EVIDENCE-BASED CLINICAL MEDICINE

Clinical Utility of Interferon Gamma Assay in the Diagnosis of Tuberculosis

Miguel G. Madariaga, MD, Ziba Jalali, MD, PhD, and Susan Swindells, MBBS

Newly developed assays that measure the production of cellular interferon gamma are useful diagnostic tools for the diagnosis of tuberculosis and may potentially replace or complement the tuberculin skin test in some circumstances. Importantly, interferon gamma release assays are more specific than tuberculin skin tests. Unfortunately the tests do not differentiate between active or latent infection. In addition, immunocompromised patients are more likely to have indeterminate results. The current interferon gamma release assays test approved in the United States is costly and requires drawing blood and processing within 12 hours of collection. This study discusses the potential benefits and drawbacks in patients, including those who are immunocompromised. (J Am Board Fam Med 2007;20:540–547.)

Tuberculosis is the leading cause of death from a curable infectious disease and a major cause of morbidity in the world.[1] Almost 9 million new cases were estimated worldwide in 2004, but only half of them were reported.[2]

In the United States, a significant decline in new cases of tuberculosis was seen in the 1950s, thanks to an accurate national notification system, sanitation improvements, and the availability of specific antibiotics.[3] However, a reemergence of the disease, including multidrug-resistant cases, occurred in the 1980s, correlating with the acquired immune deficiency syndrome epidemic and with an overall deterioration in the public health measures for tuberculosis control. The peak incidence during those years seems to have

been curtailed since the early 1990s.[4] Nevertheless, the constant migratory flux of people from high-prevalence countries makes tuberculosis a problem that is by no means extinct.

The tuberculin skin test (TST) has been used for more than 100 years for the diagnosis of both active and latent tuberculosis.[5] The test is based on the intradermal injection of purified protein derivative (PPD), a crude mixture of mycobacterial antigens, including some from the vaccine strain *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) and from nontuberculous mycobacteria.[6] TST is inexpensive and does not require additional infrastructure to be performed, hence its popularity. However, TST has many drawbacks including a high rate of false-positive results (because it includes mycobacterial antigens that are not specific for *M. tuberculosis*), a high rate of false-negative results among immunosuppressed patients, a need for patient compliance to return for the test to be read, and subjectivity and interpersonal variability among health care workers when interpreting the results.[7] TST also has the potential disadvantage of boosting an anamnestic response with successive tests.[8]

New tests that measure the production of interferon gamma (IFN-γ) by T-cells after sensitization with *M. tuberculosis* antigens are available and, at least theoretically, may overcome some of the limitations of the TST.[9] The 2 commercially available tests are the T SPOT-TB assay, an enzyme-linked immunosorbent spot or ELISPOT test that uses

Table 1. Comparison of QuantiFERON-TB Gold and Tuberculin Skin Testing

	QuantiFERON-TB Gold	TST
How is the test performed?	Laboratory measurement of interferon-gamma in whole blood	Intradermal injection of purified protein derivative and measurement of resulting induration
What does the test measure?	In vitro release of interferon gamma after exposure to specific *M. tuberculosis* antigens	In vivo type intradermal hypersensitivity after injection of a mixture of mycobacterial antigens (not only *M. tuberculosis*)
Ability to detect tuberculous infection	Yes	Yes
Ability to distinguish latent and active tuberculosis	No	No
False positive results	Yes, but less frequently than with the TST. False-positive results may occur with exposure to selected nontubercular mycobacteria, but not after BCG vaccination.	Yes. False-positive results may occur after exposure to nontubercular mycobacteria or after BCG vaccination.
False negative results	Possible among immunosuppressed individuals	Possible among immunosuppressed individuals
Effect of BCG vaccination	None	May give a false-positive result after BCG vaccination
Need for patient to return for second visit	No	Yes, for reading of the skin reaction
Required infrastructure	Yes. Special equipment required in the laboratory	No special infrastructure required. Easy to perform even in unsophisticated facilities
Subjectivity in reporting results	No	Yes, it may vary depending on training of individual
Cost	More expensive	Less expensive
Time to obtain result	At least 24 hours, but longer if run in batches	At least 48 hours
Boosting an anamnestic response with successive tests	No	Yes

TST, tuberculin skin test; BCG, Bacillus Calmette-Guérin.

peripheral blood mononuclear cells, and the QuantiFERON-TB Gold, an enzyme-linked immunosorbent assay (ELISA) that uses whole blood.[10,11] The QuantiFERON-TB Gold involves the use of more specific antigens and has replaced the first generation QuantiFERON-TB. QuantiFERON-TB Gold is the only test of its class licensed by the Food and Drug Administration for use in the United States, and consequently will be the focus of this review.[8] An excellent review of other IFN-γ assays used for the diagnosis of tuberculosis has been published elsewhere.[12] Key differences between the ELISA T-cell–based IFN-γ release assay (ELISA interferon gamma release assays [IGRA]) and the TST are outlined in Table 1.

ELISA T-Cell–based INF-γ Release Assay
The test is based on the principle that T-cells from a whole blood sample, when exposed and incubated with a specific *M. tuberculosis* antigen, will produce

IFN-γ.[13] The ELISA-IGRA uses the culture filtrate protein 10 (CFP-10) and the early secretory antigenic target 6 (ESAT-6) as stimulating antigens.[12] CFP-10 and ESAT-6 are encoded within the region of difference 1 of the *M. tuberculosis* genome, and they are more specific for *M. tuberculosis* than PPD because they are not shared with BCG strains or common nontuberculous mycobacteria.[14] Therefore, the advantage of this test is the potential ability to discriminate between tuberculosis infection and previous use of BCG vaccine or atypical mycobacteria reactivity.[15]

The test is performed by obtaining whole heparinazed blood and incubating it with peptide cocktails simulating ESAT-6 and CFP-10 proteins. Other antigens, such as phytohemaglutinin and saline, are used as controls. The former is a mitogen that, after stimulating plasma, serves as an IFN-γ positive control for each specimen; the latter is a control that adjusts for heterophile antibodies in
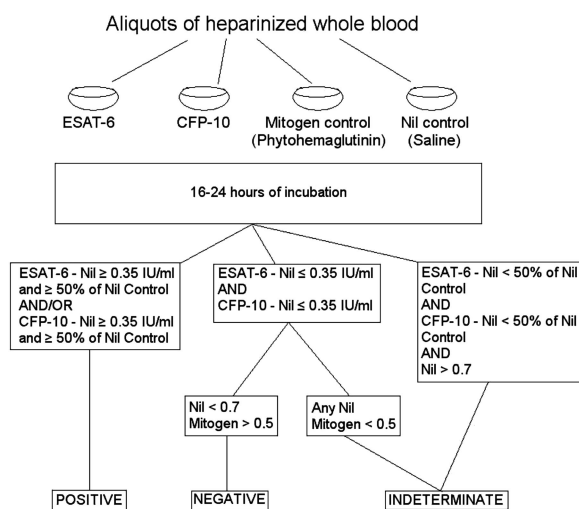
Figure 1. Procedure and result interpretation of ELISA T-cell–based gamma release essay. ESAT-6 and CFP-10 refer to specific antigens coded by the *esat-6* and *lhp* genes of *Mycobacterium tuberculosis*. For indeterminate results, the test may be repeated if there is evidence of potential technical errors, such as storage at incorrect temperature, pipetting errors, etc.

serum or plasma, which are known to cause interference with immunoassays.[8,16] After 16 to 24 hours, the production of IFN-γ is measured by ELISA. The test is reported as positive, negative, or indeterminate (Figure 1). Indeterminate results can be caused by reduced lymphocyte count in the blood sample or reduced lymphocyte activity because of an intercurrent illness (such as human immunodeficiency virus [HIV] infection, malignancy, or renal dysfunction); prolonged specimen transport or improper specimen handling; and incorrect addition of the mitogen.[16,17] A significant drawback of the test is that the sample needs to be processed within 12 hours of collection; otherwise, the white cells will lose viability.[16] A variant of the ELISA-IGRA called "QuantiFERON-TB Gold in tube" uses blood-collection tubes (which are precoated with control and test antigens) as incubation vessels, thus minimizing blood handling and simplifying the blood stimulation step. This device is only approved in Europe, Asia, and Australia.[16,18]

When Is the IFN-γ Assay Indicated?
The test is licensed by the Food and Drug Administration as an aid in the diagnosis of both active tuberculosis and latent infection.[8] The test can be used on all occasions when a TST is used, including

contact investigations, evaluation of immigrants who have received BCG vaccination, and TB screening of health workers.[18–21] Consequently, the test should prompt the same response as a positive TST: basically exclusion of active tuberculosis and/or consideration of treatment of latent tuberculosis.[8]

The ELISA-IGRA cannot differentiate between latent or active tuberculosis. That differentiation needs to be done in the context of a thorough clinical and radiologic evaluation to determine which of the 2 conditions is present. In addition, indeterminate and false-negative test results may occur in immunosuppressed patients.[17,22] A study of 318 patients showed that the majority of the 21.4% with indeterminate results were very young or very old (younger than 3 or older than 80 years old); were receiving immunosuppressive therapy (cancer chemotherapy, systemic steroids, or antitumor necrosis factor α agents); or were patients with cancer, HIV infection, or renal failure.[17]

Although the proteins used in the assay are specific for *M. tuberculosis*, they are also seen in some nontuberculous mycobacteria, including *M. kansasii*, *M. szulgai*, and *M. marinum*. False-positive results in the setting of these infections is likely.[8] For example, in a small study that included 7 patients infected with *M. marinum*, 5 patients infected with *M. kansasii*, and 7 long-term owners of tropical fish tanks (likely exposed to *M. marinum*), more than 50% of them had a detectable IFN-γ response to either ESAT-6 or CFP-10 as measured by either ELISA or ELISPOT.[23] It also seems that L-ESAT, a *M. leprae* antigen homolog to the T-ESAT-6 seen in *M. tuberculosis*, has significant cross-reactivity and may induce the production of IFN-γ, therefore making the test less useful in populations in which leprosy is endemic.[24] Leprosy is considered endemic, but with low prevalence, in Louisiana and Texas, particularly among South East Asian immigrants.[25] The ESAT gene cluster is also present in *M. avium*; however, evidence of false-positive ELISA-IGRA tests with this infection is lacking.[26]

How Does the Test Behave in the General Population?
Both the ELISA-IGRA and TST tests are useful in the identification of tuberculous infection, but they measure different responses: the former detects the in vitro release of IFN-γ, the later measures an in vivo-type intradermal response.[6,11] Both tests have

a good level of agreement, at least among patients at risk in industrialized countries. The largest prospective comparison study to date, done using the QuantiFERON-TB test, included 1226 patients and showed 83.1% concordance between the 2 tests ($\kappa = 0.60$).[27] It seems that the ELISA-IGRA results and the size of induration of the TST are directly correlated. People with a TST of <5 mm are more likely to have indeterminate ELISA-IGRA results when compared with patients with a larger induration.[17] In addition, the concordance of both tests is lower among BCG-vaccinated people as compared with unvaccinated individuals ($\kappa = 0.09$ vs $\kappa = 0.56$, respectively).[17] In countries with a higher prevalence of nontubercular mycobacterial infections, the correlation between the tests may decline; for example, a report from Korea described a poor correlation between the test in healthy volunteers with and without risk factors for tuberculosis exposure ($\kappa = 0.08 – 0.17$), even when correcting the cutoff value for TST to >15 mm.[20]

ELISA-IGRA seems to be highly specific for active tuberculosis, as defined by the number of patients without tuberculosis who have negative ELISA-IGRA. In 4 studies that addressed this issue, the specificity ranged between 97% and 100%.[6,15,28,29] Although it has been suggested that IGRA detects IFN-γ released from T-cells that have recently encountered antigens in vivo rather than from persistent memory T-cells, the use of this test for follow-up purposes and to document the cure of tuberculosis remains controversial.[30,31]

In areas where tuberculosis is endemic, it has been suggested that ELISA-IGRA offers increased specificity in the diagnosis of *M. tuberculosis* infection at the cost of some sensitivity.[32] In fact, in head-to-head comparisons between the TST and IFN-γ assays, the highest sensitivity corresponded to the TST, followed by IFN-γ assays based on PPD, and lastly IFN-γ assays based on specific region of difference 1 antigens (such as ELISA-IGRA).[12] The sensitivity of ELISA-IGRA in people with active tuberculosis, based on 4 case-control studies and 1 cross-sectional study, varies between 72% and 89%.[6,15,28,29]

The first study that directly compared ELISA-IGRA with conventional microbiologic methods for the diagnosis of active tuberculosis was published in 2005.[29] Previous studies calculated sensitivity and specificity in patients with microbiologically proven active tuberculosis, but this study enrolled patients prospectively and analyzed data without knowing the final diagnoses. The sensitivity for ELISA-IGRA was 85%, compared with only 42% for microscopy and 59% for culture. ELISA-IGRA was of particular use in patients with active extrapulmonary tuberculosis who were microscopy-negative and culture-negative. On the other hand, among healthy controls without exposure to tuberculosis, ELISA-IGRA had a specificity of 97% (so the majority of uninfected patients did have a negative ELISA-IGRA). The specificity dropped to 60% when patients who were being ruled out for tuberculosis were tested because a high number of them had latent tuberculosis. In conclusion, in a clinical setting, the ELISA-IGRA will be highly sensitive but unable to differentiate between latent and active disease.

Because of the absence of a criterion standard for the diagnosis of latent tuberculosis, it is difficult to determine whether the ELISA-IGRA performs better than the TST.[27] Studies of sensitivity in latent tuberculosis are mainly based on previous information obtained for the old QuantiFERON-TB. In fact, one of the first reports that assessed the liberation of IFN-γ after exposure to tuberculin was performed in Australia and showed a specificity of 98% and a sensitivity of 90%.[33] An outbreak of tuberculosis in a secondary school with low prevalence of latent tuberculosis in Leicestershire, United Kingdom, gave investigators the opportunity to compare the sensitivity of the IFN-γ–based test with TST.[34] Although the IFN-γ test used was an ELISPOT, the antigens used were the same as those used in the QuantiFERON-TB Gold assay. The agreement between TST and ELISPOT was high ($\kappa = 0.72$; 95% CI, 0.64–0.80; $P < .0001$); however, for students with discordant results, ELISPOT was a stronger predictor of *M. tuberculosis* exposure compared with an isolated positive TST. The conclusion was that ELISPOT was more accurate than TST for the detection of latent tuberculosis, and presumably more sensitive. The performance characteristics of the ELISA-IGRA are listed in Table 2.

ELISA-IGRA in Patients with Immune Dysfunction
HIV Co-Infection
The importance of the relationship between AIDS and tuberculosis cannot be overemphasized. In immunocompetent patients with latent tuberculosis, the lifetime risk of developing active disease is ap-

Table 2. Sensitivity and Specificity of QuantiFERON-TB Gold and Agreement Between QuantiFERON-TB Gold and Tuberculin Skin Testing

Agreement with TST	79–94
Specificity in latent tuberculosis	89–100
Sensitivity in latent tuberculosis	90
Specificity in active tuberculosis	97–100
Sensitivity in active tuberculosis	72–89

All values shown as percentages. TST, tuberculin skin test.

proximately 10%. However, in people with HIV infection it becomes 10% per year.[35] In addition, active tuberculosis is more difficult to treat in HIV-infected patients because of complex drug–drug interactions between antiretroviral and antituberculous medications, increased pill burden, and increased risk of toxicity.[36] Unfortunately, people with HIV infection may be prone to indeterminate IFN-γ test results as a consequence of the inability to mount a satisfactory T-cell response.[26,37] In addition, in patients with AIDS treated with antiretroviral therapy, the functional capacity of the CD4$^+$ cells to produce IFN-γ may remain impaired even after immune recovery and increase in the absolute and relative CD4$^+$ cell count.[38] Studies of the role of IGRA in patients with HIV/tuberculosis co-infection are lacking.

A study using ELISPOT in Zambia showed that the test had high sensitivity in HIV-infected patients; however, 4 patients with active tuberculosis did not have any response. There was no significant difference in the mean lymphocyte count of these patients compared with responders, but no comparison of CD4$^+$ cell counts was performed.[39] In this study, the sensitivity for active tuberculosis dropped from 100% in healthy people to 90% in HIV-infected patients, and the sensitivity for latent tuberculosis among HIV-infected patients was poor, falling from 80% in healthy people to 36% among HIV-infected patients.

Other Immunosuppressive Conditions
Very limited information is available for other immunosuppressed populations. Conditions such as hematologic and solid organ malignancies, diabetes mellitus, chronic renal failure, and the use of corticosteroids, antineoplastic drugs, immunosuppressants (for chemotherapy and organ transplantation), and tumor necrosis α antagonists may affect the response to TST and also may affect the result

of ELISA-IGRA because of decreasing production of IFN-γ.[8] For example, when evaluating the routine use of ELISA-IGRA among unselected patients in an Italian hospital, those who were receiving immunosuppressive therapies had higher odds ratio (odds ratio, 3.35; 95% confidence interval, 1.84–6.08) of having indeterminate results compared with patients who were not receiving such therapy.[17] Although it may be disappointing that ELISA-IGRA is associated with indeterminate results, the lack of mitogen response may warn about the possibility of an underlying immunosuppressant (a piece of information that cannot be obtained with a TST) and may alert the clinician of the need to search more extensively for tuberculosis infection among patients at risk.[37]

The performance of the IGRA has not been evaluated in patients with impaired immune function.

In summary, ELISA-IGRA should be used with caution among HIV-infected and other immunosuppressed patients. A positive or negative result is helpful, but an indeterminate result does not rule out tuberculosis infection. An indeterminate result in the absence of a mitogen response may suggest anergy rather than absence of infection.

Potential Drawbacks
Although ELISA-IGRA has several advantages when compared with TST, it may be several times more expensive.[20] In a study in San Francisco, the cost of an ELISA-IGRA was calculated at approximately $37.39 per patient.[39] Most of the cost was incurred by the laboratory, but additional costs such as phlebotomy, specimen transport, and processing were included. This is in comparison with $9.79 for a TST as estimated by the Center for Medicare Services.[40] However, the price listed for TST does not take into consideration the cost of a second visit to read the results and the transportation and work-force related costs for the patient.

One of the main values of the ELISA-IGRA is its high negative predictive value. A negative test in a healthy patient excludes tuberculosis infection. However, as with other diagnostic tests, the positive predictive value depends on the prevalence of *M. tuberculosis* in the population that is being tested.[8,29]

ELISA-IGRA does not differentiate between active or latent tuberculosis. This determination

should be based on the presence of symptoms (chronic cough, fever, hemoptysis) and the findings on a chest radiograph.

The threshold for deciding when a TST is positive depends on the risk factors of the patient population. For example HIV-infected patients have a positive TST at 5 mm, health care workers at 10 mm, and the general public at 15 mm. Current guidelines for interpretation of the results for the ELISA-IGRA are the same for all patient populations. The results are reported as positive, negative, or indeterminate. An indeterminate result is unhelpful in the diagnosis of tuberculosis infection (although, as mentioned before, the absence of mitogen response may suggest anergy).

Unanswered Questions and Further Directions

The advantage of ELISA-IGRA is that by improving specificity it will reduce the number of false-positive results among BCG-vaccinated people or those exposed to nontuberculous mycobacteria, thus avoiding additional costs and potential toxicity of drugs used for the treatment of latent tuberculosis. In addition, it may increase the identification of more cases of latent tuberculosis before progression to active disease. These advantages need to be balanced with increased laboratory costs, which are a result of dedicated equipment and personnel training requirements.[41] Performing the test in diagnostic laboratories in developed countries is feasible, as documented by experience in the United States and Europe,[9,17,40] but also in some resource-poor settings such as South Africa and India.[42,43] In San Francisco, among 4143 patients, usable results (either positive or negative) were available from 92%, compared with only around 47% to 60% (at most 84% in selected locations) of patients returning for the reading of a TST.[40] Additional studies in economic evaluation and decision analyses are required in this area.

It has been hypothesized that after active tuberculosis treatment the levels of IFN-γ production will decrease. However, several reports using ELISPOT have been contradictory, with some suggesting a decrease in the ESAT-6 response and others showing persistent elevation or even increased response.[31,44] Measurement changes of ELISA-IGRA after treatment of both latent and active tuberculosis, and the ability of the test to

Table 3. Take-Home Points

IGRA can be used in any evaluation for tuberculosis infection (active or latent).

IGRA are more specific than TSTs, which may be positive because of previous BCG vaccination or exposure to non-tuberculosis Mycobacterium.

A positive IGRA indicates tuberculosis infection but does not differentiate between active or latent infection.

A negative IGRA excludes tuberculosis in immunocompetent patients.

Immunosuppressed patients are more likely to have indeterminate results.

IGRA does not require a second visit to read the results.

IGRA is more costly and requires processing within 12 hours of collection.

IGRA, interferon gamma release assays; TST, tuberculin skin test; BCG, Bacillus Calmette-Guérin.

detect reinfection, are areas of research that have been encouraged by the Centers for Disease Control and Prevention.[8] Finally, large prospective longitudinal studies are needed to clearly identify factors that influence test performance, particularly among immunosuppressed patients and children.

Conclusions

"Take home" points are outlined in Table 3. In summary, the ELISA-IGRA is a new assay based on in vitro detection of IFN-γ that may replace PPD as a diagnostic tool for latent and active tuberculosis. The test is more specific and does not require patients to return for a second visit (as TST demands). Cost, technical limitations, and limited data in the most vulnerable groups, such as children and immunosuppressed patients, may prevent the immediate replacement of the TST. In addition, the test can only aid in the diagnosis of active tuberculosis, which must still be made by clinical and microbiologic evaluations.

References

1. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. Lancet 2003;362:887–99.
2. Dye C. Global epidemiology of tuberculosis. Lancet 2006;367:938–40.
3. Huebner RE, Castro KG. The changing face of tuberculosis. Annu Rev Med 1995;46:47–55.
4. Murray JF. A century of tuberculosis. Am J Respir Crit Care Med 2004;169:1181–6.

5. Lee E, Holzman RS. Evolution and current use of the tuberculin test. Clin Infect Dis 2002;34:365–70.

6. Brock I, Weldingh K, Lillebaek T, Follmann F, Andersen P. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. Am J Respir Crit Care Med 2004;170:65–9.

7. Chaparas SD, Vandiviere HM, Melvin I, Koch G, Becker C. Tuberculin test. Variability with the Mantoux procedure. Am Rev Respir Dis 1985;132:175–7.

8. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Morb Mortal Wkly Rep 2005;54:49–55.

9. Ferrara G, Losi M, D'Amico R, et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. Lancet 2006;367:1328–34.

10. Meier T, Eulenbruch HP, Wrighton-Smith P, Enders G, Regnath T. Sensitivity of a new commercial enzyme-linked immunospot assay (T SPOT-TB) for diagnosis of tuberculosis in clinical practice. Eur J Clin Microbiol Infect Dis 2005;24:529–36.

11. Barnes PF. Diagnosing latent tuberculosis infection: turning glitter to gold. Am J Respir Crit Care Med 2004;170:5–6.

12. Pai M, Riley LW, Colford Jr JM. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 2004;4:761–76.

13. Orme IM, Roberts AD, Griffin JP, Abrams JS. Cytokine secretion by CD4 T lymphocytes acquired in response to Mycobacterium tuberculosis infection. J Immunol 1993;151:518–25.

14. Goletti D, Vincenti D, Carrara S, et al. Selected RD1 peptides for active tuberculosis diagnosis: comparison of a gamma interferon whole-blood enzyme-linked immunosorbent assay and an enzyme-linked immunospot assay. Clin Diagn Lab Immunol 2005;12:1311–6.

15. Brock I, Munk ME, Kok-Jensen A, Andersen P. Performance of whole blood IFN-gamma test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. Int J Tuberc Lung Dis 2001;5:462–7.

16. Cellestis Limited. QuantiFERON-TB Gold (in-tube method) [package insert]. Available at http://www.cellestis.com/IRM/Company/ShowPage.aspx?CPID=1023. Accessed 29 August 2007.

17. Ferrara G, Losi M, Meacci M, et al. Routine hospital use of a new commercial whole blood interferon-gamma assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2005;172:631–65.

18. Diel R, Nienhaus A, Lange C, Meywald-Walter K, Forssbohm M, Schaberg T. Tuberculosis contact investigation with a new, specific blood test in a low-incidence population containing a high proportion of BCG-vaccinated persons. Respir Res 2006;7:77.

19. Brock I, Weldingh K, Leyten EM, Arend SM, Ravn P, Andersen P. Specific T-cell epitopes for immunoassay-based diagnosis of Mycobacterium tuberculosis infection. J Clin Microbiol 2004;42:2379–87.

20. Kang YA, Lee HW, Yoon HI, et al. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005;293:2756–61.

21. Pai M, Joshi R, Dogra S, et al. Serial testing of health care workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med 2006;174:349–55.

22. Brock I, Ruhwald M, Lundgren B, Westh H, Mathiesen LR, Ravn P. Latent tuberculosis in HIV positive, diagnosed by the M. tuberculosis specific interferon-gamma test. Respir Res 2006;7:56.

23. Arend SM, van Meijgaarden KE, de Boer K, et al. Tuberculin skin testing and in vitro T cell responses to ESAT-6 and culture filtrate protein 10 after infection with Mycobacterium marinum or M. kansasii. J Infect Dis 2002;186:1797–807.

24. Geluk A, van Meijgaarden KE, Franken KL, et al. Identification and characterization of the ESAT-6 homologue of Mycobacterium leprae and T-cell cross-reactivity with Mycobacterium tuberculosis. Infect Immun 2002;70:2544–8.

25. Taylor JP, Vitek I, Enriquez V, Smedley JW. A continuing focus of Hansen's disease in Texas. Am J Trop Med Hyg 1999;60:449–52.

26. Van Pittius NCG, Gamieldien J, Hide W, Brown GD, Siezen RJ, Beyers AD. The ESAT-6 gene cluster of Mycobacterium tuberculosis and other high G+C Gram-positive bacteria. Genome Biol 2001;2:44.

27. Mazurek GH, LoBue PA, Daley CL, et al. Comparison of a whole-blood interferon gamma assay with tuberculin skin testing for detecting latent Mycobacterium tuberculosis infection. JAMA 2001;286:1740–7.

28. Mori T, Sakatani M, Yamagishi F, et al. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med 2004;170:59–64.

29. Ravn P, Munk ME, Andersen AB, et al. Prospective evaluation of a whole-blood test using Mycobacterium tuberculosis-specific antigens ESAT-6 and CFP-10 for diagnosis of active tuberculosis. Clin Diagn Lab Immunol 2005;12:491–6.

30. Lalvani A. Counting antigen-specific T cells: a new approach for monitoring response to tuberculosis treatment? Clin Infect Dis 2004;38:757–9.

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

32. Hill PC, Brookes RH, Fox A, et al. Large-scale evaluation of enzyme-linked immunospot assay and

skin test for diagnosis of Mycobacterium tuberculosis infection against a gradient of exposure in The Gambia. Clin Infect Dis 2004;38:966–73.

33. Streeton JA, Desem N, Jones SL. Sensitivity and specificity of a gamma interferon blood test for tuberculosis infection. Int J Tuberc Lung Dis 1998;2: 443–50.

34. Ewer K, Deeks J, Alvarez L, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. Lancet 2006;361:1168–73.

35. Corbett EL, Watt CJ, Walker N, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med 2003; 163:1009–21.

36. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 2006;368:1575–80.

37. Pai M, Lewinsohn DM. Interferon-gamma assays for tuberculosis: is anergy the Achilles' heel? Am J Respir Crit Care Med 2005;172:519–21.

38. Sutherland R, Yang H, Scriba TJ, et al. Impaired IFN-gamma-secreting capacity in mycobacterial antigen-specific CD4 T cells during chronic HIV-1 infection despite long-term HAART. AIDS 2006;20: 821–9.

39. Chapman AL, Munkanta M, Wilkinson KA, et al. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of Mycobacterium tuberculosis-specific T cells. AIDS 2002;16:2285–93.

40. Dewan PK, Grinsdale J, Liska S, Wong E, Fallstad R, Kawamura LM. Feasibility, acceptability, and cost of tuberculosis testing by whole-blood interferon-gamma assay. BMC Infect Dis 2006;6:47.

41. Richeldi L. An update on the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2006;174: 736–42.

42. Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 2004;364:2196–203.

43. Pai M, Gokhale K, Joshi R, et al. Mycobacterium tuberculosis infection in health care workers in rural India: comparison of a whole-blood interferon gamma assay with tuberculin skin testing. JAMA 2005;293:2746–55.

44. Aiken AM, Hill PC, Fox A, et al. Reversion of the ELISPOT test after treatment in Gambian tuberculosis cases. BMC Infect Dis 2006;30:66.