratification at a $CD4^+$ cell count of $250/mm^3$ is shown in Figure 4). This was observed regardless of the cutoff point for $CD4^+$ cell count. With T-SPOT.*TB*, 48% (25), 54% (13), and 44% (9) at $CD4^+$ cell count < 350, < 250, and $< 200/mm^3$, respectively, were identified as positive and QFT-G identified 33% (24), 33% (12), and 26%, respectively (Figure 4). Tests for trend were, however, not statistically significant. When including the HIV-uninfected group for comparison, the proportion positive clearly fell for the TST ($p < 0.001$ for 5 and 10 mm, and $p = 0.053$ for 15 mm) but remained relatively constant for both IFNGRAs ($p =$ nonsignificant, regardless of whether indeterminate results were factored as negative; Figure 4).

Agreement between Tests

In the combined HIV-infected and HIV-uninfected groups, overall test agreement was significant but moderate with κ values ranging from 0.31 to 0.40 (Table 4). In HIV-infected persons agreement between the TST and both forms of IFNGRA was fair at the 5- and 10-mm TST cutoffs ($\kappa = 0.52$–0.6, $p < 0.001$) but only moderate at the 15-mm cutoff ($\kappa = 0.31$–0.49, $p \leq 0.006$). QFT-G and T-SPOT.*TB* were also in moderate agreement in the HIV-infected group ($\kappa = 0.37$, $p < 0.0001$). Overall, 69% of HIV-infected people were scored positive by one or more test.

TABLE 3. EFFECT OF HIV INFECTION ON SKIN TEST POSITIVITY* ADJUSTED FOR BASELINE FACTORS

Adjusted Factor	OR for HIV Infection (95% CI)†	p Value	p Value (LRT)‡
No adjustment	0.2 (0.10–0.45)	< 0.0001	—
Deltoid scarring	0.2 (0.09–0.45)	< 0.0001	0.72
Female sex	0.2 (0.09–0.46)	< 0.0001	0.97
TB contact	0.21 (0.09–0.47)	< 0.0001	0.77

Definition of abbreviations: CI = confidence interval; LRT = likelihood ratio test; OR = odds ratio; TB = tuberculosis.

* ⩾ 10 mm.

† OR estimated by logistic regression

‡ LRT p values for the simple model showing the association between HIV infection and skin test positivity compared with the model in which a baseline factor is adjusted. Nonsignificant LRT p values indicate that the simpler, unadjusted model best explains the data.

T-Spot.*TB* against QFT-G

A

	QFT-G+	QFT-G-
T-Spot.*TB*+	28	***17***
T-Spot.*TB*-	***8***	21

McNemar p=0.07

TST positivity > 10mm

B

	T-spot.*TB*+	T-Spot.*TB*-
TST+	40	***21***
TST-	***5***	8

(Exact McNemar's p=0.003)

C

	QFT-G+	QFT-G-
TST+	32	***29***
TST-	***4***	9

(Exact McNemar's p<0.0001)

TST positivity > 15mm

D

	T-Spot.*TB*+	T-Spot.*TB*-
TST+	30	***12***
TST-	***15***	17

(McNemar's p=0.56)

E

	QFT-G+	QFT-G-
TST+	26	***16***
TST-	***10***	22

(McNemar's p=0.24)

Figure 3. Pair-wise analysis of test results in HIV-uninfected persons: 2×2 tables show concordant and discordant test results for the HIV-uninfected group.

In contrast, generally poor agreement between the TST and IFNGRA was observed in the HIV-uninfected group. In this group κ values for QFT-G against TST at cutoff levels of 5 and 10 mm were poor ($\kappa = 0.07$ and 0.12, $p = 0.19$ and 0.08, respectively); against the TST at a cutoff of 15 mm, agreement was slightly better with $\kappa = 0.30$ ($p = 0.004$). Although the T-SPOT.*TB* showed marginally better agreement (65%, $p \leq 0.04$, $\kappa = 0.17$ and 0.18 for 5 and 10 mm, respectively) with the lower grades of TST, the pattern was similar with best (albeit still only moderate to poor) agreement with TST at 15 mm (64%, $\kappa = 0.24$, $p = 0.02$). Poor agreement was contributed to by

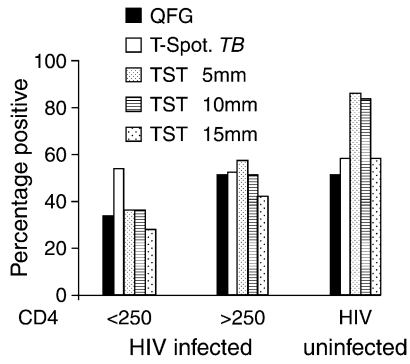

Figure 4. Proportion positive in each test stratified by HIV status and $CD4^+$ cell count. Although the proportion scored positive in lower $CD4^+$ cell count strata tended to be consistently higher for T-SPOT.*TB* compared with QFT-G, this effect was not statistically significant for any $CD4^+$ cell count cutoff. When including the HIV-uninfected group for comparison, the proportion positive clearly fell for the TST ($p <$ 0.001 for 5 and 10 mm, and $p = 0.053$ for 15 mm) but remained relatively constant for the IFNGRA ($p =$ nonsignificant, regardless of whether indeterminate results were factored as negative).

TABLE 4. AGREEMENT BETWEEN TUBERCULIN SKIN TEST AT VARIOUS CUTOFFS, T-SPOT.*TB* TEST, AND QuantiFERON-TB GOLD TEST

	Combined Groups			HIV Infected			HIV Uninfected		
	Agreement (%)	κ	p Value	Agreement (%)	κ	p Value	Agreement (%)	κ	p Value
QFT-G vs. T-SPOT.*TB*	68	0.37	< 0.001	67	0.34	0.002	70	0.40	< 0.001
QFT-G vs. TST 5 mm	65	0.31	< 0.001	79	0.58	< 0.001	53	0.07	0.189
QFT-G vs. TST 10 mm	65	0.31	< 0.001	76	0.52	< 0.001	55	0.12	0.078
QFT-G vs. TST 15 mm	65	0.31	< 0.001	66	0.31	0.006	65	0.30	0.005
T-SPOT.*TB* vs. TST 5 mm	72	0.40	< 0.001	80	0.60	< 0.001	65	0.17	0.035
T-SPOT.*TB* vs. TST 10 mm	72	0.40	< 0.001	80	0.60	< 0.001	65	0.18	0.034
T-SPOT.*TB* vs. TST 15 mm	68	0.37	< 0.001	74	0.49	< 0.001	64	0.24	0.016

both IFNGRA$^-$ TST$^+$ pairs and IFNGRA$^+$ TST$^-$ pairs (Figures 3B–3E).

DISCUSSION

This is the first study to compare three methods to ascertain LTBI in comparable groups of HIV-infected and uninfected young adults in an area where there is a high incidence of tuberculosis and prevalence of HIV. The results indicate a high prevalence ($\sim$ 69%) of LTBI among HIV-infected young adults in this township, a finding that is of considerable public health significance.

In studies of the TST, it is desirable to ascertain BCG vaccination status. However, we found an inconsistent variety of scars in both the left and right deltoid regions. Some of these were characteristic of variolation, others of percutaneous or intradermal BCG, and yet others that could not be reliably classified. The median age of persons enrolled to this study was 30 years. In 1976 and 1981 the geographic Eastern Cape birthplace of many participants in this study transiently became the "independent" apartheid homelands of Transkei and Ciskei, respectively. Others were born in what was considered South Africa. It follows that childhood immunization procedures, if in place at all, were variable indeed and it was not therefore possible for us to ascertain BCG status with confidence. Deltoid scarring was not associated with positivity for any test of tuberculosis sensitization. Our results are consistent with other studies in adults from endemic countries that demonstrate no relationship between TST positivity and the presence of a BCG scar (28, 29).

Although 90% of TST determinations could be made, the proportion of IFNGRAs that yielded an interpretable result was higher (95.6% for QFT-G and 99.4% for T-SPOT.*TB*). The difference in proportion was significant for the T-SPOT.*TB* when compared with both tests. Thus both forms of the IFNGRA are robust in a developing country setting albeit in the context of research conducted by experienced personnel. Even if the patient does not reattend for IFNGRA results the determination will remain valid, which is not the case for the TST. One issue that has arisen with the QFT-G test is postmarketing refinement of the manufacturer's criteria of what constitutes an unacceptable "nil" (i.e., unstimulated blood) value for IFN-γ detected by ELISA (9, 30). High nil values complicate the interpretation of the antigen-stimulated increment, but in this study we found a lower proportion of QFT-G assays indeterminate than in other studies of immunocompromised patients (16, 20).

Our study showed a clear decrement in TST reactivity in HIV-infected persons when compared with HIV-uninfected persons from the same community. This is consistent with many previous evaluations of the TST (31, 32). There was no similar HIV-related decrement in response in the HIV-infected subjects tested by IFNGRA. This highly encouraging finding therefore suggests that the IFNGRA could better identify HIV-infected persons at risk of tuberculosis. However, the median CD4$^+$ cell count of our patients was relatively high at 392/mm^3 and there was a trend toward decreased positivity for QFT-G in those with counts less than 250/mm^3 (Figure 4). It is also arguable that in an area where the incidence of tuberculosis is 1,612 per 100,000, a high proportion of positive IFNGRAs was expected and might be expected to be higher still in the HIV-infected group, the group most susceptible to tuberculosis. As there is no "gold standard" for the diagnosis of latent tuberculosis infection, the manufacturers of both forms of IFNGRA have pragmatically based estimation of sensitivity on the detection frequency of HIV-uninfected patients with active tuberculosis in developed countries. Whether this is generalizable to sensitivity to detect latent tuberculosis could be questioned. A correlation between antigen load and the extent of IFN-γ secretion in response to ESAT-6 and CFP-10 has been observed both in experimental systems and in humans (33–35). Further evidence in favor of this relationship comes from observations that demonstrate a falling response during successful chemotherapeutic treatment (3, 33, 36). The bacillary burden in LTBI is many logs lower than in active disease and so it may not be possible to conclude with confidence that a negative IFNGRA excludes LTBI. In this respect addition of further species-specific antigens to test formulations might incrementally improve sensitivity (29, 37). Cutoffs for novel diagnostic tests are often defined by receiver operator characteristic (ROC) analysis. However, in the absence of a gold standard to diagnose latent tuberculosis, the division of our groups into affected and unaffected would have been arbitrary. In addition, both IFNGRAs under investigation have manufacturer-defined cutoffs that therefore defined our analysis. It would be possible to define ROC curves for these tests only by comparing microbiologically confirmed cases with similar people who were definitely not latently infected. Even were the latter possible to ascertain, the appropriateness of such ROC analysis is questionable given the previously cited considerations.

Overall agreement between the TST and IFNGRAs tended to be lower than has been reported in similar studies from lower tuberculosis incidence environments (15). Our results are consistent with similar poor agreement in high-prevalence areas found between generations of the QuantiFERON test and the TST in a nearby area of South Africa (29), and with others made in Korea (38). In the HIV-infected group, discordant pair comparisons of IFNGRA and TST at a cutoff of 5 mm or more suggest that the sensitivities of the tests may be similar. At a higher skin test cutoff, there were significantly more positive IFNGRA results with negative TST results. This may be interpreted as higher sensitivity of IFNGRA for tuberculosis infection. By contrast, in the HIV-uninfected group there were significantly more positive TST results that were not detected by IFNGRA, resulting in poor agreement even at the 15-mm TST cutoff

(Figures 3D and 3E). Our analysis indicated such reactions are unlikely to arise via BCG vaccination. Another possibility is the existence of a strongly sensitizing environmental mycobacterium: the better agreement between the TST and IFNGRA in the HIV-infected group reflecting the inability of this group to maintain such a response. The other alternative, as discussed previously, is that the TST-positive, IFNGRA-negative responses reflect true sensitization by *M. tuberculosis* that is undetected by the IFNGRA. In this respect the greater proportion of positive T-SPOT.*TB* results in both HIV-infected and uninfected persons (Table 2 and Figure 3) accords with an analysis suggesting greater sensitivity of this method when compared with QFT-G (20).

Our data suggest that there may be a role for IFNGRA in the context of high HIV/TB prevalence. However, it is clear from our analysis of agreement that the information on immune status provided by these tests differs substantially from the TST and even between tests. Furthermore, in their current format, both IFNGRAs still require relatively specialized laboratories removed from the point of care and cost is another limiting factor. It is our opinion that sensitivity to detect latent tuberculosis cannot be assigned and therefore meaningfully discussed when the positive predictive value (i.e., of subsequent active tuberculosis) is not known for either method. Despite the cost and ethical difficulties that will need to be overcome, what is needed are large prospective studies to determine whether either of these methods more accurately predicts the risk of subsequent active tuberculosis better than the TST.

Conflict of Interest Statement: M.X.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.A.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.V.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.A.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.G.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.J.W. is cosignatory to a patent pending on diagnostic use of adenylate cyclase as a delivery system for diagnostic antigens.

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