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Meta-analysis: New Tests for the Diagnosis of Latent Tuberculosis Infection: Areas of Uncertainty and Recommendations for Research

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Background: Until recently, the tuberculin skin test was the only test for detecting latent tuberculosis (TB) infection, but 2 ex vivo interferon-γ release assays (IGRAs) are now commercially licensed.

Purpose: To estimate sensitivity, specificity, and reproducibility of IGRAs (commercial or research versions of QuantiFERON [QFT] and Elispot) for diagnosing latent TB infection in healthy and immune-suppressed persons.

Data Sources: The authors searched MEDLINE and reviewed citations of all original articles and reviews for studies published in English.

Study Selection: Studies had evaluated IGRAs using *Mycobacterium tuberculosis*–specific antigens (RD1 antigens) and overnight (16- to 24-h) incubation times. Reference standards had to be clearly defined without knowledge of test results.

Data Extraction and Quality Assessment: Specific criteria for quality assessment were developed for sensitivity, specificity, and reproducibility.

Data Synthesis: When newly diagnosed active TB was used as a surrogate for latent TB infection, sensitivity of all tests was suboptimal, although it was higher with Elispot. No test distinguishes active TB from latent TB. Sensitivity of the tuberculin skin test and IGRAs was similar in persons who were categorized into clinical gradients of exposure. Pooled specificity was 97.7% (95% CI, 96% to 99%) and 92.5% (CI, 86% to 99%) for QFT and for Elispot, respectively. Both assays were more specific than the tuberculin skin test in samples vaccinated with bacille Calmette–Guérin. Elispot was more sensitive than the tuberculin skin test in 3 studies of immune-compromised samples. Discordant tuberculin skin test and IGRA reactions were frequent and largely unexplained, although some may be related to varied definitions of positive test results. Reversion of IGRA results from positive to negative was common in 2 studies in which it was assessed.

Limitations: Most studies used cross-sectional designs with the inherent limitation of no gold standard for latent TB infection, and most involved small samples with a widely varying likelihood of true-positive and false-positive test results. There is insufficient evidence on IGRA performance in children, immune-compromised persons, and the elderly.

Conclusions: New IGRAs show considerable promise and have excellent specificity. Additional studies are needed to better define their performance in high-risk populations and in serial testing. Longitudinal studies are needed to define the predictive value of IGRAs.

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The tuberculin skin test is 1 of the few tests that has been in use for nearly 100 years in clinical medicine (1). Therefore, it is not surprising that the test has important limitations. The tuberculin skin test uses a relatively crude mix of antigens from *Mycobacterium tuberculosis*. As a result, false-positive reactions can occur because of previous bacille Calmette–Guérin (BCG) vaccination or sensitization to nontuberculous mycobacteria. False-negative results on tuberculin skin tests can occur because of severe illness, including active tuberculosis (TB), or immune suppression, often due to HIV infection. Initial testing can affect results of subsequent tests because of anamnestic recall of immunity (the booster effect). Errors in administration or reading can lead to incorrect results. The variability of the tuberculin skin test can be minimal with well-trained personnel using meticulous techniques (2–8), although such personnel are not always available. Interpretation of test results requires a thorough understanding of the test.

In the past decade, 2 new T-cell–based tests for diagnosing latent TB infection have been developed and licensed for commercial distribution in many countries (**Appendix Table 1**, available at www.annals.org). One test, QuantiFERON (QFT)-TB Gold (Cellestis, Victoria, Australia), uses enzyme-linked immunosorbent assay to measure antigen-specific production of interferon-γ (IFN-γ) by circulating T cells in whole blood. The other test, the T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom), uses the Elispot technique to measure peripheral blood mononuclear cells that produce IFN-γ. Current versions of both IFN-γ release assays (IGRAs) use more specific *M. tuberculosis* antigens—ESAT-6, CFP-10, and TB7.7. The genes encoding these antigens are found in the region of difference 1 (RD1) of the *M. tuberculosis* genome, which is deleted from the genome of *M. bovis* BCG, and certain nontuberculous mycobacteria, such as *M. avium*. The U.S. Centers for Disease Control and Prevention (9) has recommended that QFT replace the tuberculin skin

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test, whereas the U.K. National Institute for Clinical Excellence has suggested that IGRAs are useful adjuncts to the tuberculin skin test (10).

Interferon γ–release assays have several important advantages over the tuberculin skin test: Testing requires only 1 patient visit and these assays are ex vivo tests, which reduce the risk for adverse effects and eliminate potential boosting when testing is repeated. However, IGRAs have important disadvantages, including higher material cost, need for an equipped laboratory, and a requirement to draw blood with subsequent careful handling to maintain viability of lymphocytes. Although boosting will not occur, the variability of these tests when repeated after months or years, such as in serial testing of exposed populations, has not been well studied. The greatest disadvantage is the lack of prospective data regarding the future risk for active TB in persons with positive results on IGRAs. This has been established for different-sized tuberculin skin test reactions in many large-scale cohort and experimental studies (11–20), which permits the estimation of risk for disease and benefit of therapy.

In this review, we compared sensitivity, specificity, and reproducibility of commercially available IGRAs (or in-house equivalent tests) with the tuberculin skin test for diagnosing latent TB infection in healthy and immunesuppressed adults and children. Because there is no gold standard for testing latent TB infection, we estimated sensitivity from studies of 1) patients with active TB (who must have infection), 2) persons in contact with patients with active TB who were categorized into gradients of exposure, and 3) concordance of IGRA and the tuberculin skin test. We estimated specificity from studies of healthy persons with a very low likelihood of exposure. Reproducibility was estimated from studies of repeated IGRAs, with or without treatment for active or latent TB.

Context

Do new ex vivo interferon-γ release assays (IGRAs) detect latent tuberculosis (TB) more accurately than tuberculin skin tests (TSTs)?

Contribution

This meta-analysis of 59 studies found that no test distinguished active TB from latent TB, no test had high sensitivity, IGRAs were more specific than the TST in populations vaccinated with bacille Calmette–Guérin (BCG), and the results of IGRAs and the TST were frequently discordant.

Cautions

Studies had many limitations, including no gold standard for diagnosing latent TB.

Implications

New IGRAs have good specificity and show promise for detecting latent TB, particularly in BCG-vaccinated patients.

—The Editors

METHODS

Search Strategy

We conducted a MEDLINE search for articles published between 1966 and October 2006 on 4 December 2005 and updated the search on 2 July 2006 and 31 October 2006. Search terms included TB infection, or TB disease, AND Quantiferon, or Elispot, or interferon-gamma assays, or interferon-gamma release assays, or T cell assays, AND ESAT-6, or CFP10, or RD1 antigens. The searches were limited to human studies published in English.

We identified additional studies from the reference lists of articles identified in this manner and performed a hand search of all issues of the *International Journal of Tuberculosis and Lung Disease* that were published over the past 5 years. We contacted the authors of some studies for clarification.

Study Selection

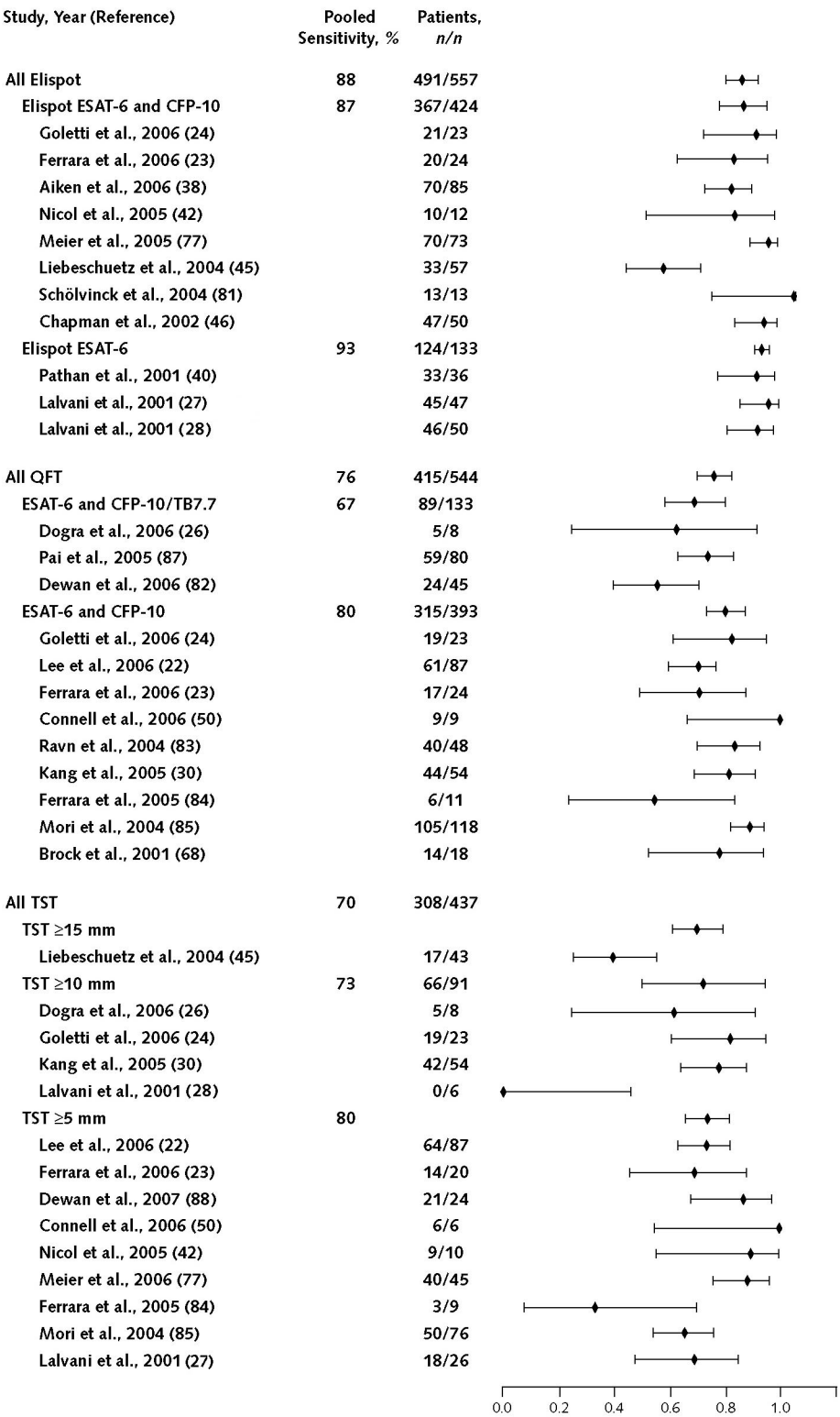
Studies were included if they used QFT or Elispot tests with overnight (16 to 24 h) in vitro incubation of peripheral blood lymphocytes stimulated with RD1 antigens. This meant research, in-house, or commercial versions of QFT or Elispot tests that used ESAT-6 with or without CFP-10 and with or without TB7.7. Commercial versions that were included were the QuantiFERON-TB Gold (QFT-G), QuantiFERON-TB Gold In-Tube (QFT-IT), and the T-SPOT.TB.

For studies assessing sensitivity, the study sample had to have active TB or contact with a person with active TB and a clearly defined gradient of exposure of at least 2 categories (such as high or low exposure). For studies assessing specificity, the population had to be healthy, lifelong residents of low-incidence countries with an average age younger than 40 years without occupational, travel, or other exposure to TB. In studies assessing concordance of 2 or more tests, all tests had to be done simultaneously in all persons. We excluded studies that performed sequential testing, in which the second test was done only in persons selected on the basis of the results of the first test. Studies assessing reproducibility had to perform at least 2 tests in the same persons. Two independent reviewers assessed titles and abstracts to select studies for the review. A list of excluded studies is available from the authors.

Extraction of Data and Assessment of Study Quality

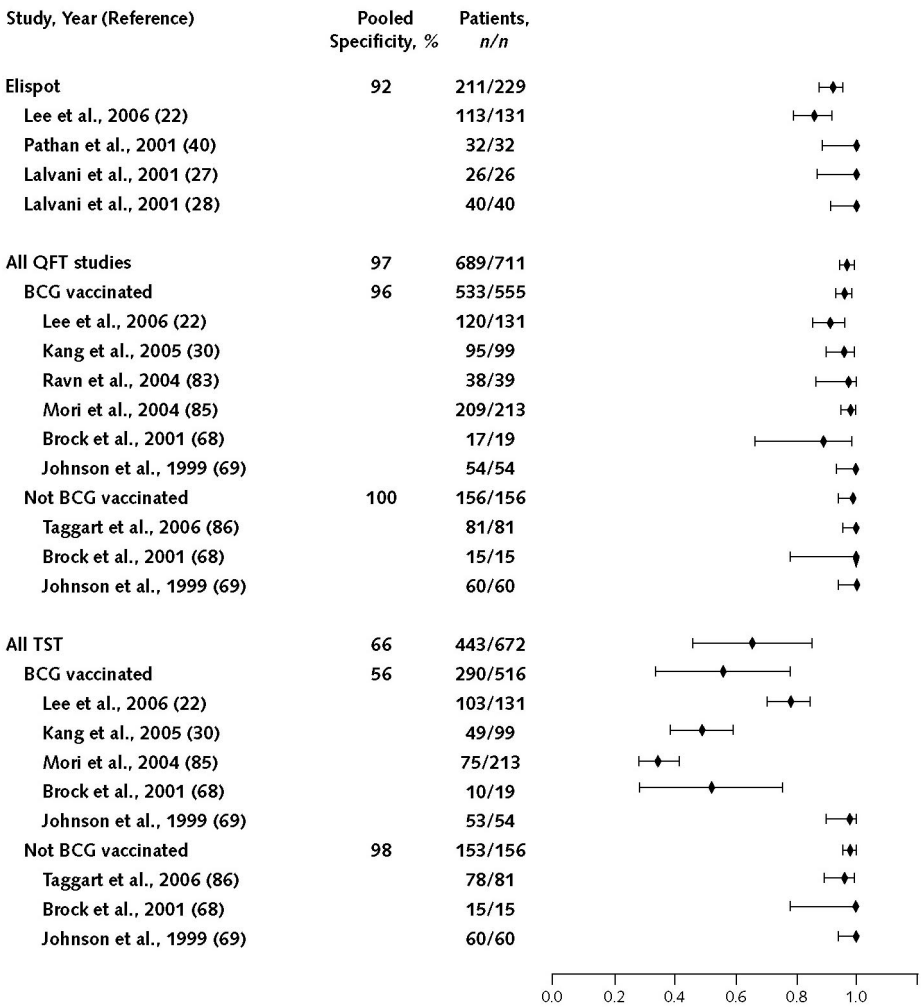
One reviewer reviewed all studies and abstracted data regarding test characteristics and study quality (**Appendix Tables 2** through **6**, available at www.annals.org). A second reviewer assessed a random sample of 20% of the full-text articles to determine concordance in assessment of data quality and accuracy of abstracted data. We assessed 2 quality criteria in all studies: The technicians performing

Figure 1. **Forest plot of studies estimating sensitivity of the 3 tests in patients with active tuberculosis as a surrogate for latent tuberculosis infection.**



Point estimates for sensitivity and 95% CIs are shown. QFT = QuantiFERON; TST = tuberculin skin test.

Figure 2. **Forest plot of studies estimating specificity of the 3 tests in populations at very low risk for latent tuberculosis infection.**



Point estimates for specificity and 95% CIs are shown. BCG = bacille Calmette–Guérin; QFT = QuantiFERON; TST = tuberculin skin test.

the tuberculin skin test and IGRAs were blinded to the patients' clinical status, other test results and timing of IGRA relative to the tuberculin skin test, and treatment (if applicable). In all studies estimating sensitivity, the clinical gold standard had to be determined without knowing or using results of the tests that were being evaluated. In addition, high-quality studies of active TB (as a surrogate for latent TB infection) confirmed the diagnosis microbiologically or histologically. High-quality studies of reproducibility had high rates of participation of eligible persons, and high rates of completion of testing protocols and assessed treatment adherence or outcomes in studies of treated persons.

Data Synthesis and Analysis

Methods of studies estimating sensitivity among patients with active TB as a surrogate for latent TB infection and estimating specificity in low-risk populations were similar enough to allow pooled estimates. In particular, the same diagnostic thresholds were used for in-house assays and commercial assays. Different cut-points were used for the tuberculin skin test; these were analyzed separately.

For each study, we calculated sensitivity or specificity (and 95% CIs) and summarized the results in forest plots. A fixed-effects meta-analysis was done by using Meta-DiSc, version 1.2 (Ramon y Cajal Hospital, Madrid, Spain) (21): Studies were weighted by total sample size to pool (summarize) estimates of sensitivity and specificity across the studies. Presence of statistically significant heterogeneity across studies was evaluated by using the chi-square test for heterogeneity. To account for heterogeneity due to between-study variability, we corrected for overdispersion.

We did not pool estimates of test sensitivity on the basis of gradients of exposure, concordance among high-risk persons, and effects of immune suppression or treat-

Table 1. **Summary of Sensitivity from Pooled Estimates from All Studies***

Variable	Studies, *n*	Sensitivity (95% CI)†	Chi-Square Test for Heterogeneity
Tuberculin skin testing			
All studies	14	0.71 (0.65–0.74)	61.4 (0.001)
Size of reaction, *mm*			
5	9	0.74 (0.66–0.82)	23.5 (0.001)
10	4	0.72 (0.50–0.95)	18.0 (0.01)
15	1	0.40 (0.25–0.56)	–
Sample			
Pediatric	4	0.55 (0.43–0.67)	17.4 (0.01)
Adult	10	0.73 (0.68–0.78)	35.7 (0.001)
QuantiFERON			
All studies	13	0.76 (0.7–0.83)	38 (0.001)
Antigens			
ESAT-6 only	1	0.58 (0.34–0.80)	–
ESAT-6/CFP-10	9	0.80 (0.73–0.87)	20.9 (0.001)
ESAT-6/CFP-10 and TB7.7	3	0.67 (0.56–0.78)	6.8 (0.05)
Sample			
Pediatric	4	0.66 (0.5–0.83)	11.0 (0.01)
Adult	10	0.76 (0.7–0.83)	32.5 (0.001)
Elispot or T-SPOT.TB			
All studies	12	0.88 (0.81–0.95)	57.3 (0.001)
Antigens			
ESAT-6	3	0.93 (0.91–0.96)	0.8 (NS)
ESAT-6/CFP-10	9	0.87 (0.78–0.95)	51.7 (0.001)
Sample			
Pediatric	2	0.62 (0.43–0.81)	3.0 (0.08)
Adult	10	0.92 (0.88–0.95)	17.1 (0.001)

* Patients with active tuberculosis were used as surrogates for latent tuberculosis. NS = not significant.
† All 95% CIs are corrected for overdispersion.

ment, because of the heterogeneity of ascertainment and classification of exposure and study samples.

Role of the Funding Source

No funding sources directly supported the study. Dr. Menzies receives salary support from the Fonds de la Recherche en Santé du Québec, which had no role in the conduct of or in the decision to publish the study.

RESULTS

Studies Identified

A total of 275 studies were identified from PubMed, of which 83 were selected for full-text review (**Appendix Figure**, available at www.annals.org). From these original articles and reviews, 21 additional articles were identified and 6 more were identified at the time of the last update, for a total of 110 studies for full review. Of these studies, 52 were excluded because they did not meet the review criteria, leaving 58 studies included in this review. All studies used cross-sectional designs, except for 8 that performed serial testing in cohorts. Studies ranged from fewer than 10 participants to more than 500 participants. The expected prevalence of latent TB infection and the prevalence and policy of BCG vaccination also varied widely.

Twenty-two studies evaluated sensitivity of IGRA among newly diagnosed patients with active TB as a surrogate for latent TB infection. Of these studies, 3 tested both IGRAs and the tuberculin skin test, 12 tested 1 IGRA and the tuberculin skin test, and 7 tested 1 IGRA only. Specificity of IGRA in populations at very low risk for latent TB infection was assessed in 11 studies, of which 6 performed the tuberculin skin test. Ten studies compared a single IGRA with the tuberculin skin test in contacts with a gradient of exposure, and 14 studies examined concordance of IGRA with the tuberculin skin test. Serial testing was performed in 7 treated cohorts and in 1 additional untreated cohort. Five studies examined the impact of immune suppression on IGRA performance; 3 of these compared results with those of the tuberculin skin test.

Sensitivity Using Tuberculosis as a Surrogate for Latent Infection

Figure 1 shows that all 3 tests, particularly the tuberculin skin test, have suboptimal sensitivity among patients with newly diagnosed, active TB. Pooled estimates of sensitivity were lowest for the tuberculin skin test, higher for QFT, and highest for Elispot. However, only 3 studies, with a total of 143 participants, conducted direct head-to-head comparisons (22–24). There were some differences in sensitivity between studies using different versions of QFT. QuantiFERON with ESAT-6 alone was used only once in an older study with earlier assay and antigens. This study reported substantially lower sensitivity but higher specificity (**Figure 2**). Sensitivity of QFT-G was somewhat higher than with QFT-IT (**Tables 1** and **2**). Sensitivities of Elispot assays that incorporated ESAT-6 alone were similar to those of assays with ESAT-6 plus CFP-10. The IGRA test procedures were well standardized, but tuberculin skin test procedures were not. As shown in **Table 1**, different cut-points were used, and this had some effect on sensitivity. In 4 studies, which used a dose of tuberculin that was 50% or less than the recommended amount (26–28, 85), sensitivity of the tuberculin skin test averaged 0.63 (range, 0.46 to 0.80) compared with a sensitivity of 0.73 (range, 0.62 to 0.84) in studies that used a tuberculin dose recommended by the World Health Organization (2).

Sensitivity Using a Gradient of Exposure as an Indicator of the Likelihood of Latent Tuberculosis Infection

Table 3 summarizes studies that compared IGRA and the tuberculin skin test with a clinically defined gradient of exposure. It is difficult to ensure equivalence of the exposure categories because each study characterized exposure differently. Nevertheless, overall findings were similar. The prevalence of positive results on IGRA and the tuberculin skin test was highest in the most-exposed groups. In the less-exposed groups, the prevalence of positive results on the tuberculin skin test was higher than that of IGRA in studies that involved populations that had received BCG vaccination at an older age (30, 31). In 1 study, the expected prevalence of latent TB infection was 30% in the

least-exposed group, yet only 4% had a positive result on IGRA (30).

Specificity of Tests

In all studies of healthy populations considered at very low risk for latent TB infection, IGRA with RD1 antigens have had high specificity (**Figure 2**). Pooled average specificity was 97.7% and 92.2% in QFT and Elispot, respectively (**Tables 1** and **2**), and was unaffected by BCG vaccination. A limitation of these studies is that persons at low risk could still have had latent TB infection, but this could not be verified because of the absence of a true gold standard for testing for latent TB infection.

Discordance between Interferon-γ Release Assays and Tuberculin Skin Test: Reflection of Uncertainty on Sensitivity and Specificity of These Tests

Tables 4 and 5 summarize studies of dual tuberculin skin testing and IGRA testing of populations considered at risk for TB infection. In 3 studies, discordance was greater in persons with BCG vaccination than in those who were not vaccinated (30, 32, 33). In 2 studies in which very few persons had been BCG vaccinated, almost half of all persons with positive results on tuberculin skin test had negative results on IGRA (34, 35). Discordant positive IGRA reactions and negative tuberculin skin test reactions occurred in 6% to 7% of all persons and accounted for 23% of all positive results on QFT and 26% of all positive results on Elispot. In 3 studies summarized in **Table 6**, agreement between QFT-G and Elispot was good: κ values were 0.57 to 0.70. Of note, Elispot results were more often positive than those of QFT, which is consistent with higher sensitivity or lower specificity as noted earlier. In a fourth study (80), QFT-G was compared with QFT-IT. Concordance was moderate ($\kappa = 0.5$): QFT-IT had a greater prevalence of positive reactions. The differences in these 2 versions of the same test may reflect poor reproducibility under field conditions, higher sensitivity of QFT-IT because of the addition of the third antigen (TB7.7), or that QFT-IT was more sensitive because of its greater technical simplicity.

Serial Testing

Very few published studies have assessed reproducibility of IGRAs. In studies conducted by the manufacturer, the test-related coefficient of variation for the QFT was 8.7% by using replicate serum samples from well-characterized patients (37). Among healthy volunteers from Gambia in whom Elispot was repeated 1 week apart, 12 initially had negative results and none had conversion to positive results, but of the 11 who initially had positive results, 1 (9%) reverted to having negative results (38). In a study of health care workers in India who were tested 18 months apart, 11.6% had QFT conversion, but 24% of those with initial positive results on QFT reverted to having negative results (39). QuantiFERON reversion was associated with negative results on the initial tuberculin skin test or an initial QFT result close to the manufacturer's suggested cut-point for a positive result.

As seen in **Table 7**, in 4 studies of patients treated for active TB, serial Elispot assays decreased in 3 studies (38, 40, 41), increased after 1 month in a fourth study, then decreased with continued therapy (42). In patients who were treated for latent TB infection, IGRA results did not change in 2 studies (26, 43) but reverted to negative results

Table 2. **Summary of Specificity from Pooled Estimates from Studies of Persons at Very Low Risk for Tuberculosis Infection***

Grouping	Studies, *n*	Specificity (95% CI)	Chi-Square Test for Heterogeneity	*P* Value
Tuberculin skin testing				
All studies	8	0.66 (0.46–0.86)	251	0.001
BCG vaccination				
Not vaccinated	3	0.98 (0.96–1.0)	4.0	NS
Vaccinated	5	0.56 (0.34–0.78)	122	0.001
Criteria				
Positive ≥10 mm	6†	0.58 (0.37–0.79)	155	0.001
Positive ≥15 mm	3†	0.87 (0.7–1.0)	31.4	0.001
QuantiFERON				
All studies	9‡	0.97 (0.95–0.99)	25.4	0.01
ESAT-6	2	1.0 (0.94–1.0)	0	
ESAT-6 and CFP-10	7	0.96 (0.94–0.99)	17.6	0.01
BCG vaccination				
Not vaccinated	3	1.0 (0.94–1.0)	0	
Vaccinated	6	0.96 (0.93–0.99)	14.3	0.02
Elispot or T-SPOT.TB				
All studies	4	0.92 (0.88–0.95)	21.3	0.01

* BCG = bacille Calmette–Guérin; NS = not significant.
† In 1 study (30), data for 2 tuberculin skin test cut-points are given.
‡ In each of 2 studies (68, 69), 2 different very-low-risk populations were tested. These were counted as separate studies.

Table 3. **Studies Comparing Interferon-γ Release Assay (IGRA) with Tuberculin Skin Test (TST) against the Clinical Gold Standard of Exposure Gradients***

Study, Year (Reference)	Country	Patients with a History of BCG vaccination, *n* (%)	Antigens for IGRA-Positive and TST-Positive Type	Results by Exposure Gradient†		
				High Exposure		
				Participants in Exposure Category, *n*	IGRA-Positive, %	TST-Positive, %
Elispot						
Lalvani et al., 2001 (31)	United Kingdom	59 (most)	ESAT-6 HEAF (PPD)	20	73	65
Ewer et al., 2003 (41)	United Kingdom	535 (most)	ESAT and CFP-10 HEAF (PPD)	20	100	90
Richeldi et al., 2004 (70)	Italy	92 (10)	ESAT-6 and CFP-10 TST: 5TU-PPD	4	25	0
Hill et al., 2004 (71)	Gambia	735 (45)	ESAT-6 and CFP-10 TST: 2TU-RT23	149	38	62
Zellweger et al., 2005 (72)	Switzerland	91 (86)	ESAT-6 and CFP-10 TST: 2TU-RT23	54	22	50
Shams et al., 2005 (73)	United States	413 (49}	ESAT-6 and CFP-10 TST: 5TU-PPDS	104	32	28
Hill et al., 2006 (36)	Gambia	718 (46)	ESAT-6 and CFP-10 TST: 2TU-RT23	163	46	53
QuantiFERON						
Brock et al., 2004 (34)	Denmark	85 (0)	ESAT-6 and CFP-10 TST: 2TU-RT23	45	46	53
Kang et al., 2005 (30)	Korea	273 (81)	ESAT-6 and CFP-10 TST: 2TU-RT23	48	44	71
Nakaoka et al., 2006 (51)	Nigeria	207 (90)	ESAT-6 and CFP-10 TST: 5TU-PPDS	72	74	53

* BCG = bacille Calmette–Guérin; PPD = purified protein derivative; PPDS = purified protein derivative standard.
† The percentage is the proportion in that exposure category with a positive response to IGRA or TST.
‡ This group represented a random sample from the general population. On the basis of historical data, the authors estimated that the prevalence of latent tuberculosis infection should have been 30%. On the basis of the average age of 25 years, the incidence of smear-positive tuberculosis (40/100 000) (74), and the Styblo equation (75), the estimated prevalence of latent tuberculosis infection should have been 18.5%.

in 16% of treated persons in the third study (44). Of importance, in this last study, 7 of 25 (28%) persons who were not treated had reversion—a phenomenon seen in those who initially had positive results on Elispot and negative results on tuberculin skin test (44).

Impact of Immune Suppression

To date, few studies have assessed IGRA in these high-risk populations. In 2 studies (45, 46), responses of IGRA were slightly reduced in immune-compromised patients compared with those who were not immune-compromised. In 3 studies with direct comparison, the prevalence of positive results on Elispot was substantially higher than that of positive results on the tuberculin skin test (46–48), particularly in persons with greater immune suppression (47). The only study to evaluate QFT noted a low prevalence of positive results in asymptomatic HIV-infected patients and that indeterminate results were more common if the patient's CD4 count was less than 100 cells/mm^3 (49).

Results in Pediatric Populations

Studies of IGRA response in pediatric samples are more heterogeneous because the populations selected were suspected of having active TB (23, 26, 50), had contact with infected persons (23, 36, 51), or were school children (52). Some were healthy (36, 52, 53), whereas others were hospitalized (26, 50); some studies were conducted in low-income countries (26, 36, 51, 52), and others were conducted in middle-income (53) or high-income (23, 50) countries. Confirming or excluding active TB is also more difficult in children. Hence, information is currently insufficient to estimate sensitivity, specificity, and reproducibility of IGRAs in children, although the occurrence of indeterminate QFT (23, 50) and failed phlebotomy (51, 52) may be important potential limitations of IGRAs in this population.

DISCUSSION

Principal Findings

Commercially available IGRAs have evolved rapidly over the past decade. The latest versions use more specific *M. tuberculosis* antigens and are simpler to perform. These tests are promising and have been successfully used by independent investigators in many settings, including low-income countries. In our review, the sensitivity of the tuberculin skin test compared with QFT, using active TB as a surrogate for latent TB infection, was low but similar for both tests, whereas the sensitivity of Elispot using this sur-

Table 3—Continued

Results by Exposure Gradient†								
Moderate Exposure			Low Exposure			Very Low Exposure		
Participants in Exposure Category, *n*	IGRA-Positive, %	TST-Positive, %	Participants in Exposure Category, *n*	IGRA-Positive, %	TST-Positive, %	Participants in Exposure Category, *n*	IGRA-Positive, %	TST-Positive, %
8	38	43	12	0	31	7	0	33
81	53	51	47	38	40	387	17	20
9	67	11	16	6	6	63	14	3
340	30	41	246	24	28	–	–	–
–	–	–	37	5	35	–	–	–
103	28	26	103	21	25	103	19	20
372	33	32	182	20	15	–	–	–
–	–	–	40	5	10	–	–	–
72	10	60	99	4‡	51‡	–	–	–
81	10	16	39	10	15	–	–	–

rogate was somewhat superior. When a gradient of exposure among contacts was used as the gold standard, sensitivity of IGRA and the tuberculin skin test were similar. None of these tests can distinguish between latent and active TB.

Specificity of IGRA is excellent because neither IGRA is affected by BCG vaccination, although the effect of nontuberculous mycobacteria on IGRA response is poorly studied. However, BCG vaccination has a greater effect on the specificity of the tuberculin skin test, particularly if BCG was given after infancy. There is substantial discordance of IGRA with the tuberculin skin test in populations with varying likelihood of latent TB infection. Although some discordance may be explained by superior specificity of IGRA, it would be overly simplistic to assume that the results of IGRA tests were always correct and that those of tuberculin skin test were always incorrect. Some discordance may be explained by 1 test being close to the criteria for positive test results, but this phenomenon has not been analyzed in most studies and thus is not well-understood.

In 3 studies of immune-compromised patients, sensitivity of Elispot was superior to that of the tuberculin skin test. Studies in pediatric populations were too heterogeneous to draw firm conclusions. Studies of the effect of treatment on IGRA response are contradictory. However, the true effect cannot be ascertained until the biological and random variability of IGRA response has been better characterized. In 2 studies, a substantial number of persons with positive results on IGRA reverted to having negative results without treatment. This was associated with initial negative results on the tuberculosis skin test (that is, discordant results) (39, 44) and IGRA response close to the cut-point for positive test results (39).

Limitations

The major limitation of estimation of sensitivity or specificity was the use of a cross-sectional design in all studies reviewed. With this design, there is no gold standard for latent TB infection. The only certain measure that latent TB infection exists is when the risk for active TB associated with a particular test result has been defined. This requires large-scale cohort studies with long-term follow-up of untreated populations with positive results at baseline. In addition to being expensive and complex, such studies are ethically impossible in most high-income countries, where the standard of care is to offer treatment to such persons.

With cross-sectional designs, surrogates for true infec-

tion must be used. The most commonly used proxy is active TB, because persons with disease must be infected. However, with active TB, the cell-mediated immune response is often diminished, particularly at the time of diagnosis, in patients with more advanced disease (54–56), malnutrition (57), or older age (55, 58). Intuitively, one would expect reduced performance of tests, such as the tuberculin skin test or IGRA, because the cell-mediated immunity they measure must have failed, to some extent, in any person with active disease. Of interest, the greater sensitivity of Elispot in active TB is paralleled by findings in immune-suppressed patients. We speculate that this may reflect the technical requirement for standardization of the number of peripheral blood mononuclear cells in each assay well.

An additional important limitation is the heterogeneity of the studies reviewed, which were conducted with different timing of testing and in different settings with samples that had markedly different risks for exposure and infection. Because of these methodological differences, pooling of estimates could be made only for results of sensitivity among patients with active TB (as a surrogate) and of specificity in low-risk populations. The estimation of the sensitivity of these tests among contacts who were categorized into gradients of exposure was limited by the differences in degree and categorization of exposure. Thus, differences in results may have reflected differences in exposure or exposure definition rather than differences in test performance. There were too few studies that evaluated IGRAs in immune-compromised persons and in pediatric patients to make firm conclusions.

To date, most studies of the new IGRA have included the investigators who developed these assays. This is inevitable because of their recent introduction, but could result in 2 problems. First, highly complex tests could achieve initially excellent results in research laboratories, but test performance may be reduced in the hands of independent investigators who lack the specialized expertise to overcome

Table 4. **Concordance of QuantiFERON and Tuberculin Skin Test (TST) in Healthy Populations with Varying Risk for Latent Tuberculosis Infection***

Study, Year (Reference)	Country	Risk Group	Total Participants, *n*	BCG, %	Age at BCG Vaccination	Concordant Results		Discordant Results	
						TST-Positive and IGRA-Positive, *n* (%)	TST-Negative and IGRA-Negative, *n* (%)	TST-Negative and IGRA-Positive, *n* (%)	TST-Positive and IGRA-Negative, *n* (%)
Brock et al., 2004 (34)	Denmark	Contacts of persons with tuberculosis	45	0		23 (51)	19 (42)	1 (2)	2 (4)
Pai et al., 2005 (76)	India	Health care workers	719	71	Infancy	223 (31)	360 (50)	65 (9)	72 (10)
Kang et al., 2005 (30)	Korea	Close and casual contacts of persons with tuberculosis	120	73	Older	24 (20)	40 (33)	4 (3)	53 (44)
Porsa et al., 2006 (35)	U.S.	Prisoners	409		All ages†	8 (2)	360 (88)	12 (3)	29 (7)
Harada et al., 2006 (32)	Japan	Health care workers	304	91	Older‡	24 (8)	18 (6)	1 (1)	261 (86)
Ferrara et al., 2006 (23)	Italy	Hospitalized adults	286	18	All ages†	67 (23)	143 (50)	21 (7)	55 (19)
Dogra et al., 2006 (26)	India	Hospitalized children	97§	82	Infancy	3 (3)	89 (92)	3 (3)	2 (2)
Mahomed, 2006 (80)	South Africa	Healthy adults	358‖	81	Infancy	189 (53)	57 (16)	12 (3)	100 (28)
Tsiouris et al., 2006 (52)	South Africa	Pediatric contacts	184	73	Infancy	51 (28)	94 (51)	10 (5)	29 (16)
Lee et al., 2006 (22)	Korea	Healthy students	131	100	Older	3 (2)	95 (73)	8 (6)	25 (19)
Nakaoka et al., 2006 (51)	Nigeria	Pediatric contacts	179	37	Infancy	40 (22)	106 (59)	19 (11)	14 (8)
Connell et al., 2006 (50)	Australia	Pediatric contacts	75	49	All ages†	10 (13)	38 (51)	4 (5)	23 (31)
Total QuantiFERON			3216 (100)	1890 (59)		693 (21.5)	1586 (49.3)	163 (5.1)	774 (24.1)

* BCG = bacille Calmette–Guérin; IGRA = interferon-γ release assay.
† In these 3 studies, the participants who had received BCG vaccination were immigrants from many countries in which BCG vaccination policy differed.
‡ Study participants had previous multiple BCG vaccinations and TSTs.
§ Eight participants with active tuberculosis were excluded from this analysis.
‖ Results from QuantiFERON-Gold In-Tube (ESAT-6/CFP-10/TB7.7) are shown. Results with QuantiFERON-Gold (ESAT-6/CFP-10) were less concordant with TST.

Table 5. **Concordance of Elispot or T-SPOT.TB with Tuberculin Skin Test (TST) in Healthy Populations with Varying Risk for Latent Tuberculosis Infection***

Study, Year (Reference)	Country	Risk Group	Participants, *n*	BCG Vaccination, %	Age at BCG Vaccination	Concordant Results		Discordant Results	
						TST-Positive and IGRA-Positive, *n (%)*	TST-Negative and IGRA-Negative, *n (%)*	TST-Positive and IGRA-Negative, *n (%)*	TST-Negative and IGRA-Positive, *n (%)*
Chapman et al., 2002 (46)	Zambia	Adults (29% were HIV infected)	49	67	Infancy	22 (45)	8 (16)	11 (22)	8 (16)
Ewer et al., 2003 (41)	United Kingdom	Contacts in high school outbreak	535	87	Older	118 (22)	353 (66)	32 (6)	27 (5)
Richeldi et al., 2004 (66)	Italy	Nosocomial contacts	92	10	Mixed†	2 (2)	73 (79)	2 (2)	15 (16)
Hill et al., 2004 (71)	Gambia	Contacts	735	45	Infancy	162 (22)	382 (52)	139 (19)	58 (8)
Shams et al., 2005 (73)	United States	Contacts	413	49	Mixed†	132 (32)	174 (42)	74 (18)	29 (7)
Zellweger et al., 2005 (72)	Switzerland	Contacts	91	86	Mixed†	11 (12)	48 (53)	27 (32)	3 (3)
Ferrara et al., 2006 (23)	Italy	Hospitalized patients	308	18	Mixed†	90 (29)	146 (47)	42 (14)	30 (10)
Hill et al., 2006 (36)	Gambia	Contacts	693	46	Infancy	165 (24)	413 (60)	60 (9)	55 (8)
Total ELISPOT, *n (%)*			2916 (100)	1493 (51)		702 (24.1)	1597 (54.8)	387 (13.3)	225 (7.7)

* BCG = bacille Calmette–Guérin; IGRA = interferon-γ release assay.
† The participants who had received BCG vaccination were immigrants from many countries where BCG vaccination policy differed.

technical challenges. Second, investigators could have a conflict of interest if they retained financial interest in the assays.

The strengths of this review include the focus on commercially available versions of the new IGRAs or in-house versions that used identical techniques, making the results more relevant to practicing clinicians. In addition, most of the studies we included were published in the past 2 years, making this review as up-to-date as possible. However, because this is an active field of investigation, estimated sensitivity, specificity, and reproducibility may change with additional publications, particularly where evidence is limited.

Implications

The most consistent finding in this review is the high specificity of IGRA, which was unaffected by BCG vaccination, in all populations. This reflects the antigens used in these assays, which are not present in BCG or in certain nontuberculous mycobacteria (59). In the studies reviewed, the effect of BCG vaccination on the results of the tuberculin skin test varied, which may reflect differences in patients' ages when the vaccination was administered. In an earlier review of 24 studies that involved 240 243 persons who had been vaccinated with BCG in infancy and a similar number of persons who were not vaccinated, false-positive results on tuberculin skin tests (≥10 mm) attributable to BCG vaccination occurred in 6.3% persons overall and only in 1% of those who were tested after more than 10 years (60). In the same review, in the 12 studies involving 12 728 persons who were vaccinated with BCG at 2 years of age or older, BCG was responsible for false-positive results on tuberculin skin tests in 40% of all persons and in 20% after 10 or more years (60). It is clear that IGRA will be most useful in BCG-vaccinated populations, particularly in those that received BCG vaccination repeatedly or after infancy. This practice was common in Europe and Latin America for many years, but now global vaccination policy is to vaccinate infants only (61). In populations that received BCG vaccinations as infants, the IGRA will be slightly more specific in childhood but no more specific in adolescence or in adult life (60).

None of the reviewed studies assessed the effect of nontuberculous mycobacteria on IGRA response. Persons with disease caused by *M. marinum* and *M. kansasei* can have positive results on RD1-IGRA (62), which is of interest because the genomes of these nontuberculous mycobacteria organisms contain the RD1 region (59). It would be interesting to perform RD1 on the basis of IGRA and dual skin testing with purified protein derivative (or RT23) plus nontuberculous mycobacteria antigens, such as *M.*

Table 6. **Concordance of T-SPOT.TB and QuantiFERON***

Study, Year (Reference)	Country	Risk Group	Participants, *n*	BCG Vaccination, %	Age at BCG Vaccination	Concordant Reactions		Discordant Reactions	
						T-SPOT.TB–Positive and QFT-G–Positive, *n* (%)	T-SPOT.TB–Negative and QFT-G–Negative, *n* (%)	T-SPOT.TB–Positive and QFT-G–Negative, *n* (%)	T-SPOT.TB–Negative and QFT-G–Positive, *n* (%)
Ferrara et al., 2006 (23)	Italy	Persons with suspected TB	383	18	All ages	94 (25)	233 (61)	50 (13)	6 (2)
Lee et al., 2006 (22)	Korea	Persons with active TB and those at low risk for TB	218	81	All ages	62 (28)	104 (48)	34 (16)	10 (5)
Goletti et al., 2006 (24)	Italy	Persons with suspected TB	32	25	All ages	12 (37)	18 (56)	1 (3)	1 (3)
Total			633			168 (27)	355 (56)	85 (13)	17 (3)

* BCG = bacille Calmette–Guérin; TB = tuberculosis.

marinum, *M. kansasei, M. szulgai,* or *M. flavescens*, from organisms containing the RD1 region. In our earlier review, we found that for every 100 persons with nontuberculous mycobacteria sensitivity, only 2 (2%) would have false-positive results, measuring 10 to 14 mm, on tuberculin skin tests. This effect was reasonably consistent in all countries where dual testing was performed, despite the wide variation in prevalence in nontuberculous mycobacteria sensitivity (60). This means that even in populations in which every person was sensitized to nontuberculous mycobacteria, tuberculin skin test specificity would still be 98%. This modest effect will be clinically important only in populations with a high prevalence of nontuberculous mycobacterial sensitivity and a very low prevalence of true TB infection (such as in the southern United States [63, 64]).

For the diagnosis of latent TB infection, the sensitivity of IGRA is less clear, although in study samples with a gradient of exposure, the prevalence of positive test results was similar in the most-exposed categories. The biggest problem in estimating sensitivity is the lack of a proper gold standard for latent TB infection. In cross-sectional studies, the most commonly used surrogate of latent TB infection has been newly diagnosed, active TB. However, this is a poor surrogate because of the known reduction in cell-mediated response in such patients. The ideal gold standard in cross-sectional studies would be healthy persons known to have TB infection. The only patients who meet these criteria are those being treated for active TB, who have clinically recovered. In an older multicenter study of more than 1000 such patients, tuberculin skin test reactions were remarkably similar in all populations, and overall sensitivity exceeded 93% (29). In 3 earlier studies of such patients, sensitivity of purified protein derivative–based IGRA ranged from 59% to 71%; however, tuberculin skin test sensitivity was 95% (65). Does this mean that the IGRA is less sensitive or that treatment changes the immune response to the IGRA measure? The results of our review indicate that IGRA response generally decreased with treatment in patients who had active TB, although this phenomenon was not seen in patients with latent TB infection. But, why and how would patients with active TB differ from those with untreated latent TB infection after 3 to 6 months of treatment?

This issue is unresolved. Unfortunately, there are no recent studies using RD1-based IGRA in patients who have recovered from active TB, although these would be of great interest. The effect of treatment on IGRA response also cannot be ascertained without understanding the inherent biological variability in IGRA response over months or years. Short-term variability was approximately 9% in 2 studies (37, 38). However, in the only 2 studies with follow-up of 1 to 2 years, spontaneous reversion of IGRA was documented in 24% to 28% of persons and was associated with discordant and weaker initial IGRA responses (39, 44). Until the variability of IGRA response has been studied, the effect of treatment on IGRA response cannot be ascertained. Without this information, the accuracy of evaluation of IGRA sensitivity will not be known in patients with active TB who have recovered clinically.

The numerous studies that have used active TB as a surrogate for latent infection may give rise to the misconception that these tests are useful for diagnosing active TB. However, they do not have optimal sensitivity, and more important, cannot distinguish active from latent TB, which severely reduces their specificity for active TB. The only exception is diagnosis of active TB in children, because tuberculin skin testing is commonly used for this indication. Results from our review indicate that sensitivity of QFT and Elispot are somewhat lower in children than in adults. There are too few studies to make definitive statements, but the results of this review support the urgent

need for further evaluation of IGRA in the diagnosis of TB in pediatric patients.

We hope that in the near future, the gold standard will be longitudinal studies that determine the incidence of active TB in persons with positive and negative results on IGRA and tuberculin skin tests. Results from these studies will establish the risk for TB in persons with positive results on IGRA—particularly those with discordant negative results on tuberculin skin testing. As noted in our review, these discordant reactions are common and poorly understood at present, rendering clinical management of persons with such reactions very difficult. Some discordance may be explained by random variation with 1 or both tests being close to the cut-point for positivity (66, 67).

One important use of tuberculin skin testing is serial testing of exposed populations to detect new TB infection. The variability of tuberculin skin testing has been well established, but the variation in IGRA response has not. Two studies have shown that similar to the tuberculin skin test, IGRA shows nonspecific variations and reversions from positive to negative results during serial testing (39, 44). Until information is available to define IGRA conversion, results of serial IGRA testing will be largely uninterpretable.

Recommendations for Research

On the basis of the data presented, we recommend further research in the following areas. Independent studies of the reproducibility of IGRA should be done. To estimate test variability, repeated assays should be performed on the same samples by the same technician, by different technicians in the same laboratory, or in different laboratories. The effect of minor modifications in the field testing protocol, such as time from phlebotomy to incubation, time of incubation, or temperature of storage, should be tested in the same way. To estimate biological variability, repeated tests should be repeated in unexposed and un-

Table 7. **Effect of Treatment on Interferon-γ Release Assay (IGRA) Response in All Cohort Studies Using RD1 Antigens and Overnight Assays Only***

Study, Year (Reference)	Country	Test Type (Incubation Time)	Participants	Days When Tested	Change or Difference†	Details
Pathan et al., 2001 (40)	United Kingdom	Elispot (14 h)	Persons with active TB: 12 tested before, during, and after treatment	0 and 30–240	Decrease	62% had a decrease in mean levels over an average of 19 wk
Carrara et al., 2004 (78)	Italy	Elispot	Persons with active TB: 18 were treated	0, 90, and 180	Decrease	13 of 18 had reversion to negative results
Nicol et al., 2005 (42)	South Africa	Elispot (18 h)	Pediatric patients with active TB: 15 had probable or possible disease	0 and 30	Increase then decrease	Mean levels increased by 45% after 1 mo of therapy compared with before therapy
Aiken et al., 2006 (38)	Gambia	Elispot (6–14 h)	Persons with active TB: 82 tested before and after treatment	0 and 365	Decrease	82% had positive results before treatment, and 46% had positive results 6 mo after treatment
Ewer et al., 2006 (44)	United Kingdom	Elispot	Persons with latent TB infection: 38 with positive TST and IGRA results were treated; 11 with positive TST and IGRA results were not treated; 14 with negative TST results and positive IGRA results were not treated	0, 180, 365, and 640	Decrease, no change, and decrease	6 of 38 (16%) had reversion after treatment; 0 of 11 (0%) had reversion who were not treated; and 7 of 14 (50%) had reversion without treatment
Wilkinson et al., 2006 (79)	United Kingdom	Elispot (14 h)	Persons with latent TB infection: 33 received INH/RIF; 8 received no treatment	0, 26, and 82	No change	Mean levels increased during treatment but decreased at the end of treatment; no change was seen in untreated persons
Pai et al., 2006 (43)	India	QFT-IT (16–20 h)	Persons with latent TB infection: 10 received INH	0, 365, and 640	No change	Median levels 10 U → 5 U → 7.9 U; percentage positive (>0.35): 100% → 90% → 90%

* In this study, differences between group means were less marked and less significant after 18 hours than after 6 days incubation, but trends were similar. INH = isoniazid; RIF = rifampin; QFT-IT = QuantiFERON-Gold In-Tube; TB = tuberculosis; TST = tuberculin skin test.
† Change or difference comparing results on or after treatment relative to before treatment.

treated persons at intervals ranging from days to years. This information is crucial to distinguish random variation from conversions attributable to new TB infection and to study the effect of treatment on IGRA response.

More studies are needed that compare the sensitivity of the tuberculin skin test with IGRA in HIV-infected and other immune-compromised groups, intravenous drug users, and pediatric and elderly populations.

More data are needed to understand discordant tuberculin skin test and IGRA reactions, including the effect of changes in cut-points, the role of nontuberculous mycobacteria, and time to conversion after exposure and infection.

Now that both IGRAs are widely commercially available, independent field studies can evaluate the feasibility, utility, and costs of these tests in different populations and under different conditions. Such studies should report on the actual completion of tests and the subsequent evaluation and treatment for patients with positive test results. Much of the value of a test is lost if persons who are tested do not return to learn the significance of their test result or if those with positive results are not evaluated further. With these results, the cost-effectiveness of IGRA and the tuberculin skin test can be compared in real-world settings.

Finally, large-scale cohort studies are needed that estimate risk for progression to active disease in persons who have had tuberculin skin test and IGRA. Of particular interest is the risk for disease in persons with discordant reactions.

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Appendix Table 1. **Characteristics of the 3 Tests for Latent Tuberculosis Infection***

Variable	Tuberculin Skin Test	QuantiFERON-Gold and QuantiFERON-Gold In-Tube	Elispot T-SPOT.TB
Administration	In vivo (intradermal)	Ex vivo, ELISA-based	Ex vivo, Elispot-based
Antigens	PPDS or RT-23	ESAT-6, CFP-10, and TB7.7	ESAT-6 and CFP-10
Standardized	Mostly	Yes	Yes
Units of measurement	Millimeters of induration	Units of IFN-γ	IFN-γ spot-forming cells
Definition of positive test results	5, 10, and 15 mm	Patient's IFN-γ ≥0.35 U/mL (after subtracting IFN-γ response in nil control)	≥6 spot-forming cells in the antigen wells, with 250 000 cells/well, and at least double-negative well
Indeterminate	If anergy (rarely tested)	Poor response to mitogen (<0.5 U/mL in positive control) or high background response (>8.0 U/mL in nil well)	Poor response to mitogen (<20 spot-forming cells in positive control well) or high background (>10 spot-forming cells in negative well)
Time to result	48–72 h	16–24 h (but longer if run in batches)	16–24 h (but longer if run in batches)
Cost per test, $†			
Materials		19 (A3)	63 (A3)
Labor/other		22 (A1, A3)	22 (A1)
Total cost	12.73 (A1, A2)	41	85

* ELISA = enzyme-linked immunosorbent assay; IFN = interferon; IGRA = interferon-γ release assay; PPDS = purified protein derivative standard.
† All costs are in Canadian dollars ($1 Canadian = $0.91 U.S.). The numbers in parentheses are references. For the IGRA tests, the materials cost is based on quotes from the manufacturers for shipment to Canadian centers in September 2006. The cost for IGRA labor, shipping, and handling is taken from published field experience with QuantiFERON testing, as reported in reference A1. Costs may vary widely in different countries.

Appendix Table 2. Summary of Methodological Aspects of Studies Included in the Review*

Study, Year (Reference)	Country	Age, y†	Diagnostic Criteria	Interval from Start of TB Therapy until IGRA, d	Blinding‡	Results on QFT or Elispot			Results on TST			
						Positive, n	Total, n	Sensitivity, %	Cut-point, mm§	Positive, n	Total, n	Sensitivity, %
ESAT-6 only												
Johnson et al., 1999 (A4)	Australia	Adults	M,H,C	0–12	NS	11	19	58	–	–	–	–
ESAT–6 and CFP-10 (QFT-G)												
Brock et al., 2001 (A5)	Denmark	37	M	0–30	NS	14	18	72	–	–	–	–
Mori et al., 2004 (A6)	Japan	53	M	0–6	NS	105	119	89	NS	50	76	66‖
Kang et al., 2005 (A7)	Korea	43	M, H	NS	Yes	44	54	81	10	42	54	78
Ravn et al., 2005 (A8)	Denmark	41	M, H, C	0–7	Yes	40	48	85	NS	20	24	88
Ferrara et al., 2005 (A9)	Italy	NS	NS	NS	NS	6	9	67	5	3	9	33
Connell et al., 2006 (A10)	Australia	4	M, TST	NS	NS	9	9	100	5	6	6	100
Ferrara et al., 2006 (A11)	Italy	43	M	NS	Yes	17	24	71	5	14	20	70
Lee et al., 2006 (A12)	Korea	48	M, C	NS	NS	61	87	70	5	64	87	74
Goletti et al., 2006 (A13)	Italy	33	M	0	Yes	19	23	83	10	19	23	83
ESAT-6 and CFP-10 and TB 7.7 (QFT-IT)												
Dogra et al., 2006 (A14)	India	6	M, H	NS	NS	5	8	63	10	5	8	63**
Pai et al., 2005 (A15)	India	36	M	0	Yes	44	60	73	–	–	–	–
Dewan et al., 2007 (A16)	United States	45	M, H	0–14	NS	25	45	55	5	21	24	88
Elispot (ESAT-6 only)												
Lalvani et al., 2001 (A17)	United Kingdom	35	M	0–180	NS	45	47	96	5	18	26	69¶
Pathan et al., 2001 (A18)	United Kingdom	34	M, H	0–180	Yes	33	36	92	–	–	–	–
Lalvani et al., 2001 (A19)	India	33	M	0–180	No	46	50	92	10	0	6	0¶
Elispot–ESAT-6 and CFP 10												
Chapman et al., 2002 (A20)	Zambia	33	M	0–30	No	46	50	92	–	–	–	–
Schölvinck et al., 2004 (A21)	United Kingdom	Adults	M, H	NS	NS	13	13	100	–	–	–	–
Liebeschuetz et al, 2004 (A22)	South Africa	4	M	0	Yes	46	57	81	15	17	43	40
Nicol et al., 2005 (A23)	South Africa	3	M	0	NS	10	12	83	5	9	10	90
Aiken et al., 2006 (A24)	Gambia	32	M	0	NS	70	85	82	–	–	–	–
T-SPOT (ESAT-6 and CFP-10)												
Meier et al., 2005 (A25)	Germany	Adults	M, C, TST	7–21	NS‖	70	73	96	5	40	45	89
Lee et al., 2006 (A12)	Korea	48	M, C	NS	NS	83	87	95	5	64	87	74
Ferrara et al., 2006 (A11)	Italy	43	M	NS	Yes	20	24	83	5	14	20	70
Goletti et al., 2006 (A13)	Italy	33	M	0	Yes	21	23	91	10	10	12	83

* Active tuberculosis was used as a surrogate marker for latent tuberculosis infection. C = clinical; H = histologic; IGRA = interferon-γ release assay; M = microbiologic; NS = not stated; QFT-Q = QuantiFERON-Gold; TB = tuberculosis; TST = tuberculin skin test.
† If given, the median or mean age is shown.
‡ If yes, the technicians performing the IGRAs were blinded to the clinical condition of the patients. If no, the technicians were aware of the clinical condition of the patients.
§ A 5-mm definition was used when the results were available, and a 10-mm definition was used if only those results were given.
‖ 3TU-PPD was used instead of 5TU-PPD.
¶ The Heaf test with 1TU-RT23 was used.

Appendix Table 3. **Specificity of QuantiFERON, Elispot, and Tuberculin Skin Test (TST) in Populations at Very Low Risk for Latent Tuberculosis Infection***

Study, Year (Reference)	Country	Age, y†	Population Risk Ascertained‡	Blinding§	BCG Verification‖	BCG Status	QFT or Elispot			TST¶			
							Positive, *n*	Total, *n*	Specificity, %	Cut-point, *mm*	Positive, *n*	Total, *n*	Specificity, %
QFT (ESAT-6 only)													
Johnson et al., 1999 (A4)	Australia	20	C	NS	D	No BCG	0	60	100	10	0	60	100
						BCG	0	54	100	15	1	54	98
QFT (ESAT-6 and CFP-10)													
Brock et al., 2001 (A5)	Denmark	35	C	NS	NS	BCG	0	19	100	–	–	–	–
		26	C		NS	No BCG	0	15	100				
Mori et al., 2004 (A6)	Japan	20	C	NS	H	BCG	4	213	98	10	73	113	36
Kang et al., 2005 (A7)	Korea	25	C	Yes	Scar	BCG	4	99	96	10	50	99	49
Ravn et al., 2004 (A8)	Denmark	37	I	NS	H	BCG	1	39	97	–	–	–	–
Taggart et al., 2006 (A27)	United States	38	C	NS	H	No BCG	0	81	100	15	3	81	96¶
Lee et al., 2006 (A12)	Korea	15	C	NS	H	BCG	11	131	92	10	28	131	79
Elispot (ESAT-6 only)													
Lalvani et al., 2001 (A17)	United Kingdom	Adults	I	NS	H, S	BCG	0	26	100	–	–	–	–
Lalvani et al., 2001 (A19)	United Kingdom	32	C	No	–	BCG	0	40	100	–	–	–	–
Pathan et al., 2001 (A18)	United Kingdom	32	I	NS	H	BCG	0	32	100	–	–	–	–
Elispot (ESAT-6 and CFP-10)													
Lee et al., 2006 (A12)	Korea	15	C	NS	H	BCG	18	131	86	10	28	131	79

* BCG = bacille Calmette-Guérin; NS = not stated.
† If given, the median or mean age is shown.
‡ Risk ascertainment: C = complete (all factors questioned); I = incomplete (only some factors ascertained).
§ If yes, the technicians performing the interferon-γ release assays were blinded to the clinical condition of the patients. If no, the technicians performing the assay were aware of the clinical condition of the patients.
‖ D = documented; H = history; scar = the patient was examined for a scar.
¶ TST cut-point: A 10-mm definition was used for participants at low risk, but a 15 mm definition is used if only those results were given.

Appendix Table 4. Results of Serial Testing for Patients Receiving Treatment*

Study, Year (Reference)	Country	Patients, *n*	Conditions	Definition	Participation, Completed Follow-Up, *n* (%)†	Treatment Adherence Assessed/ Status‡	Treatment Outcomes Provided§
Elispot							
Pathan et al., 2001 (A18)	United Kingdom	12	ATB	M, C	33 (NS)	NS	Not provided
Carrara et al., 2004 (A28)	Italy	18	ATB	M	100 (100)	NS	Yes
Nicol et al., 2005 (A23)	South Africa	70	ATB	M, C	60‖ (23)	NS	Not provided
Aiken et al., 2006 (A24)	Gambia	89	ATB	M	59 (100)	NS	Yes
Wilkinson et al., 2006 (A29)	United Kingdom	33	LTBI	TST- and IGRA-positive	48 (100)	Yes	NA
Ewer et al., 2006 (A30)	United Kingdom	63	LTBI	TST- and IGRA-positive	35 (100)	NS	NA
QuantiFERON							
Pai et al., 2006 (A31)	India	10	LTBI	TST- and IGRA-positive	63 (100)	Yes	NA

* The intervals between testing were clearly stated in all studies. ATB = active tuberculosis; C = clinical diagnosis; IGRA = interferon-γ release assay; LTBI = latent tuberculosis infection; M = microbiologic confirmation; NA = not applicable; NS = not stated; TST = tuberculin skin test.
† The value in parentheses is the percentage of participants who completed all assays.
‡ Adherence was assessed during treatment.
§ If yes, treated outcomes were analyzed and provided.
‖ In this study, of all eligible participants, 60% were retested at 1 month, 36% were retested at 3 months, and 14% were retested at 6 months.

Appendix Table 5. Studies That Estimated Sensitivity among Contacts for Whom a Gradient of Exposure was Defined*

Study, Year (Reference)	Country	Exposure Defined a Priori	Interval from TST to IGRA	Blinding	
				TST	IGRA
Elispot					
Lalvani et al., 2001 (A32)	United Kingdom	Yes	Same day	No	NS
Ewer et al., 2003 (A33)	United Kingdom	Yes	2–3 m	NS	Yes
Richeldi et al., 2004 (A34)	Italy	Yes	Same day	Yes	NS
Hill et al., 2004 (A35)	Gambia	Yes	Same day	NS	Yes
Zellweger et al., 2005 (A36)	Switzerland	NS†	NS	NS	NS
Shams et al., 2005 (A37)	United States	NS†	0–14 d (60%) >14 d (33%)	NS	Yes
Hill et al., 2006 (A38)	Gambia	Yes	Same day	NS	Yes
QuantiFERON					
Brock et al., 2004 (A39)	Denmark	Yes	3 d after TST	No	NS
Kang et al., 2005 (A7)	Korea	Yes	Same day	Yes	Yes
Nakaoka et al., 2006 (A40)	Nigeria	Yes	Same day	Yes	NS

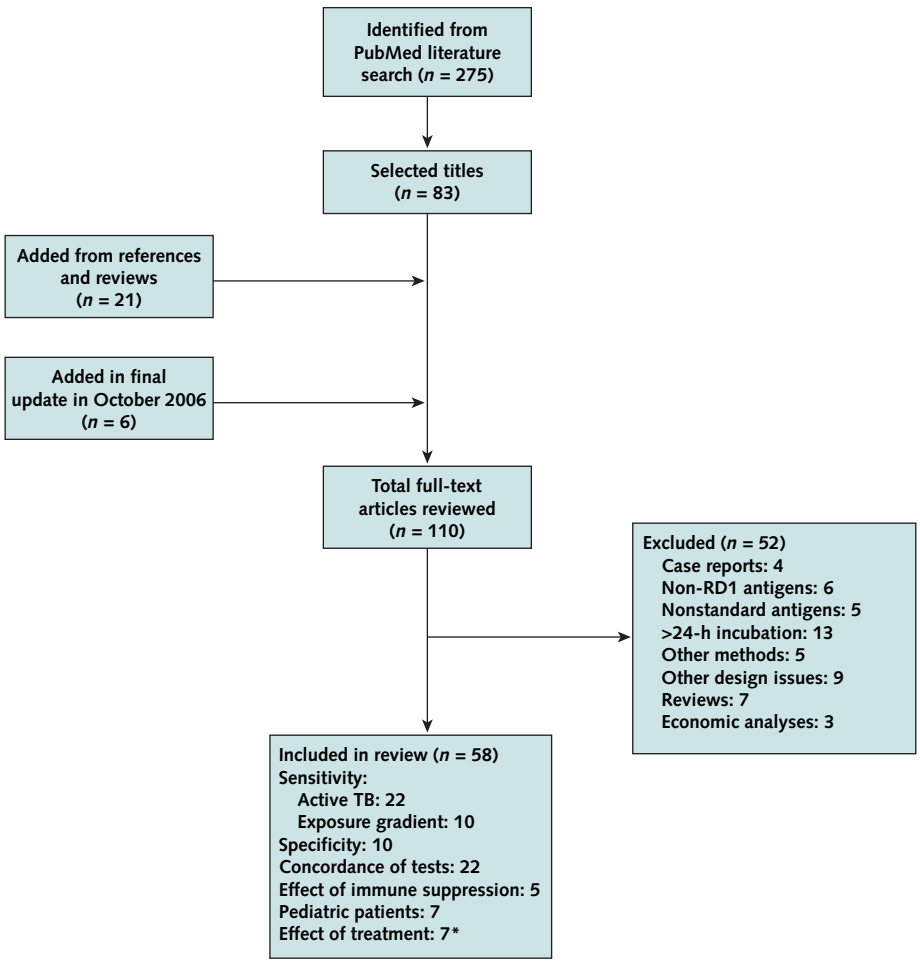
* IGRA = interferon-γ release assay; NS = not stated; TST = tuberculin skin test.
† Exposure scores were calculated on the basis of type, closeness, and duration of contact. In all other studies, exposure was defined as a single criterion: smear-positive or smear-negative index cases, and same house or room versus different house.

Appendix Table 6. **Studies That Examined Concordance of Interferon-γ Release Assay (IGRA) and Tuberculin Skin Test (TST)***

Study, Year (Reference)	Country	Patients, *n*	BCG Verification†	TST and IGRA Same Day‡	Blinding§	
					TST	IGRA
No BCG vaccination						
Brock et al., 2004 (A39)	Denmark	45	H	Yes	NS	NS
Diel et al., 2006 (A41)	Germany	309	H, scar	Yes	Yes	Yes
Porsa et al., 2006 (A42)	United States	409	H	Yes	NS	Yes
BCG vaccination in infancy						
Pai et al., 2005 (A43)	India	719	H	Yes	NS	NS
Dogra et al., 2006 (A14)	India	97	Scar	Yes	Yes	NS
Nakaoka et al., 2006 (A40)	Nigeria	207	Scar	Yes	NS	Yes
Tsiouris et al., 2006 (A44)	South Africa	184	Scar	Yes	Yes	NS
Mahomed et al., 2006 (A45)	South Africa	358	Scar	Yes	NS	NS
BCG vaccination after infancy (or varying or unspecified age of vaccination)						
Kang et al., 2005 (A7)	Korea	120	Scar	Yes	Yes	Yes
Lee et al., 2006 (A12)	Korea	131	H	Yes	NS	NS
Connell et al., 2006 (A10)	Australia	98	Scar	NS	NS	NS
Ferrara et al., 2006 (A11)	Italy	286	Scar	NS	NS	Yes
Harada et al., 2006 (A46)	Japan	304	H	Yes	Yes	NS
Diel et al., 2006 (A41)	Germany	309	H, scar	Yes	Yes	Yes

* BCG = bacille Calmette–Guérin; H = history; NS = not stated; TST = tuberculin skin test.
† The participants were examined for the presence of a scar.
‡ If yes, the 2 tests were performed on the same day.
§ If yes, the technicians performing the tests were blinded to the clinical condition of the patients. If no, the technicians performing the tests were aware of the clinical condition of the patients.

Appendix Figure. **Summary of literature search and study selection.***



* TB = tuberculosis.