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REVIEW

Diagnosis of latent tuberculosis infection: The potential role of new technologies

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Summary Tuberculosis (TB) is a major cause of morbidity and mortality worldwide. TB control programmes need improvement in the diagnosis of latent TB infection. The tuberculin skin test (TST) is far from a 'gold' standard as it often gives false results. Interferon-gamma assays are newly available tests to detect latent TB infection, but they are currently not routinely used. They are based on immune responses to purified protein derivative (PPD) or to region of difference 1 (RD1) specific antigens. Assays based on RD-1 specific antigens perform better than both PPD based assays and TST. They correlate with TB exposure and are less likely to give false results in non-tuberculous mycobacterial disease, Bacille Calmette-Guerin (BCG) vaccination and immunosuppression. More accurate diagnosis of latent TB infection with RD-1 specific antigen based interferon-gamma assays may allow targeting of chemoprophylaxis to reduce the burden of active TB while decreasing wastage of health care resources due to false results associated with TST. However, further research and development is required to verify that new tests can predict the risk of later development of active TB and to make it feasible to perform these tests in a reproducible fashion at low cost, particularly in developing countries.

Contents

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Introduction

One-third of the world's population has latent tuberculosis (TB) infection.[1] This develops into active disease in 5–10% of cases and annually 2 million people die from TB.[2] Diagnosing people with latent TB infection is important for control of the disease. World Health Organisation's (WHO) "global plan to stop tuberculosis" intends to improve diagnostic tests and to make them available for use in high TB prevalence countries[2] so that active disease can be prevented by chemoprophylaxis. Testing should be of sufficiently high accuracy to prevent the unwanted consequences of false results. For example, false-positive results can lead to inappropriate initiation of chemoprophylaxis with potential toxic side-effects, while false-negative results can lead to inappropriate non-use of chemoprophylaxis with progression to active TB. Both lead to unnecessary morbidity and use of health care resources.

Mycobacterium tuberculosis is an intracellular pathogen, which is difficult to recover from infected subjects and humoral responses in TB infection are weak.[3] These factors have resulted in difficulties when developing useful microbiological and serological tests. Tuberculin skin test (TST) remains the most commonly used test despite being insufficiently accurate. Interferon-gamma assays based on immune responses to purified protein derivative (PPD) were first developed to detect latent TB infection. Recently more specific, region of difference 1 (RD-1) based antigen assays have become available. Several studies have been conducted on comparing whole blood enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot (ELISPOT) assay with TST, which have recently been reviewed.[4–6] What are the characteristics of these new tests and what is their comparative value vis-a-vis traditional tests?

Tuberculin skin test

TST is currently the standard tool to detect latent TB infection, though it is far from a 'gold' standard. TST is based on the detection of delayed-type hypersensitivity to PPD, a mixture of antigens shared by several mycobacteria that gives rise to a skin reaction. Two visits are required for the test, one for PPD inoculation (the Mantoux technique uses intracutaneous injection by needle and syringe) and another after 48–72 h for interpretation of the result based on the size of the skin reaction (Fig. 1).

TST is a simple test with low material costs and can be performed without the need for a specialist laboratory. This is important in high burden, resource-limited countries where even quality sputum microscopy may be difficult to access. TST has been extensively used in resource-limited settings for epidemiological field surveys on annual

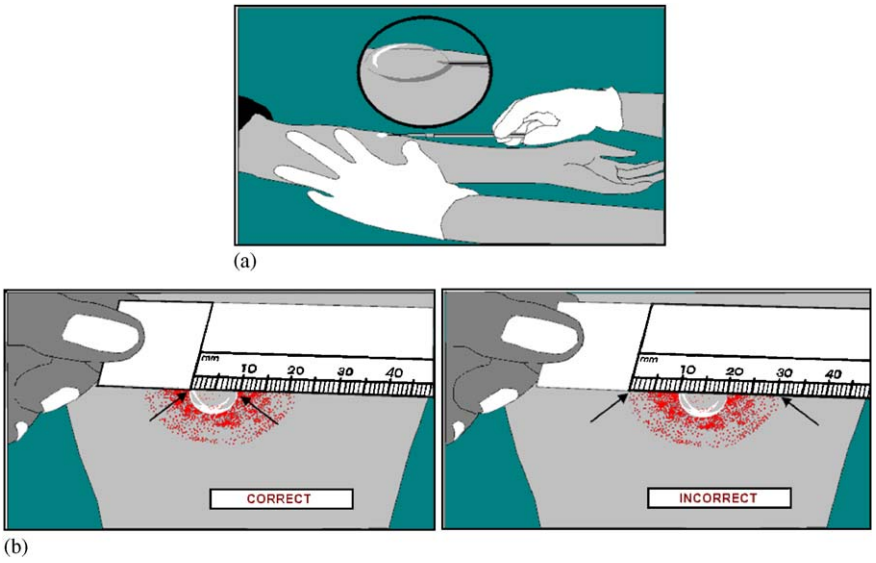

Figure 1 Inoculation and interpretation of Tuberculin skin test. (a) Inoculation of purified protein derivative using syringe and needle; (b) measurement of skin induration 48–72 h after inoculation. *Source*: www.phppo.cdc.gov.

risk of infection.[7,8] These often involve large numbers of healthy children and conducting surveys with a skin test is relatively straight forward. Several longitudinal studies have demonstrated a positive association between TST response and subsequent risk of active TB.[9,10] Randomized trials have shown that treatment of latent infection, diagnosed on the basis of positive TST, reduces the risk of active TB. This experimental evidence has lead to the policy of tuberculin testing and treatment of latent TB infection.[11–13]

Difficulties in test administration and interpretation often lead to false results. There are many practical difficulties in conducting TST. The second of the two visits might pose a compliance problem for people who live in remote settings and in some patient groups, e.g. in urban human immunodeficiency virus (HIV) clinics return rates are low.[14] The inoculation may induce a painful skin inflammation sometimes with induration[15] and with scarring at the injection site, which may be unacceptable to certain population groups. The test might not be possible in individuals with skin disorders.

Dose of PPD, method of application and criteria for interpretation vary between countries. Weak PPD doses increase the likelihood of false-negative results and strong doses increase the likelihood of false-positive results. A 1.5 mm difference of reaction size may be seen when a 10 TU dose is compared to 5 TU.[16] The technique for inoculating PPD doses may cause false results.[17] Different tests are available e.g. Heaf test, used in the UK, and Mantoux test, used in most other countries. The Heaf test, however, has been recently discontinued in the UK, since small metal fragments were found in the test site following use of Heaf test heads.[18] Different cut-offs are used for positivity of TST, as there is no general consensus on this issue. Criteria of 5, 10 or 15 mm for skin reaction have been recommended depending on the clinical situation.[11] There can be false TST results from operator variability in both inoculation and reading of the test.[19] Digit preference e.g. rounding measures of TST induration to the nearest multiple of 5 mm and interpretation bias can significantly affect TST results.[20]

There are many reasons for false-positive TST results (Table 2). PPD contains a poorly defined mixture of mycobacterial antigens. Because antigens are shared with other mycobacteria, tuberculin reactivity leading to a positive TST can result from Bacille Calmette-Guerin (BCG) vaccination with a live attenuated mycobacterial strain derived from *M. bovis* or from exposure to non-tuberculous mycobacteria.[21–24] Whereas in developed countries BCG vaccination is not always routine, in developing countries it is generally included in the immunisation scheme and is usually given at birth or in infancy. The effect of BCG vaccination on TST can persist as long as 15 years after vaccination.[17] The degree of BCG induced delayed-type hypersensitivity varies with country, timing of vaccination, time elapsed since vaccination and whether vaccination is repeated.[22,25,26] There is considerable loss of hypersensitivity over time in several subtropical and tropical countries as studies in Uganda, India, Malawi, Sri Lanka, the Gambia and the Solomon Islands have shown.[22,27–32] Reaction to TST in cases of BCG vaccination tends to be small, however this is not always consistent.[11]

Specificity problems of PPD can be addressed by simultaneous skin testing with *M. tuberculosis* PPD and sensitins, which are PPD-like products derived from non-tuberculous mycobacteria. This approach can help to discriminate patients with TB from those who are infected with *M. avium complex*.[33–35] Repeated TST's may induce booster responses leading to false-positive results.[36] Energy associated with HIV infection, disseminated TB or immunosuppression due to haemodialysis, transplantation or medication can give rise to false-negative reactions.[17,37,38]

There have been many responses to the above problems, none of which are ideal. Some countries have stopped using BCG vaccination, since prevention of adult tuberculosis with chemoprophylaxis may be superior than using BCG vaccination. However in view of the persistent increase of TB in the UK compared to other European countries, BCG vaccination is still continued.

Interferon-gamma assays

Interferon-gamma assays have been developed as tests to replace TST. They operate on a T-cell based approach as TB infection evokes a strong T-helper 1 type cell-mediated immune response.[39] Release of interferon-gamma from *M. tuberculosis* specific T-cells is used as a marker of infection. In vitro studies of interferon-gamma production in response to mycobacterial antigens can be used to detect latent TB infection.[40] The antigens used to illicit an interferon-gamma response define the main types of existing commercial tests: assays based on PPD and those based on RD-1 specific antigens (Figs. 2 and 3). Three commercial interferon-gamma assays have been developed, the QuantiFERON-TB assay (Cellestis Limited, Carnegie, Victoria, Australia), the T SPOT-TB assay (Oxford Immunotec, Oxford, UK) and the Quantiferon Gold assay (Cellestis Limited, Carnegie, Victoria, Australia).

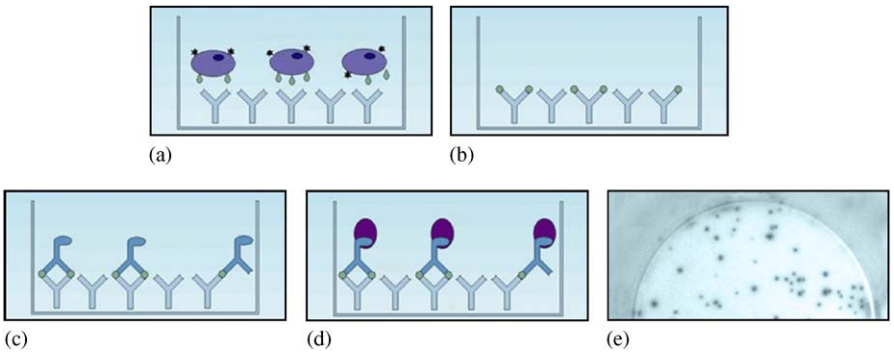

Figure 2 Principle of ELISPOT. (a) Microtiter plate wells are coated with monoclonal antibodies. T-cells secreting interferon-gamma are added and incubated in the presence of tuberculosis antigens; (b) interferon-gamma is captured by monoclonal antibodies; (c) conjugated second antibody is added to interferon-gamma followed by incubation and washing of wells; (d) substrate is added and after colour change, spots can be counted; (e) spots in well correspond to T-cells that have released interferon-gamma. *Source*: Adapted from manufacturer's websites.

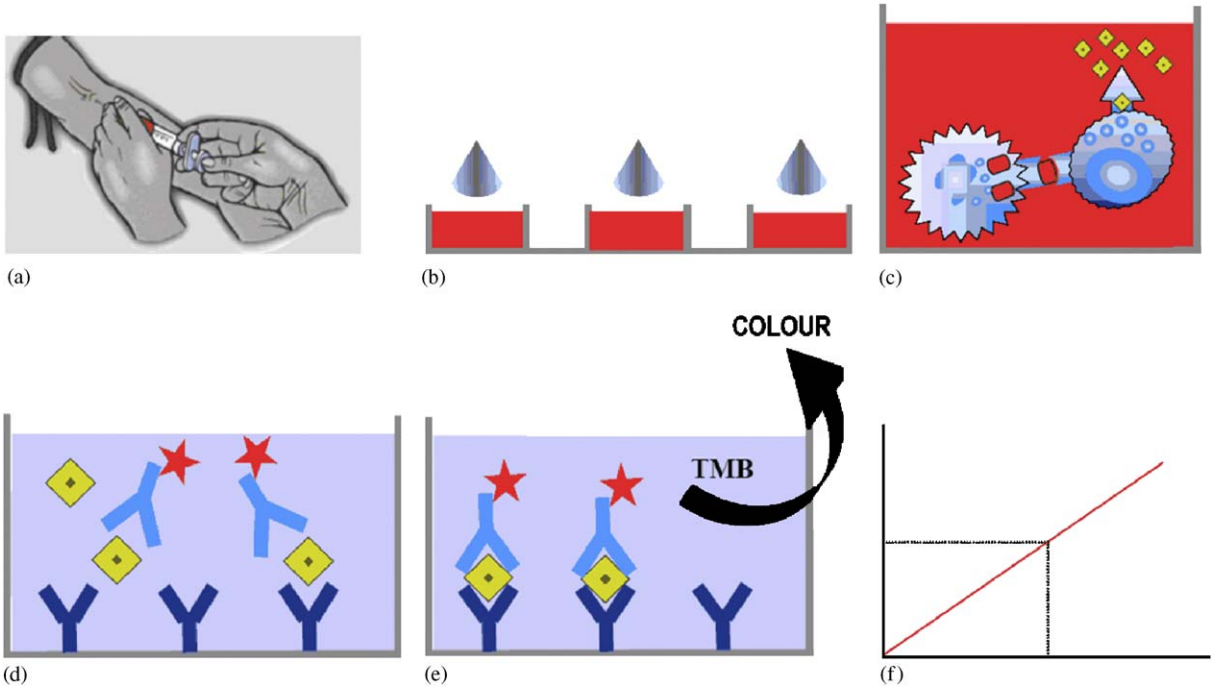

Figure 3 Principle of whole blood interferon-gamma assay ELISA. (a) Aliquots of undiluted whole blood are taken; (b) antigens (PPD, ESAT-6, CFP10) are added and incubated overnight; (c) antigen-specific T-cells produce interferon-gamma; (d) plasma is harvested and incubated in ELISA; (e) bound interferon-gamma is equivalent to colour development; (f) Interferon-gamma is measured as IU/ml. *Source*: Adapted from manufacturer's websites.

The interferon-gamma tests have several advantages over TST (Tables 1 and 2). They involve having a blood test at a single visit and a return visit might not be needed in some settings depending on the test result. Patients may need to come back to obtain the result and counselling about the implications of the results in other settings. Automated testing has the advantage of reducing reader bias as interpretation is objective. A booster phenomenon does not occur and therefore screening of people who are repeatedly exposed to TB (e.g. health care workers) becomes feasible and these tests should prove a better marker of latent TB infection than TST.[41]

Interferon-gamma assays also have some limitations: The need to perform a blood test might not

Table 1 Characteristics of tests for latent tuberculosis infection with regard to test administration.

	Tuberculin skin test	Interferon-gamma assays
Patient visit	Two visits are required, one for administration and one for interpretation	Only one visit may be required, return visit not always necessary in certain settings
Laboratory	No laboratory required, easy administration	Laboratory and experience with assay required
Reagents	Reagents not standardised and vary between countries	Reagents not standardised, several assays available within one country
Site of test	Skin test may cause inflammation and scarring Contraindicated in certain skin conditions	Blood test required
Patient acceptability	Painful on skin site, may not be acceptable to some patient groups	Obtaining blood sample may not be acceptable to some patient groups especially children
Operator variability	Operator variability in inoculation and reading of test, false results may arise due to improper placement or wrong measurements	No reader bias, reduced risk of misclassification
Effect of repeated testing	Repeated tests can cause booster responses	No booster response
Cost	Cheap test	Expensive test
Time of test	48–72 h	24 h or longer depending on resources

Abbreviations: PPD = purified protein derivative, ESAT = early secretory antigen target, CFP = culture filtrate protein.

Table 2 False results among tests for latent tuberculosis infection.

	Tuberculin skin test	Interferon-gamma assays based on PPD	Interferon-gamma assays based on RD1 specific antigens
Use in patients with BCG vaccination	False-positive due to BCG vaccination	Likely less false-positive due to BCG vaccination	Potentially even less false-positive due to BCG vaccination
Effect of non-tuberculous mycobacteria on interpretation of test	False-positive due to exposure to non-tuberculous mycobacteria	Whole blood ELISA discriminates between *M. avium* and *M. tuberculosis*, however not other non-tuberculous mycobacteria	Discriminates between many non-tuberculous mycobacteria however not *M. kansasii*, *M. szulgai, M. marinum, M.gastrii and M. flavescens*
Use in patients with immuno-suppression (e.g. HIV infection)	False-negative due to anergy secondary to immunosuppression	Possibly less false-negative due to anergy	Potentially even less false-negative due to anergy (limited data available)

Abbreviations: PPD = purified protein derivative, ESAT = early secretory antigen target, CFP = culture filtrate protein, BCG = Bacille Calmette-Guerin, HIV = human immuno-deficiency virus.

be desirable in certain patient groups especially children. The blood often needs to be processed within 12 h after collection and laboratories need to gain expertise in technology like isolation of mononuclear cells.[37] Although some laboratories in developing countries have been run these tests with simple equipment like centrifuge, incubator and microscope,[3] interferon-gamma assays are

currently not in widespread use since they are expensive and require at least a basic laboratory. There are no reports of longitudinal studies showing an association between positive interferon-gamma response and subsequent risk of active TB. There is also lack of evidence, whether withholding treatment for latent TB infection based on a positive TST, but negative interferon-gamma response is safe.

Interferon-gamma assays based on PPD

The whole blood interferon-gamma ELISA, the Quantiferon TB test[42] was the first commercially available test. Although it is inferior to RD1 specific antigen based assays it is of relevance to mention since many countries have used this whole blood ELISA. This assay measures interferon-gamma production with ELISA, after in vitro stimulation of whole blood cells with PPD from *M. tuberculosis* and control antigens.[43,44] It is able to discriminate between *M. tuberculosis* and *M. avium intracellulare complex* infection.[45] It responds to multiple antigens spontaneously.[43,46–48] It does not boost anamnestic immune responses.[48] Moreover the whole blood ELISA interferon-gamma assay is comparable with the TST in its ability to detect latent TB infection.[43,44,47] Disadvantages of a PPD-based assay such as the whole blood ELISA is, that it can give false-positive results in BCG vaccinated people and that it does not discriminate between most of the non-tuberculous mycobacteria and *M. tuberculosis*.[43,49–51] Tuberculin reactivity seems to be more detectable by the whole blood interferon-gamma assay than by TST in HIV infection, however studies have shown conflicting results and it is not clear if the whole blood ELISA is superior than TST.[52–54]

Interferon-gamma assays based on RD-1 specific antigens

Interferon-gamma assay based on RD-1 specific antigens, early secretory antigen target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), can overcome some of the above disadvantages. Comparative genomics has identified several genetic regions in *M. tuberculosis* and *M. bovis* that are deleted in all tested BCG strains.[55] The identified region, the so-called region of difference 1 (RD1) region, is present in *M. kansasii*, *M. szulgai* and *M. marinum*[56,57] however it is also encountered in rarer mycobacteria such as *M. flavescens* and *M. gastrii*. Proteins encoded in these regions have formed the basis of new specific T-cell-based blood tests that do not cross-react with BCG, but only two antigens, ESAT-6 and CFP 10 have been studied in detail in humans.[58] ESAT-6 is a secreted antigen that is expressed in *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, and *M. africanum*), but it is compared to PPD absent from BCG and many non-tuberculous mycobacteria.[56,59] ESAT-6 and CFP-10 share the same messenger RNA transcript, which suggest that CFP-10 and ESAT-6 may interact with one another and serve a common function in detection of *M. tuberculosis*.[60] All stimulated T-lymphocytes secrete interferon-gamma, but ESAT-6 and CFP-10 assays can only detect interferon-gamma secreted from T-lymphocytes produced as a result of exposure to ESAT-6 and CFP-10 antigen. In vivo and in vitro experiments have shown that the combination of ESAT-6 and CFP-10 has a higher sensitivity and specificity than PPD in diagnosis of TB infection.[49–51]

There are two commercial assays available incorporating the two RD1-based antigens ESAT-6 and CFP-10. The T SPOT-TB assay[61,62] is an ELISPOT assay, whereas Quantiferon Gold[63] is an ELISA test. Both ELISPOT[64,65] and the whole blood interferon-gamma ELISA[66] have shown in outbreak investigations that *M. tuberculosis* exposure is more strongly associated with RD-1 specific antigen based assays than with TST. Recently, an attempt has been made to estimate the likelihood of latent TB infection by calculating a contact score that quantified exposure to the index case. The likelihood of a positive interferon-gamma assay increased significantly with a rising contact score.[67] Most studies using intensity of exposure as a surrogate measure to predict latent TB infection were conducted in developed countries. However, studies in countries with high prevalence of TB showed that accuracy of interferon-gamma assays in predicting latent TB infection was not as good as that observed in low prevalence countries.[28,68,69]

Assays based on RD1-specific antigens have shown to cause less confounding by BCG vaccination than TST and are therefore more reliable to use in BCG vaccinated individuals.[59,64–67,69–71] ELISPOT has shown improved accuracy over TST in children with suspected TB who are of young age, malnourished and those with HIV infection. Interferon-gamma assays might also improve diagnostic accuracy in latently infected people with greatest risk of progression in whom TST is often false-negative especially people with HIV infection.[72] Their superiority in patients who are on immunosuppressive medication is unclear since indeterminate interferon-gamma results limit their clinical usefulness.[73–75] In haemodialysis patients, ESAT-6 has been shown to be unaffected by uraemia-

induced immunosuppression and therefore may be a better marker of LTBI than TST.[41] Nevertheless, very few studies have been published on the use of these assays in HIV-infected individuals, patients on immunosuppressive medications, and those with other immunocompromising conditions. Thus there is lack of evidence to recommend RD-1 specific antigen based assays in immunosuppressed patients at the moment.

There is a lot of heterogeneity in the interferon-gamma assays with regard to antigens, format, type and source of antigens, cut-off points used for positivity, type of blood specimen use, and incubation period. There are several technical issues, including impact of specimen collection, storage, transport and time from collection to incubation. Unexplained high background interferon-gamma response and indeterminate results especially in immunosuppressed patients have also been reported.[74,75] The repeatability of the interferon-gamma assay results over time in the same individual is largely unknown. Inter-laboratory variability is also poorly studied. A new interferon-gamma assay based on ESAT-6 and CFP-10 fusion protein has become available in addition to that based on peptides of these antigens.[76] Whether it is preferable to conduct the interferon-gamma assay with recombinant protein or pools of peptides, or whether increased sensitivity could be achieved through measurement of a combined peptide and protein response, needs to be established.[77]

Future direction

More research and development is needed to determine the efficacy of the new tests in detecting latent TB in both developed and developing countries. There is a need to determine whether high responses to RD-1 specific antigens predict a higher risk of developing active TB. Assays using additional antigens apart from ESAT-6 and CFP-10 need to be evaluated.[28,78] There is a need to discover more specific antigens which do not cross react with non-tuberculous mycobacteria. Recombinant proteins of existing antigens[77] (in addition to their peptide pools), should be evaluated further. Studies comparing performance of the ELISA and ELISPOT assay will have to be done.

Current interferon-gamma assays based on RD-1 specific antigen assays have shown improved performance over both PPD-based assays and TST. Despite the expense involved, they may be cost-effective as they may reduce unnecessary chemoprophylaxis and use of health care resources associated with false results. They have the potential to lessen the number of active TB cases, if they prove to be more accurate than TST. In the future, longitudinal studies of the association between interferon-gamma response and subsequent risk of development of active TB will allow us to determine whether or not these tests are superior to TST.

Conflict of interest

None.

Search strategy and selection criteria

Electronic searches of MEDLINE (from 1966 to present), EMBASE (from 1980 to present) were undertaken to identify citation of studies comparing interferon-gamma assays with tuberculin skin test and those providing diagnostic information on the value of these tests in latent tuberculosis infection. Experts in the field were contacted and manufacturers' websites were visited. Reference lists of all relevant reviews and primary articles were searched. There were no language restrictions in the search or selection process.

References

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *J Am Med Assoc* 1999;**282**:677–86.
2. The global plan to stop tuberculosis http://www.who.int/gtb/publications/stb/cds_stb_2003_23/globalplan_en.pdf. 2003.
3. Lalvani A. Spotting latent infection: the path to better tuberculosis control. *Thorax* 2003;**58**:916–8.
4. Dheda K, Udwadia ZF, Huggett JF, Johnson MA, Rook GA. Utility of the antigen-specific interferon-gamma assay for the management of tuberculosis. *Curr Opin Pulm Med* 2005;**11**:195–202.
5. 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.
6. Lalvani A, Richeldi L, Kunst H. Interferon gamma assays for tuberculosis. *Lancet Infect Dis* 2005;**5**:322–4.
7. Tupasi TE, Radhakrishna S, Pascual ML, Quelapio MI, Villa ML, Co VM, et al. BCG coverage and the annual risk of tuberculosis infection over a 14-year period in the Philippines assessed from the Nationwide Prevalence Surveys. *Int J Tuberc Lung Dis* 2000;**4**:216–22.
8. Zhang LX, Tu DH, He GX, Ma ZQ, Nagelkerke NJ, Borgdorff MW, et al. Risk of tuberculosis infection and *Tuberculous meningitis* after discontinuation of Bacillus Calmette-Guerin in Beijing. *Am J Respir Crit Care Med* 2000;**162**:1314–7.
9. Christie CD, Constantinou P, Marx ML, Willke MJ, Marot K, Mendez FL, et al. Low risk for tuberculosis in a regional pediatric hospital: nine-year study of community rates and the mandatory employee tuberculin skin-test program. *Infect Control Hosp Epidemiol* 1998;**19**:168–74.

10. MacIntyre CR, Plant AJ. Longitudinal incidence of tuberculosis in South-East Asian refugees after re-settlement. *Int J Tuberc Lung Dis* 1999;**3**:287–93.
11. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. *MMWR Recomm Rep* 2000;**49**:1–51.
12. Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. *Am J Respir Crit Care Med* 2000;**161**:S221–47.
13. Control and prevention of tuberculosis in the United Kingdom: code of practice 2000. Joint Tuberculosis Committee of the British Thoracic Society. *Thorax* 2000;**55**:887–901.
14. Chaisson RE, Keruly JC, McAvinue S, Gallant JE, Moore RD. Effects of an incentive and education program on return rates for PPD test reading in patients with HIV infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**11**:455–9.
15. Nardell EA, Fan D, Shepard JA, Mark EJ. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 22-2004. A 30-year-old woman with a pericardial effusion. *N Engl J Med* 2004;**351**:279–87.
16. Stuart RL, Bennett N, Forbes A, Grayson ML. A paired comparison of tuberculin skin test results in health care workers using 5 TU and 10 TU tuberculin. *Thorax* 2000;**55**:693–5.
17. Comstock GW. False tuberculin test results. *Chest* 1975;**68**:465–9.
18. Recall of Bignell Heaf testing device http://www.hpa.org.uk/cdr/archives/archive05/News/news1005.htm. 2005.
19. Pouchot J, Grasland A, Collet C, Coste J, Esdaile JM, Vinceneux P. Reliability of tuberculin skin test measurement. *Ann Intern Med* 1997;**126**:210–4.
20. Bearman JE, Kleinman H, Glyer VV, Lacroix OM. A study of variability in tuberculin test reading. *Am Rev Respir Dis* 1964;**90**:913–9.
21. Fine PE, Bruce J, Ponnighaus JM, Nkhosa P, Harawa A, Vynnycky E. Tuberculin sensitivity: conversions and reversions in a rural African population. *Int J Tuberc Lung Dis* 1999;**3**:962–75.
22. Floyd S, Ponnighaus JM, Bliss L, Nkhosa P, Sichali L, Msiska G, et al. Kinetics of delayed-type hypersensitivity to tuberculin induced by bacille Calmette-Guerin vaccination in northern Malawi. *J Infect Dis* 2002;**186**:807–14.
23. Huebner RE, Schein MF, Bass Jr JB. The tuberculin skin test. *Clin Infect Dis* 1993;**17**:968–75.
24. Kwamanga DO, Swai OB, Agwanda R, Githui W. Effect of non-tuberculous Mycobacteria infection on tuberculin results among primary school children in Kenya. *East Afr Med J* 1995;**72**:222–7.
25. Menzies R, Vissandjee B. Effect of bacille Calmette-Guerin vaccination on tuberculin reactivity. *Am Rev Respir Dis* 1992;**145**:621–5.
26. Saito M, Bautista CT, Gilman RH, Bowering A, Levy MZ, Evans CA. The value of counting BCG scars for interpretation of tuberculin skin tests in a tuberculosis hyperendemic shantytown, Peru. *Int J Tuberc Lung Dis* 2004;**8**:842–7.
27. Baily GV. Tuberculosis prevention Trial, Madras. *Indian J Med Res* 1980;**72**(Suppl):1–74.
28. Pai M, Gokhale K, Joshi R, Dogra S, Kalantri S, Mendiratta DK, et al. *Mycobacterium tuberculosis* infection in health care workers in rural India: comparison of a whole-blood interferon gamma assay with tuberculin skin testing. *J Am Med Assoc* 2005;**293**:2746–55.
29. Lienhardt C, Fielding K, Sillah J, Tunkara A, Donkor S, Manneh K, et al. Risk factors for tuberculosis infection in sub-Saharan Africa: a contact study in The Gambia. *Am J Respir Crit Care Med* 2003;**168**:448–55.
30. Mudido PM, Guwatudde D, Nakakeeto MK, Bukenya GB, Nsamba D, Johnson JL, et al. The effect of bacille Calmette-Guerin vaccination at birth on tuberculin skin test reactivity in Ugandan children. *Int J Tuberc Lung Dis* 1999;**3**:891–5.
31. Eason RJ. Tuberculin sensitivity. *Ann Trop Paediatr* 1987;**7**:87–90.
32. Karalliedde S, Katugaha LP, Uragoda CG. Tuberculin response of Sri Lankan children after BCG vaccination at birth. *Tubercle* 1987;**68**:33–8.
33. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000;**356**:1099–104.
34. Von Reyn CF, Green PA, McCormick D, Huitt GA, Marsh BJ, Magnusson M, et al. Dual skin testing with *Mycobacterium avium* sensitin and purified protein derivative: an open study of patients with *M. avium* complex infection or tuberculosis. *Clin Infect Dis* 1994;**19**:15–20.
35. Von Reyn CF, Williams DE, Horsburgh Jr CR, Jaeger AS, Marsh BJ, Haslov K, et al. Dual skin testing with *Mycobacterium avium* sensitin and purified protein derivative to discriminate pulmonary disease due to *M. avium* complex from pulmonary disease due to *Mycobacterium tuberculosis*. *J Infect Dis* 1998;**177**:730–6.
36. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. *Am J Respir Crit Care Med* 1999;**159**:15–21.
37. Barnes PF. Diagnosing latent tuberculosis infection: the 100-year upgrade. *Am J Respir Crit Care Med* 2001;**163**(4):807–8 (2001;**117**:807–8).
38. Chin DP, Osmond D, Page-Shafer K, Glassroth J, Rosen MJ, Reichman LB, et al. Reliability of anergy skin testing in persons with HIV infection. The pulmonary complications of HIV infection study group. *Am J Respir Crit Care Med* 1996;**153**:1982–4.
39. Munk ME, Kern P, Kaufmann SH. Human CD30+ cells are induced by *Mycobacterium tuberculosis* and present in tuberculosis lesions. *Int Immunol* 1997;**9**:713–20.
40. Lein AD, Von Reyn CF. In vitro cellular and cytokine responses to mycobacterial antigens: application to diagnosis of tuberculosis infection and assessment of response to mycobacterial vaccines. *Am J Med Sci* 1997;**313**:364–71.
41. Sester M, Sester U, Clauer P, Heine G, Mack U, Moll T, et al. Tuberculin skin testing underestimates a high prevalence of latent tuberculosis infection in hemodialysis patients. *Kidney Int* 2004;**65**:1826–34.
42. Guidelines for using the Quantiferon -TB test for diagnosing latent *Mycobacterium tuberculosis* infection http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5202a2.htm. Centers for disease control and prevention . 31-1-2003.
43. Fietta A, Meloni F, Cascina A, Morosini M, Marena C, Troupioti P, et al. Comparison of a whole-blood interferon-gamma assay and tuberculin skin testing in patients with active tuberculosis and individuals at high or low risk of *Mycobacterium tuberculosis* infection. *Am J Infect Control* 2003;**31**:347–53.
44. Pottumarthy S, Morris AJ, Harrison AC, Wells VC. Evaluation of the tuberculin gamma interferon assay: potential to replace the Mantoux skin test. *J Clin Microbiol* 1999;**37**:3229–32.
45. 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.

46. Desem N, Jones SL. Development of a human gamma interferon enzyme immunoassay and comparison with tuberculin skin testing for detection of *Mycobacterium tuberculosis* infection. *Clin Diagn Lab Immunol* 1998;**5**:531–6.
47. Mazurek GH, LoBue PA, Daley CL, Bernardo J, Lardizabal AA, Bishai WR, et al. Comparison of a whole-blood interferon gamma assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. *J Am Med Assoc* 2001;**286**:1740–7.
48. Mazurek GH, Villarino ME. Guidelines for using the QuantiFERON-TB test for diagnosing latent *Mycobacterium tuberculosis* infection. Centers for disease control and prevention. *MMWR Recomm Rep* 2003;**52**:15–8.
49. Arend SM, Ottenhoff TH, Andersen P, van Dissel JT. Uncommon presentations of tuberculosis: the potential value of a novel diagnostic assay based on the *Mycobacterium tuberculosis*-specific antigens ESAT-6 and CFP-10. *Int J Tuberc Lung Dis* 2001;**5**:680–6.
50. 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.
51. van Pinxteren LA, Ravn P, Agger EM, Pollock J, Andersen P. Diagnosis of tuberculosis based on the two specific antigens ESAT-6 and CFP10. *Clin Diagn Lab Immunol* 2000;**7**:155–60.
52. Bellete B, Coberly J, Barnes GL, Ko C, Chaisson RE, Comstock GW, et al. Evaluation of a whole-blood interferon-gamma release assay for the detection of *Mycobacterium tuberculosis* infection in 2 study populations. *Clin Infect Dis* 2002;**34**:1449–56.
53. Converse PJ, Jones SL, Astemborski J, Vlahov D, Graham NM. Comparison of a tuberculin interferon-gamma assay with the tuberculin skin test in high-risk adults: effect of human immunodeficiency virus infection. *J Infect Dis* 1997;**176**:144–50.
54. Kimura M, Converse PJ, Astemborski J, Rothel JS, Vlahov D, Comstock GW, et al. Comparison between a whole blood interferon-gamma release assay and tuberculin skin testing for the detection of tuberculosis infection among patients at risk for tuberculosis exposure. *J Infect Dis* 1999;**179**:1297–300.
55. Lalvani A, Nagvenkar P, Udwadia Z, Pathan AA, Wilkinson KA, Shastri JS, et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. *J Infect Dis* 2001;**183**:469–77.
56. Harboe M, Oettinger T, Wiker HG, Rosenkrands I, Andersen P. Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. *Infect Immun* 1996;**64**:16–22.
57. Pollock JM, Andersen P. The potential of the ESAT-6 antigen secreted by virulent mycobacteria for specific diagnosis of tuberculosis. *J Infect Dis* 1997;**175**:1251–4.
58. Liu XQ, Dosanjh D, Varia H, Ewer K, Cockle P, Pasvol G, et al. Evaluation of T-cell responses to novel RD1- and RD2-encoded *Mycobacterium tuberculosis* gene products for specific detection of human tuberculosis infection. *Infect Immun* 2004;**72**:2574–81.
59. Lalvani A, Pathan AA, Durkan H, Wilkinson KA, Whelan A, Deeks JJ, et al. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Lancet* 2001;**357**:2017–21.
60. Berthet FX, Rasmussen PB, Rosenkrands I, Andersen P, Gicquel B. A *Mycobacterium tuberculosis* operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10). *Microbiology* 1998;**144**(Pt 11):3195–203.
61. T Spot TB—Oxford Immunotec Limited http://www.oxfordimmunotec.com/products.htm. 2004.
62. ELISpot—Assay Procedure—Mabtech AB http://www.mabtech.com/elispot.asp. 2004.
63. Clinicians Guide to Quantiferon-TB Gold www.cellestis.com/IRM/contentAU/gold/cliniciansguide.pdf. 2004.
64. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 2003;**361**:1168–73.
65. Richeldi L, Ewer K, Losi M, Bergamini BM, Roversi P, Deeks J, et al. T Cell-based tracking of multidrug resistant tuberculosis infection following brief exposure. *Am J Respir Crit Care Med* 2004.
66. Brock I, Weldingh K, Lillebaek T, Follmann F, Andersen P. Comparison of a new specific blood test and the skin test in tuberculosis contacts. *Am J Respir.Crit Care Med* 2004.
67. Shams H, Weis SE, Klucar P, Lalvani A, Moonan PK, Pogoda JM, et al. Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis Infection. *Am J Respir Crit Care Med* 2005.
68. Hill PC, Brookes RH, Fox A, Fielding K, Jeffries DJ, Jackson-Sillah D, 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.
69. Kang YA, Lee HW, Yoon HI, Cho B, Han SK, Shim YS, 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. *J Am Med Assoc* 2005;**293**:2756–61.
70. Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K, 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.
71. Harada N, Mori T, Shishido S, Higuchi K, Sekiya Y. [Usefulness of a novel diagnostic method of tuberculosis infection, QuantiFERON TB-2G, in an outbreak of tuberculosis]. *Kekkaku* 2004;**79**:637–43.
72. Chapman AL, Munkanta M, Wilkinson KA, Pathan AA, Ewer K, Ayles H, 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.
73. Ravn P, Munk ME, Andersen AB, Lundgren B, Nielsen LN, Lillebaek T, et al. Reactivation of tuberculosis during immunosuppressive treatment in a patient with a positive QuantiFERON-RD1 test. *Scand J Infect Dis* 2004;**36**:499–501.
74. Pai M, Lewinsohn DM. Interferon-{gamma} assays for tuberculosis: is anergy the achilles' heel? *Am J Respir Crit Care Med* 2005;**172**:519–21.
75. Ferrara G, Losi M, Meacci M, Meccugni B, Piro R, Roversi P, 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**:631–5.
76. Hill PC, Jackson-Sillah D, Fox A, Franken KL, Lugos MD, Jeffries DJ, et al. ESAT-6/CFP-10 fusion protein and peptides for optimal diagnosis of *Mycobacterium tuberculosis* infection by ex vivo enzyme-linked immunospot assay in the Gambia. *J Clin Microbiol* 2005;**43**:2070–4.
77. Barnes PF. Diagnosing latent tuberculosis infection: turning glitter to gold. *Am J Respir Crit Care Med* 2004;**170**:5–6.
78. In-Tube Method for QuantiFERON-TB Gold http://www.cellestis.com/IRM/contentAU/gold/virode_introINTUBE.html. 2005.