Comparison of a Whole-Blood Interferon γ Assay With Tuberculin Skin Testing for Detecting Latent *Mycobacterium tuberculosis* Infection

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Context Identifying persons with latent tuberculosis infection (LTBI) is crucial to the goal of TB elimination. A whole-blood interferon γ (IFN-γ) assay, the QuantiFERON-TB test, is a promising in vitro diagnostic test for LTBI that has potential advantages over the tuberculin skin test (TST).

Objectives To compare the IFN-γ assay with the TST and to identify factors associated with discordance between the tests.

Design and Setting Prospective comparison study conducted at 5 university-affiliated sites in the United States between March 1, 1998 and June 30, 1999.

Participants A total of 1226 adults (mean age, 39 years) with varying risks of *Mycobacterium tuberculosis* infection or documented or suspected active TB, all of whom underwent both the IFN-γ assay and the TST.

Main Outcome Measure Level of agreement between the IFN-γ assay and the TST.

Results Three hundred ninety participants (31.8%) had a positive TST result and 349 (28.5%) had a positive IFN-γ assay result. Overall agreement between the IFN-γ assay and the TST was 83.1% (κ=0.60). Multivariate analysis revealed that the odds of having a positive TST result but negative IFN-γ assay result were 7 times higher for BCG-vaccinated persons compared with unvaccinated persons. The IFN-γ assay provided evidence that among unvaccinated persons with a positive TST result but negative IFN-γ assay result, 21.2% were responding to mycobacteria other than *M tuberculosis*.

Conclusions For all study participants, as well as for those being screened for LTBI, the IFN-γ assay was comparable with the TST in its ability to detect LTBI, was less affected by BCG vaccination, discriminated responses due to nontuberculous mycobacteria, and avoided variability and subjectivity associated with placing and reading the TST.

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TUBERCULOSIS (TB) IS THE SINGLE leading microbial killer of adults in the world with a death toll of more than 2 million persons per year. The World Health Organization estimates that one third of the world's population is infected with the causative organism, *Mycobacterium tuberculosis* complex.[1] While the majority of *M tuberculosis* infections are kept in check by the host's immune defenses and remain latent, some latent infections progress to active and contagious disease.[2]

The number of persons with latent TB infection (LTBI) in the United States is estimated to range from 10 million to 15 million and a large number of cases of active TB arise from this pool of infected persons.[3] Identifying persons with LTBI is crucial to the goal of TB elimination, because the development of active TB in these persons can effectively be prevented with treatment, thereby stopping further spread of disease.[4] In recognition of this, a recent Institute of Medicine report gave high priority to the development of tools with which to identify persons with LTBI and those at greatest risk of developing active TB.[5]

Until recently, skin testing with purified protein derivative (PPD) of tuberculin was the only practical way of detecting latent *M tuberculosis* infections. In the United States, the tuberculin skin test (TST) is used as an initial screening test for both LTBI and active TB.[6] A positive TST result is indicative of an increased risk of subsequently developing, or currently having, active TB.[7-9] However, despite its widespread use and a large body of data on its standardization, the TST is subject to considerable

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variations and other limitations. False-positive TST responses may result from contact with environmental mycobacteria that share common antigens with *M tuberculosis* or may result from prior BCG vaccinations.[7,10,11] Errors in placement and reading of the TST can also yield false-positive results. A multitude of conditions may blunt the response to tuberculin, most notably human immunodeficiency virus (HIV)–associated immunosuppression, but perhaps the largest cause of erroneous TST results lies with the subjective nature of placement and reading of the test.[6,12] Digit preference (eg, rounding measures of TST induration to the nearest multiple of 5 mm) and interpretation bias can significantly affect TST results.[13]

Discovery of the role of T lymphocytes and interferon γ in the immune process has led to the development of an in vitro assay for cell-mediated immune reactivity to *M tuberculosis*.[14] This whole-blood interferon γ (IFN-γ) assay is marketed in Australia as the QuantiFERON-TB test (Cellestis Limited, St Kilda, Australia) for the detection of LTBI. Like the TST, the IFN-γ assay detects cell-mediated immunity to tuberculin. This IFN-γ assay is based on the quantification of interferon γ that is released from sensitized lymphocytes in whole blood when it is incubated overnight with PPD from *M tuberculosis* and control antigens.

Results of animal and human studies of the IFN-γ assay conducted worldwide have been encouraging, but the test has not been widely evaluated in the United States.[15-25] Therefore, we compared the IFN-γ assay with TST results from persons at 5 sites in the United States with varying degrees of risk for *M tuberculosis* infection and in persons with documented and suspected active TB. Multivariate analysis was used to identify subject-related and test-related factors associated with test discordance.

METHODS

The study was conducted at 5 sites: Boston University School of Medicine, Mass; Johns Hopkins School of Hygiene and Public Health, Baltimore, Md; University of California at San Francisco; New Jersey Medical School, Newark; and University of California at San Diego, using a common protocol. These sites were randomly coded as A-E in the analysis. Ethical approval for the study was obtained from the institutional review boards at the Centers for Disease Control and Prevention (CDC), which supported the study, and the 5 study sites prior to enrolling any subjects. All participants provided written informed consent.

Persons recruited for the study were 18 years or older and included persons requesting a preemployment or preschool enrollment TST; persons being screened with a TST because they were considered to be at high risk for LTBI; persons in whom TB was clinically suspected and who had received fewer than 6 weeks of anti-TB therapy; and persons who previously had active TB, confirmed by a positive culture, and who had completed a course of multidrug anti-TB therapy within the prior 2 years. Subjects were excluded from the study if they self-reported as pregnant or HIV-positive; had a history of severe reaction to tuberculin; were immunocompromised due to leukemia, lymphoma, or Hodgkin disease; or had taken immunosuppressive drugs (eg, corticosteroids, methotrexate, azathioprine) during the preceding 3 months.

After providing written informed consent, enrolled persons completed a detailed questionnaire about possible risk factors for exposure to *M tuberculosis*. Subjects were also asked to indicate results of any prior TST, whether they had received BCG vaccination, details of any contact with a person having TB, any risk factors associated with HIV infection, and whether they had any other medical conditions. When applicable, data were also collected from medical records about findings on chest radiography, results and dates of cultures for mycobacteria, and details of treatment for TB. Data were collected on subjects' age, race, place of birth, residence outside of the United States, and residence or work (paid or unpaid) in a health care setting, prison, homeless shelter, drug rehabilitation unit, or other group housing. Based on responses to the questionnaire and a review of available medical records, persons were categorized into 4 study groups: (1) low-risk for LTBI, subjects receiving preemployment or preschool enrollment TST with no identified risks for LTBI; (2) high-risk for LTBI, asymptomatic subjects with risk of LTBI including contacts of patients with TB; persons from countries where tuberculosis is prevalent (>10 cases per 100 000 population)[26]; intravenous drug users; persons who lived, worked, or volunteered on a regular basis in a homeless shelter, prison, drug rehabilitation unit, hospital, or nursing home; and persons determined to be at increased risk by prior local investigations; (3) TB suspects, subjects being evaluated for active TB who had received fewer than 6 weeks of anti-TB therapy; and (4) culture-confirmed TB, subjects who completed treatment for culture-confirmed TB within the prior 2 years.

Persons enrolled during preemployment or preschool enrollment examinations were assigned to group 2 if risk factors for LTBI were identified during questioning. However, to maintain the integrity of group 1 as truly low-risk for LTBI, persons considered to be at high-risk for LTBI at enrollment were assigned to group 2 even when risk factors were denied.

Tuberculin Skin Testing

The TST was administered by the Mantoux method using 0.1 mL (5 TU) of Tubersol (Connaught Laboratories Inc, Toronto, Ontario) and interpreted by trained health care workers according to American Thoracic Society (ATS)/CDC guidelines.[6] Transverse induration at the TST site was measured 48 to 72 hours after injection of PPD. TST results were interpreted using the risk-stratified interpretation of induration, as recommended by the ATS/CDC guidelines, unless otherwise stated that the cutoff for a positive reaction was 10 mm.[6]

IFN-γ Assay

Blood for the IFN-γ assay was drawn into 10-mL heparinized tubes before a TST was placed. The assay was performed and interpreted according to the manufacturer's instructions using previously described cut-points to identify infected persons.[16,27] Specifically, within 12 hours of collection, 1-mL aliquots of heparinized whole blood were stimulated with 3 drops of the standard antigens provided in the test kit and incubated for 12 to 24 hours at 37°C. The antigens included a saline control (nil), PPD from *M tuberculosis* (human PPD), PPD from *M avium* (avian PPD), and phytohemagglutinin (mitogen). The human PPD and avian PPD included in the test kits are prepared by CSL, Limited (Parkville, Australia) specifically for IFN-γ assay testing. After incubation, plasma (200-300 µL) was collected from above the settled blood cells. Plasma samples were stored at 2°C to 8°C for up to 14 days before the concentration of IFN-γ in 50 µL of each sample was quantified by enzyme-linked immunosorbent assay. The amount of IFN-γ produced in response to the human PPD in excess of the saline control (human – nil) was calculated, as was the amount of IFN-γ produced in excess of the saline control by the avian PPD and mitogen-stimulated blood cultures, (avian – nil) and (mitogen – nil), respectively. A positive test result for *M tuberculosis* infection was defined by the following 2 criteria:

(1) [(human – nil) / (mitogen – nil) ≥ 0.15] and

(2) [{(human – nil) – (avian – nil)} / (human – nil) ≥ –0.10].

An IFN-γ assay result indicating reactivity to *M avium* complex was defined by the following criteria:

(1) (avian – nil) / (mitogen – nil) ≥ 0.20 and

(2) [(human – nil) – (avian – nil)] / (human – nil) < –0.10.

The IFN-γ assay result was considered to be "indeterminate" if (mitogen – nil) was less than 0.5 IU. All other IFN-γ assay result profiles were considered negative. Calculations and interpretations were performed using an Excel spreadsheet (Microsoft Corp, Redmond, Wash).

Data Collection and Statistical Analysis

Information from the standardized questionnaire, the TST record, and the IFN-γ assay record were entered into a dBASE IV spreadsheet (dBASE Inc, Vestal, NY) using a double data entry method for verification. Statistical analysis was conducted using SPSS statistical software (version 7.5.1, SPSS Inc, Chicago, Ill). Concordance between TST and IFN-γ assay results was assessed using κ coefficients, where κ values greater than 0.75 represented excellent agreement beyond chance; κ values less than 0.4 were considered to represent poor agreement beyond chance; and κ values between 0.4 and 0.75 were considered to represent fair to good agreement beyond chance.[28] For these comparisons, IFN-γ assay results indicating reactivity to *M avium* were categorized as assay–negative for *M tuberculosis* reactivity. We did not analyze results using receiver operating characteristic curves because there is no "gold standard" for LTBI. Bivariate and logistic regression analyses were used to identify subject-related and test-related factors associated with test discordance in the group of subjects consisting of those at low- and high-risk for LTBI combined (groups 1 and 2). Variables included age, sex, race, history of BCG vaccination, HIV risk, TB exposure, TST in the prior year, time from phlebotomy until incubation of blood with antigens, duration of incubation, delay to ELISA testing, discordance in TST and IFN-γ assay results, immune reactivity to *M avium* complex by IFN-γ assay, timing of TST reading, and site where enrolled.

Digit preference for recording TST results of 4, 5, 9, 10, 14, and 15 mm was assessed by comparing the number of measurements at each of these values with the average number of measurements around the value. For example, preference for a TST result of 5 mm was assessed by comparing the number of measurements recorded as 5 mm with the average number of measurements recorded as 3, 4, 5, 6, and 7 mm. Digit preference was considered significant if the number of measurements at a particular value exceeded the average number of surrounding measurements by more than 50%.

RESULTS

Between March 1, 1998, and June 30, 1999, a total of 1471 subjects were enrolled and information conforming to the study protocol was available for 1226 adults. For 133 subjects the TST was not placed, read, or recorded as specified. For 97 subjects the IFN-γ assay was not performed or recorded as specified. For 2 subjects, complete data for both the TST and the IFN-γ assay were unavailable. Other critical information, including results of mycobacterial culture, were missing for 11 subjects. Data from 2 subjects with an indeterminate IFN-γ assay result were not included in the analysis. Subjects included in the analysis ranged in age from 18 to 87 years (mean, 39 years). Half of the subjects were female; 72% of subjects were born in the United States; and 38% were white, 35% were black, 13% were Hispanic, 12% were Asian, and 2% were other races.

As shown in TABLE 1, 87 subjects had culture-confirmed TB and completed treatment within the prior 2 years (group 4); 94 were suspects being evaluated for active TB who had received anti-TB therapy for fewer than 6 weeks (group 3). Additionally, 947 subjects were considered to be at high-risk for infection with *M tuberculosis* (group 2), and 98 subjects were considered to be at low-risk for *M tuberculosis* infection (group 1). The TST was interpreted as positive for 390 subjects (31.8%) based on induration and risk strata. Responses less than 15 mm but more than 4 mm were considered positive for 108 subjects because of an increased risk of infection, suspected TB, or culture-confirmed TB (Table 1). Significant digit preference was observed in reporting a TST response of 10 mm at 3 study sites (sites A, B, and

C) and a TST response of 15 mm at 3 sites (sites B, C, and D).

The IFN-γ assay indicated that 349 subjects (28.5%) had immune reactivity to *M tuberculosis* and 101 subjects (8.2%) had immune reactivity to *M avium* complex. The IFN-γ assay revealed no mycobacterial immune reactivity for 776 subjects.

Overall agreement between the IFN-γ assay and the TST, using the risk-stratified interpretation of induration, was 83.1% (κ=0.60). As shown in TABLE 2, interpretation of the TST result using a single cutoff of 10 mm altered the degree of agreement minimally. Agreement between the TST and the IFN-γ assay was 91.8% (κ=0.17) for subjects with no identified risk (group 1); 84.9% (κ=0.55) for subjects at high-risk of infection (group 2); 78.7% (κ=0.41) for the TB suspects (group 3); and 69.0% (κ=0.16) for subjects with prior culture-confirmed TB (group 4).

When the analysis was confined to persons for whom the IFN-γ assay is intended, those being screened for LTBI (eg, subjects in groups 1 and 2) and not those with suspected or confirmed TB, agreement of the IFN-γ assay with the TST was 84.7% (κ=0.55) (TABLE 3). Within this group, agreement was 88.1% (κ=0.50) for subjects with no history of BCG vaccination and 70.1% (κ=0.41) for those who had received the BCG vaccine. An examination of TST and IFN-γ assay results for these persons (Table 3) reveals that BCG vaccination was associated with a disproportionate number (n=35) of positive TST/negative IFN-γ assay results. Additionally, 7 of the 33 nonvaccinated individuals (21.2%) with positive TST/negative IFN-γ assay discordance demonstrated *M avium* complex reactivity by IFN-γ assay.

Multivariable analysis was performed to identify other factors associated with TST and IFN-γ assay discordance. This analysis was confined to the intended population for the IFN-γ assay, those persons being screened for LTBI. Factors statistically associated with a positive TST but negative IFN-γ assay included history of BCG vaccination, Asian race, site of study enrollment, and evidence of *M avium* complex immune reactivity by IFN-γ assay (TABLE 4). The only factor statistically associated with a negative TST but positive IFN-γ assay was the site of enrollment (TABLE 5). Other factors that were examined but were not statistically associated with discordance of either type were age, sex, HIV risk, TB exposure, and TST in the prior year. Within the time periods stipulated by the IFN-γ assay manufacturer, there was no significant association between discordance and the time from phlebotomy until incubation of the blood with the antigens, time of incubation, or delay to ELISA testing. Similarly, there was no association between discordance and timing of TST reading within the stipulated 48 to 72 hours (data not shown).

COMMENT

The goal of this study was to evaluate the IFN-γ assay in detecting LTBI. Evaluation of diagnostic tests for LTBI in humans is hampered by the lack of a "gold standard." As a result, new tests

Table 1. Results of Tuberculin Skin Test (TST)*

	No. of Subjects With Indicated Induration (No. TST Positive)†			
Induration, mm	Group 1	Group 2	Group 3	Group 4
<5	94 (0)	703 (0)	14 (0)	4 (0)
5-9	0 (0)	16 (1)‡	1 (1)	3 (3)
10-14	2 (0)	80 (76)§	17 (17)	10 (10)
≥15	2 (2)	148 (148)	62 (62)	70 (70)
Total	**98 (2)**	**947 (225)**	**94 (80)**	**87 (83)**

*Group 1 indicates no known latent tuberculosis infection (LTBI) risk; group 2, high LTBI risk; group 3, suspected TB; and group 4, prior culture-confirmed TB. See "Methods" section for more details.
†Risk-stratified interpretation of TST induration.
‡One subject was infected with human immunodeficiency virus, hence a 5-mm cutoff was used.
§Four subjects perceived at enrollment to be high-risk for LTBI subsequently denied risk factors and their TST was interpreted as negative because induration was less than 15-mm cutoff.

Table 2. Agreement Between the Whole-Blood Interferon γ (IFN-γ) Assay and Tuberculin Skin Test (TST)*

	Group 1 (n = 98)	Group 2 (n = 947)	Group 3 (n = 94)	Group 4 (n = 87)	Overall Stratified TST Cutoff (n = 1226)	Overall 10-mm TST Cutoff (n = 1226)
Positive TST and positive IFN-γ assay	1	146	63	56	266	265
Negative TST and negative IFN-γ assay	89	649	11	4	753	751
Negative TST and positive IFN-γ assay	7	73	3	0	83	84
Positive TST and negative IFN-γ assay	1	79	17	27	124	126
Agreement, %						
Overall	91.8	84.9	78.7	69.0	83.1	82.9
κ Coefficients (95% CI)	0.17 (0.04-0.30)	0.55 (0.50-0.61)	0.41 (0.25-0.56)	0.16 (0.06-0.26)	0.60 (0.55-0.65)	0.59 (0.55-0.64)
Positive TST	50.0	64.9	78.8	67.5	68.2	67.8
Negative TST	92.7	89.9	78.6	100.0	90.1	89.9

*Group 1 indicates no known latent tuberculosis infection (LTBI) risk; group 2, high LTBI risk; group 3, suspected TB; and group 4, prior culture-confirmed TB. CI indicates confidence interval.

Table 3. Impact of BCG Vaccination on Tuberculin Skin Test (TST) and Whole-Blood Interferon γ (IFN-γ) Assay Agreement in Intended Population*

	Intended Population (n = 1045)	BCG Vaccinated (n = 157)	Unvaccinated (n = 770)	Unknown Vaccination Status (n = 118)
Positive TST and positive IFN-γ assay	147	56	60	31
Negative TST and negative IFN-γ assay	738	54	618	66
Negative TST and positive IFN-γ assay	80	12	59	9
Postive TST and negative IFN-γ assay	80	35	33	12
Agreement, %				
Overall	84.7	70.1	88.1	82.2
κ Coefficient (95% CI)	0.55 (0.50-0.60)	0.41 (0.29-0.54)	0.50 (0.44-0.56)	0.61 (0.46-0.76)
Positive TST	64.8	61.5	64.5	72.1
Negative TST	90.2	81.8	91.3	88.0

*CI indicates confidence interval. The intended population is persons being screened for latent infection.

Table 4. Factors Associated With Positive Tuberculin Skin Test (TST)/Negative Interferon γ (IFN-γ) Assay Discordance Using a Multivariable Model With Group 1 and Group 2 Subjects Combined*

Variable	No. of Subjects	Odds Ratio (95% Confidence Interval)	*P* Value
Race			
White	396	1.0	
Hispanic	103	1.24 (0.52-2.99)	.63
Black	346	1.69 (0.82-3.48)	.15
Asian	91	2.33 (1.05-5.21)	.04
Other	23	0.61 (0.07-5.36)	.66
History of BCG vaccine			
None	707	1.0	
Unknown	108	2.49 (1.07-5.76)	.03
Vaccinated	144	6.92 (3.56-13.43)	<.001
Site where enrolled			
A	201	1.0	
B	137	1.48 (0.44-5.00)	.52
C	221	3.52 (1.15-10.76)	.03
D	182	3.30 (1.10-9.95)	.03
E	218	4.29 (1.50-12.31)	.01
Mycobacterium avium complex by whole-blood IFN-γ assay			
No	866	1.0	
Yes	93	2.64 (1.28-5.42)	.01

*Odds ratios were adjusted for the variables listed and for age, sex, human immunodeficiency virus risk, tuberculosis exposure, and TST in the prior year.

Table 5. Factors Associated With Negative Tuberculin Skin Test (TST)/Positive Whole-Blood Interferon γ (IFN-γ) Assay Discordance Using a Multivariable Model With Group 1 and Group 2 Subjects Combined*

Variable	No. of Subjects	Odds Ratio (95% Confidence Interval)	*P* Value
Site where enrolled			
A	221	1.0	
B	152	1.54 (0.78-3.03)	.22
C	206	0.28 (0.10-0.76)	.01
D	174	0.55 (0.26-1.20)	.13
E	207	0.58 (0.28-1.21)	.15

*Odds ratios were adjusted for the variables listed and for age, sex, human immunodeficiency virus risk, tuberculosis exposure, and TST in the prior year.

are commonly compared with the TST, despite its well-documented limitations. Owing to the lack of a definitive standard, the IFN-γ assay was evaluated on the basis of its agreement with the TST in persons with varying degrees of risk for *M tuberculosis* infection and in persons with documented and suspected active TB. Overall agreement between the TST and IFN-γ assay was good (83.1%, κ=0.60) as was agreement when the analysis was limited to persons for whom the test is intended, those subjects being screened for LTBI, groups 1 and 2 combined (84.7%, κ=0.55). Test concordance was 65% for persons with a positive TST and 90% for those with a negative TST. Pottumarthy et al[27] found a similar level of agreement between the 2 tests in a study involving New Zealand health care workers and immigrants, whereas Streeton et al[16] reported a somewhat better concordance of 98% for persons with no known exposure and a negative TST, and 90% for untreated TST reactors.

The agreement between the IFN-γ assay and TST in this study is similar to the agreement found when multiple TSTs are administered simultaneously using different PPD preparations. Villarino et al[29] found κ coefficients of 0.46 to 0.53 when comparing Tubersol PPD (Pasteur Merieux Connaught USA, Swiftwater, Pa) with Aplisol PPD (Parkdale Pharmaceuticals, Rochester, Mich), Aplisol PPD with PPD-S1 (produced by Seibert and Glenn in 1941 and available from the Food and Drug Administration), or Tubersol with PPD-S1 (M. Elsa Villarino, MD, written communication, February 2001). This is similar to the κ coefficient of 0.60 we observed in the present study. Additionally, the IFN-γ assay and the TST measure different parameters of the immune response, which are not exclusively linked. This is demonstrated in IFN-γ knockout mice, which are able to mount a delayed-type hypersensitivity reaction to PPD despite the absence of IFN-γ production.[30] The discordant results seen for the IFN-γ assay and the TST in our

study could be due to measurement of different immune parameters by the 2 tests, but it may also be influenced by the use of different PPD preparations.

The inherent problem in comparing the IFN-γ assay with the TST is that, by virtue of the trial design, the assay cannot be perceived to perform better than the TST. Because there is no gold standard for LTBI, comparisons cannot demonstrate which test is superior for LTBI. Discrepancies encountered may be the result of limitations in the TST and not limitations in the IFN-γ assay. In recognition of this, logistic regression analysis was used to identify subject-related and test-related factors associated with test discordance. Factors examined included those known to adversely affect TST accuracy, such as BCG vaccination and reactivity to nontuberculous mycobacteria.

A history of BCG vaccination was strongly associated with positive TST/negative IFN-γ assay discordance. BCG vaccination is known to induce reactivity to PPD and can cause false-positive TST reactions.[31] Our results suggest that the IFN-γ assay may be less affected than TST by prior BCG vaccination. However, the IFN-γ assay is also affected by BCG, as was demonstrated by Johnson et al[20] in a study of medical students tested prior to and 5 months after vaccination. The effect may be relatively short-lived and may diminish with time, as shown in animals.[32-34]

Reactivity to nontuberculous mycobacteria is also known to cause false-positive TST results.[35,36] The IFN-γ assay measures the cellular immune response to both human PPD and avian PPD. Avian PPD is included in the assay as an indicator for nontuberculous mycobacterial reactivity, akin to the use of Battey Bacillus sensitin (PPD-B) in a comparative skin test. Results from the IFN-γ assay suggested that reactivity to nontuberculous mycobacteria may be the cause of a positive TST result in one fifth of the non-BCG vaccinated subjects that were positive by TST but negative by IFN-γ assay. Thus, the IFN-γ assay offers a potentially significant improvement in specificity with the consequent benefit of avoiding LTBI treatment for persons not infected with *M tuberculosis*. In the future, the use of antigens in the IFN-γ assay that are found in *M tuberculosis* but not in nontuberculous mycobacteria or BCG (such as ESAT-6) is likely to improve the assay's ability to discriminate among LTBI, BCG vaccination, and nontuberculous mycobacterial reactivity.[20]

Multivariable analysis showed that there was an association between 3 of the study sites and test discordance. Although this could possibly be explained by population differences between the sites, 2 observations suggest that differences in testing methods were involved. The first is documentation of digit preference in TST results at 4 sites, 2 of which were associated with positive TST/negative IFN-γ assay discordance. The rounding up of TST induration measures to 10 or 15 mm would lead to some persons being classified falsely as TST positive, according to the ATS/CDC interpretation criteria.[6] The evidence of digit preference is a good example of the subjectivity often encountered with interpreting the TST result. Persons reading TST reactions in the present study were not blinded to patient histories and may have been unconsciously biased toward a positive or negative result. In contrast, the IFN-γ assay is not subject to operator bias. The second observation was that the sites statistically associated with positive TST/negative IFN-γ assay discordance had less negative TST/positive IFN-γ assay discordance. Again, this may be caused by the subjectivity of reading the TST result. It is unlikely that this variation is due to differences in IFN-γ assay methods because all sites used identical standards and quality control documentation.

In the current study, more persons with no identified risk for LTBI were negative for TST and positive for IFN-γ assay than expected. Although some of these individuals may have false-negative TST results, it is likely that most of these people with no identified risk for LTBI had a false-positive IFN-γ assay. However, evidence that some negative TST/positive IFN-γ assay results reflect greater sensitivity for the IFN-γ assay comes from prior comparisons of the tests. Converse et al[19] provided evidence that the IFN-γ assay is more sensitive than the TST for injection drug users with and without HIV infection. Nine of 24 subjects who were positive by IFN-γ assay but negative by TST had a history of a positive TST in the past. Kimura et al[17] also reported finding more intravenous drug users having immune reactivity to PPD with the IFN-γ assay than with the TST. Additional evidence that the IFN-γ assay may be more sensitive for the detection of LTBI comes from studies of the bovine version of the assay; the cattle can be killed and cultures can be used as the gold standard. Wood et al[15] found that 37 of 67 cattle (55.2%) that were positive in the bovine IFN-γ assay but TST negative were culture-positive for *M tuberculosis* complex, specifically *M bovis*. In contrast, only 2 of the 53 (3.8%) animals with a positive TST and negative IFN-γ assay result were culture positive. These findings are supported by data from New Zealand, where the bovine IFN-γ assay is routinely used to detect infected cattle that are undetected by the TST.[37] Published data demonstrate that approximately 50% of negative TST/positive IFN-γ assay animals are truly infected with *M bovis*, as confirmed by culture of the organism.[38,39]

Agreement between the TST and the IFN-γ assay was less for subjects with culture-confirmed TB (both current and previously treated) as compared with those subjects without positive cultures. The observation that active TB is associated with a decrease in PPD-specific IFN-γ production may explain these differences.[40-42] Although both TST and IFN-γ assay responses can be reduced in cases of active disease, TST responses are usually restored within 2 weeks after initiation of antimycobacterial chemotherapy and nutritional supplementation.[43] Follow-

ing treatment, PPD-specific IFN-γ responses usually increase but can remain low or absent for at least a year following successful completion of therapy.[44,45] These findings suggest that the results found in the present study could be skewed in favor of the TST since all but a few individuals with culture-confirmed TB had received therapy for at least 4 weeks. In persons who have already been diagnosed with TB and begun chemotherapy, the use of either test is unlikely. To accurately compare the sensitivity of the TST with the IFN-γ assay for the diagnosis of active TB, subjects should be tested prior to the initiation of anti-TB therapy.

In this study, the IFN-γ assay was comparable with the TST in its ability to detect latent *M tuberculosis* infection. The assay has several logistic advantages over the TST. Unlike the TST, the IFN-γ assay requires a single patient visit, an important benefit because the proportion of persons returning to have their TST read is very low in some settings.[46] The IFN-γ assay assesses responses to multiple antigens simultaneously and includes avian PPD to discriminate responses due to reactivity to nontuberculous mycobacteria from those due to LTBI. The assay does not boost amnestic immune responses, eliminates the subjectivity of the TST, and can be completed in less than 24 hours. The finding that BCG vaccination has less effect on the IFN-γ assay than on the TST is promising, given that a large proportion of TB cases in the United States are in persons originating from countries where BCG vaccination is commonplace. Better performance may be seen when TB-specific antigens are included in the IFN-γ assay.

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In all my work what I try to say is that as human beings we are more alike than we are unalike.
—Maya Angelou (1928-)