ARTICLE

Skin testing for latent *Mycobacterium tuberculosis* infection has long been regarded as an inexact science. Now, guidance from the UK, Europe and the USA recommends that a simple blood test should all but replace the time-honoured but flawed procedure. Here, Chris Granger and Peter Davies review the evidence.

New tests for identifying tuberculosis infection

The tuberculosis species complex, comprising *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*, *M. microti* and *M. canettii*, all cause tuberculosis, but *M. tuberculosis* is the primary pathogen in humans.[1] The consequences of infection will vary depending on the host immune response, which is affected by factors such as age (children <4 years old are at high risk), underlying immune status, coexisting diseases and/or malnutrition, intake of corticosteroids and other immunosuppressive drugs, immunisation with the *M. bovis* Bacillus Calmette-Guérin (BCG) vaccine, the virulence of the organism, and the site of infection.[2]

In most infected individuals, immunological control contains the organism and thus the individuals have asymptomatic latent tuberculosis infection. If the immunological mechanisms fails to control the organism then typical tuberculosis (TB) symptoms occur,[2] including fever, loss of appetite, weight loss, weakness, night sweats, malaise, increased peripheral blood leucocyte count and anaemia. Symptoms specific to the site of infection also occur, so, for example, cough, purulent sputum production and chest pain are associated with pulmonary tuberculosis.

It is estimated that active disease will develop in about 10% of those who have latent tuberculosis infection over the period of a lifetime (with the risk greatest in the first two years); thus, treatment of latent disease is considered a necessary step in the elimination of tuberculosis.[2,3]

Around a third of the world's population is thought to be infected with *M. tuberculosis*.[2] The prevalence of the disease is thought to be increasing,[4] mainly due to the increased global incidence of human immunodeficiency virus (HIV) infection.[2,4] HIV-infected individuals are about 60 times more likely to develop tuberculosis than are immunocompetent individuals in industrialised countries, and six times more likely in other countries.[4] Latent TB infection progresses to active disease in most patients with concomitant HIV infection, and the presence of HIV infection also speeds the progression of active disease.[4,5]

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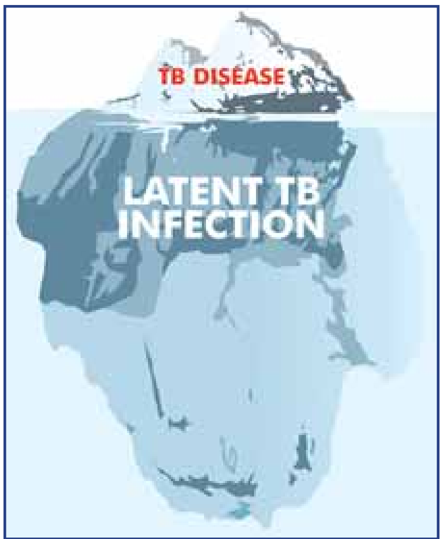
Tuberculosis is most prevalent in developing countries, but immigration is now causing an increase in the prevalence of the disease in some low-incidence countries.[2,4,6] Early detection and treatment of latent TB infection will result in significant health and economic benefits in industrialised countries.[7]



Tubercular disease is fuelled by a vast reservoir of latent *M. tuberculosis* infection.

In children, the risks of progression or dissemination of the disease are increased,[8] possibly as a result of undeveloped immune systems;[9] thus, they should be targeted for accurate detection of the disease.

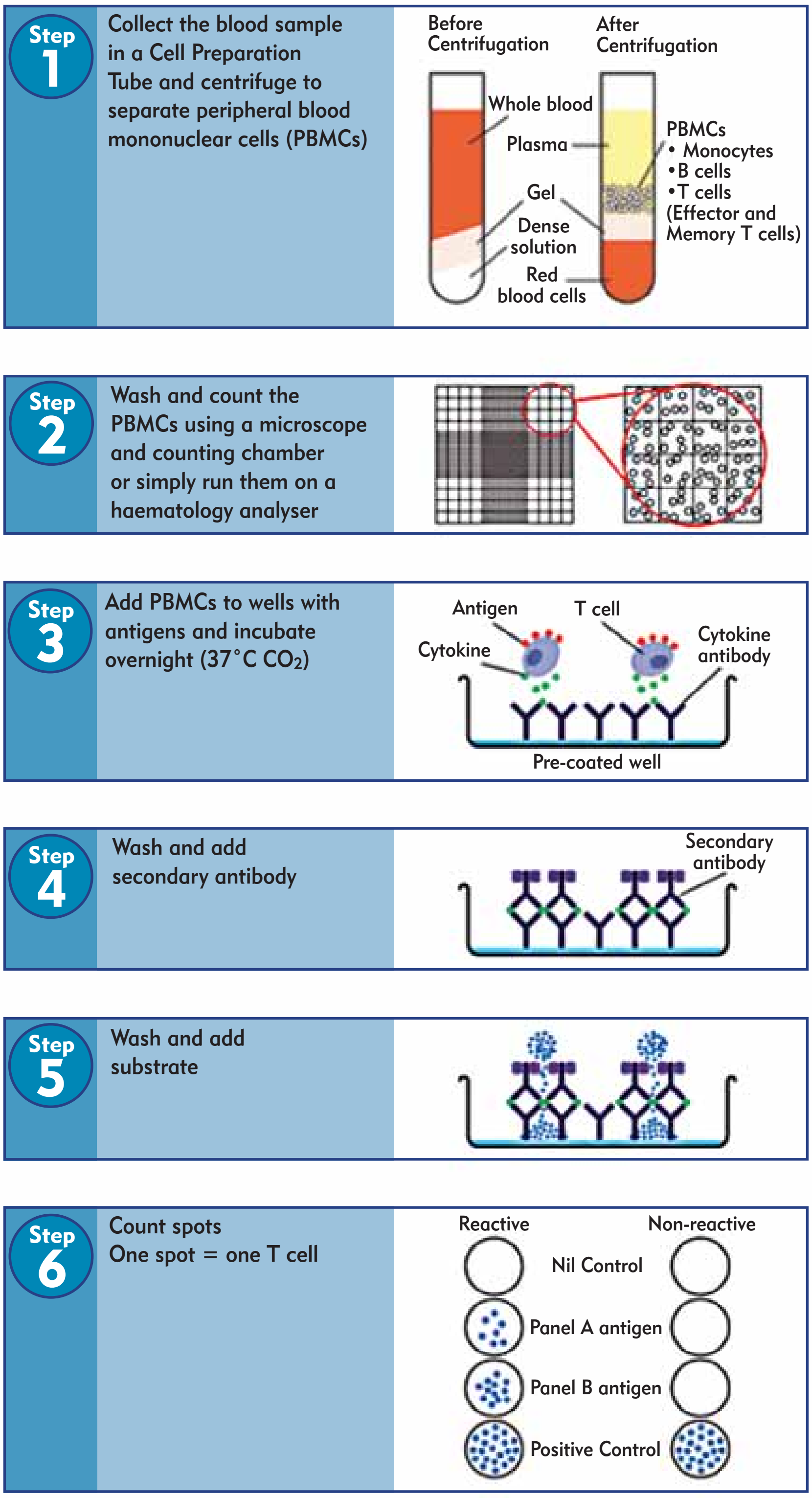
Until recently, the only method of identifying subjects infected with latent *M. tuberculosis* infection was the tuberculin (Mantoux) skin test (TST), which uses purified protein derivative (PPD), a precipitate of *M. tuberculosis* culture supernatant (containing over 200 different antigens), to produce a delayed (cellular) hypersensitivity response.[2] This PPD is injected into the skin and the extent of the subsequent skin reaction (induration) is assessed three days later.[10]

Many of the antigens in PPD occur in the widely used BCG vaccine and in common environmental mycobacteria, which can result in a false-positive TST test. Other drawbacks of the TST include the necessity for a second visit to the health clinic to assess the result (many subjects fail to return), inter-operator variation as a result of subjective assessment of the test result, the booster phenomenon in which repeated testing can result in a false-positive result, and reduced sensitivity in immunocompromised patients (eg those with HIV infection or advanced TB, transplant recipients, those taking biological cytokine-inhibiting agents such as tumour necrosis factor inhibitors for rheumatoid arthritis or other autoimmune diseases, or malnourished individuals).[10–15]

M. tuberculosis evokes a strong cell-mediated immune response in humans.[10] One of the main components of the response involves the formation of antigen-specific cytokine-secreting CD4 and CD8 T cells. Early secretory antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP 10), which are secreted proteins encoded by the *M. tuberculosis* genomic segment region of difference 1 (RD 1), are immunogenic in patients in whom *M. tuberculosis* infection has developed.[16,17]

Recently developed alternatives to the TST include assays measuring interferon-γ (IFNγ) produced by these antigen-specific T cells in response to ESAT-6/CFP 10 antigenic stimulation. These use *in vitro* enzyme-linked immunospot (ELISpot) technology, which measures the number of antigen-specific T cells, or enzyme-linked immunosorbent assay (ELISA) technology (QuantiFERON) that measures overall IFNγ production in whole blood.[11,18,19] A simplified form of the ELISpot methodology is approved for diagnostic use across Europe and is known as T-SPOT.*TB*.

The T-SPOT.*TB* enzyme-linked immunospot test comprises six straightforward steps.



Principles of the T-SPOT.*TB* test

Peripheral blood mononuclear cells (PBMC) comprising antigen-presenting cells and CD4+ and CD8+ T cells are placed in the wells of a microtitre plate. *M. tuberculosis*-specific peptides ESAT-6 and CFP 10[20] are added to the plate. T cells that recognise these peptides respond by producing cytokines, including IFNγ.[10] Anti-IFNγ antibodies capture the released IFNγ.[10] Results are available the following day, with each resultant spot, revealed by the addition of a substrate, corresponding to the specific reaction of an individual T cell.

Infection with *M. tuberculosis* is indicated if there are more than six spots relative to the negative control.[21] The highly specific antigens used in T-SPOT.*TB* are absent from most environmental mycobacteria and from the BCG vaccine.[10] T-SPOT.*TB* is capable of identifying *M. tuberculosis*, *M. bovis* and *M. africanum* infections.[21] Only three of the non-tuberculous mycobacteria (*M. marinum*, *M. szulgai*, and *M. kansasii*) contain the antigens used in T-SPOT.*TB*, and so these may give a false-positive result.

Sensitivity of the test

Overall sensitivity

Overall sensitivity of T-SPOT.*TB* test ranged from 92% to 97% in patients with confirmed active TB and mixed HIV status in three studies based in Germany, the UK, Korea and Zambia.[20,22,23]

A sensitivity rate of 97% was seen in the German study of 72 adults with confirmed active tuberculosis, 48% of whom had concomitant immunosuppressive conditions, including 2% with HIV infection.[22] Two patients with culture-confirmed *M. tuberculosis* pulmonary disease in this study had spot counts below the cut-off level for T-SPOT.*TB*. These two patients had been receiving chemotherapy for tuberculosis, in one case for over four years and in the other for 30 days, which may account for the negative results. Positive T-SPOT.*TB* results are known to decline with therapy as the numbers of *M. tuberculosis* bacilli present in the body decline.[22]

An overall sensitivity of 92% was seen in 50 adult Zambian patients (78% HIV-positive) with active tuberculosis diagnosed by clinical symptoms, radiography or sputum microscopy, all of whom were untreated or had received treatment for less than a month.[20]

Comparative sensitivity with other tests

T-SPOT.*TB* was seen to be more sensitive than TST in patients with confirmed

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tuberculosis.[22,24] In a subgroup of 45 adults with confirmed tuberculosis who received both tests in the German study, all were positive for T-SPOT.*TB* (100% sensitivity), but five (all with pulmonary tuberculosis) were negative by TST (89% sensitivity).[22]

A positive RD1 test (using ESAT-6) was significantly more predictive of future reactivation of tuberculosis than was a positive TST in a study of healthy household contacts of TB patients in Ethiopia.[25] In a study in Korea of 87 subjects with active TB (55 confirmed by culture, the remainder by clinical diagnosis), 66.7% were positive by TST, 70.1% by QuantiFERON Gold and 95.4% by T-SPOT.*TB*.[24] In a study in Rome, T-SPOT.*TB* showed a sensitivity of 91% in culture-confirmed subjects compared to a sensitivity of 83% with QuantiFERON Gold.[25]

Effect of immunosuppression

In contrast to TST, the sensitivity of T-SPOT.*TB* was not significantly affected by immunological status, age or nutritional status of children in a South African study.[23]

All 11(100%) HIV-negative and 35 out of 39 (90%) HIV-positive patients in the Zambian trial had a positive response with T-SPOT.*TB*.[20] As the diagnosis of active TB was uncertain in one of the four HIV-positive patients who did not respond to any of the peptides in the assay, the sensitivity in HIV-positive patients may have been 92% (94% overall). In the Korean study, 29 of the active TB patients were also immunosuppressed. In this subgroup the sensitivity by TST was 34.5%, by QuantiFERON Gold was 62.1% and by T-SPOT.*TB* was 96.6%.[24]

Specificity of the test

One hundred percent specificity was demonstrated for T-SPOT.*TB* in 40 healthy, low-risk adult subjects (83% BCG vaccinated) based in the UK.[20,26,27] A specificity rate of 100% was also seen in another UK-based study of 18 healthy, low-risk HIV- negative adult subjects.[28]

Of 14 patients with non-tuberculous lung disease investigated in another trial,[22] three reacted positively to both T-SPOT.*TB* and TST. Subsequently, two were found to have non-tuberculous *M. kansasii* infection. Three patients who were negative for both T-SPOT.*TB* and TST were found to have *M. xenopii* ($n=2$) or *M. avium* ($n=1$) infection and two who were T-SPOT.*TB*-negative and TST-positive had no signs of active tuberculosis.

Clinical use of the test

Contact-tracing studies using T-SPOT.*TB* have investigated contacts of index cases in a UK school,[29] a maternity unit in Italy,[30] a residential institution for alcoholics in Switzerland[31] and in children in Turkey.[33]

T-SPOT.*TB* and TST were used to screen 535 school contacts of a UK student diagnosed with pulmonary tuberculosis after having a chronic cough for nine months.[29] The correlation between a positive result and degree of exposure to the index case was significantly greater for T-SPOT.*TB* than for TST across the four stratified groups ($P<0.05$).[29]

After spending four days in a maternity unit in Italy, a woman was diagnosed with sputum smear-positive multidrug-resistant TB, resulting in possible nosocomial exposure to 41 infants and 47 adults, and nosocomial and household exposure to four additional adults.[30] These contacts were screened using T-SPOT.*TB* and TST tests 11 weeks after exposure. Of the 92 participants in this study, 17 responded positively to T-SPOT.*TB* and four to TST. All neonates had evaluable results in T-SPOT.*TB*. The odds of a positive result across the four stratified groups increased significantly for T-SPOT.*TB* ($P<0.05$), but there was no significant correlation for TST.[30]

T-SPOT.*TB* and TST were compared in 50 residents and 41 staff after a month's exposure to an index case with sputum smear-positive pulmonary TB in a residential institution for alcoholics in Switzerland.[31] Positive T-SPOT.*TB* or TST results were seen in 15% and 44% of participants, respectively. Eleven subjects were positive to both tests, three were positive only to T-SPOT.*TB* and 29 were positive only to TST. When the contacts were stratified by low or high exposure to the index case, the results of T-SPOT.*TB* were correlated significantly with exposure ($P<0.05$), but there was no such correlation with TST.[31]

In all three studies, the response to TST, but not to T-SPOT.*TB*, was strongly linked with the prevalence of BCG vaccination.[29–31]

In another community-based study, the prevalence of TB infection was assessed in 979 Turkish children aged under 17 years (median 7 years; 79% BCG vaccinated) who were household contacts of 414 adults with sputum smear-positive pulmonary TB.[33] T-SPOT.*TB* and TST showed positive results in 43% and 51% of cases, respectively. Of the 13 children with active tuberculosis at enrolment, 12 were positive by T-SPOT.*TB* and 11 were positive by TST.

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Risk factors for *M. tuberculosis* infection in this study that were significantly associated with positive results for both T-SPOT.*TB* and TST after multivariate analysis included an increasing number of index patients in the household, being the child of an index patient, and increasing age.[33] The only risk factor differentiating the tests was the absence of a BCG scar, which was significantly associated with a positive T-SPOT.*TB* result ($P<0.0001$).[3]

Prospects for the test

The studies referred to above indicate some of the many advantages of T-SPOT.*TB* when used to identify latent TB infection contacts and for targeted testing of at-risk groups, particularly children and the immunocompromised. The high sensitivity of the test means that it can be used to rule out active disease in TB suspects. ■

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Guidelines on TB testing

A number of countries have now published guidelines for *M. tuberculosis* (TB) testing. The National Institute for Health and Clinical Excellence (NICE) published its guidelines for TB control in England and Wales on 22 March and the full guidelines entitled *Tuberculosis: clinical diagnosis and management of tuberculosis, and measures for its prevention and control* are available online at www.nice.org.uk/.

The NICE guidelines recommend use of blood tests as the frontline test for latent TB infection in preference to the skin test in all immunocompromised patients. The guidelines also recommend that blood tests should be used as a confirmatory test in all cases where the tuberculin skin test (TST) is positive. In this case, the blood test is used as a means of screening out false-positive TST results. NICE also believes that blood tests have a role to play in the diagnosis of TB disease, especially in non-pulmonary TB and as a rule-out test in TB suspects.

Interestingly, the Swiss Pulmonology League Working Group has also recommended confirming positive TST results with a blood test and also recommends the use of blood tests as the frontline procedure for immunosuppressed subjects.

As part of its new guidelines, The Centers for Disease Control and Prevention (CDC) in the USA offers more straightforward advice. It states that a simple blood test should be used in all circumstances under which the traditional TST is currently used.

Chris Granger is director of global professional relations at Oxford Immunotec. Professor Peter Davies is a consultant chest physician at the Cardiovascular Centre in Liverpool.
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Further information on TB-SPOT.*TB* is available from Barbara Maniglia at Oxford Immunotec (tel +44 [0]1235 433170) or on the company's website (www.oxfordimmunotec.com).