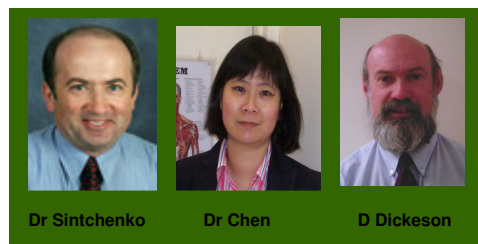


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Is it Time to Replace the Tuberculin Skin Test with Interferon-gamma Release Assays?

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Background

Tuberculosis (TB) is a major global infectious disease with an estimated 2 million deaths pa. In low prevalence countries, case detection (TB disease) as well as identification and treatment of individuals with latent tuberculosis infection (LTBI) is central to TB control and elimination. Yet, detection of active TB and LTBI remains problematic. Even with advanced microbiological tools, only 50-70% of active TB cases are bacteriologically-confirmed.

Alternate diagnostic approaches have focussed on the detection of *Mycobacterium tuberculosis*-specific cell-mediated immune responses in infected individuals, based on the premise that most (>90%) persons with TB infection develop protective immunity to *M. tuberculosis* and remain asymptomatic. Several cytokines, in particular interferon gamma (IFN- γ), play a key role in this protective immune response. The tuberculin skin test (TST), which detects delayed-type hypersensitivity to purified protein derivative prepared from *M. tuberculosis*, has long been used as a diagnostic and screening tool. However, it is insufficiently sensitive, has limited predictive value for TB disease and LTBI, and is intrinsically confounded by cross-reactive immune responses after vaccination with bacillus Calmette-Guerin (BCG) strains or infection with non-tuberculous mycobacteria (NTM).

Interferon-gamma release assays

To overcome problems with the TST, novel *in vitro* IFN- γ release assays (IGRAs), highly specific for *M. tuberculosis* have been developed. These new blood tests are widely-anticipated to eventually replace the TST for LTBI testing. Two commercial assays are available:

- T-SPOT.*TB* (Oxford Immunotec, Abingdon, UK)
- QuantiFERON-TB Gold In-Tube (QFT-IT; Cellestis Limited, Carnegie, Australia)

Both measure IFN- γ responses to *M. tuberculosis*-specific antigens but exploit different technologies - T-SPOT.*TB* is based on enzyme-linked immunospot methodology that enumerates IFN- γ secreting T-cells specific for early secretory antigen target 6 (ESAT-6), culture filtrate protein 10 (CFP-10, while the QFT-IT test measures IFN- γ production in whole blood in response to TB 7.7(p4) antigen, in addition to ESAT-6 and CFP-10.

The T-SPOT.*TB* requires the incubation of patient peripheral blood mononuclear cells (PBMC) in the presence of TB antigens using standardised numbers of PBMC in each test well. In the QFT-IT assay, stimulation of T-cell IFN- γ response in whole blood is carried out: 1 mL of blood is collected into each of 3 tubes pre-coated with *M. tuberculosis* antigens, "nil control" (no antigen) and "Mitogen", respectively (Figure 1).

Specimens should reach the laboratory within 16 h of collection. Incubation followed by centrifugation are time-critical steps; however, IFN- γ detection is time flexible and can be delayed up to 4 weeks. The "nil" control is required to adjust for background "noise" and for non-specific IFN- γ in blood. "Indeterminate" results occur in 3-10% cases and may be due to technical factors or functional T-cell defects in severely ill patients.

General advantages of IGRAs

Apart from their operational advantages (objective measurement of IFN- γ , requirement for only a single patient visit) over the TST, IGRAs have been shown to demonstrate superior specificity (approaching 100%) for TB diagnosis and screening. However, most clinical trials were performed in countries with a high TB burden and may have overestimated the accuracy of TB detection. Despite the variation in the sensitivity and specificity of IGRAs in different studies - possibly due to variations in disease severity and/or specific assay formats - IGRAs have been advocated as useful adjunctive tools for diagnosis of active TB and LTBI, investigating recent exposure and assessing the response to TB treatment.

Clinical uses of IGRAs

1. Laboratory diagnosis of symptomatic tuberculosis

IGRAs are a helpful aid for diagnosing symptomatic TB. The only systematic review of their use in this context has revealed that they are superior to the TST, for reasons outlined above, with higher specificity and better correlation with exposure to TB. More recent surveys, have shown pooled specificity rates for TB diagnosis of 97% and 93% for the QFT-IT and T-SPOT.*TB* tests, respectively.

The T-SPOT.*TB* and QFT-IT assays have at least equivalent sensitivities to the TST in patients with culture-confirmed TB (80%). Two large studies addressing the utility of the QFT-IT test reported sensitivities of 83-89% among patients with pulmonary TB and unselected patients with confirmed TB. However, though IGRAs have modest-good sensitivity in detection of symptomatic pulmonary TB, there are still insufficient data to assess their utility in extrapulmonary TB, TB in children or in immunocompromised patients, where the sensitivity appears to be lower. The results of test performance in HIV/AIDS patients are conflicting. Sensitivity of the assays may also be affected by ethnicity; both IGRAs were significantly less sensitive in Indian and Malay patients compared with ethnic Chinese and in non-Hispanic white Caucasians. The few data available suggest there is no correlation between bacterial load and the measured T-cell response.

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2. Detection of latent infection (LTBI)

Given that untreated LTBI represents a major source of life-threatening disease, identification of persons with LTBI is critical for TB control.

Indeed, initial research in the development of IGRAs focussed on improving the diagnosis of LTBI although it should be emphasised that IGRAs cannot distinguish active, from latent TB. Accurate evaluation of the T-SPOT.TB and QFT-IT assays in detecting LTBI has been hampered by the lack of a diagnostic "gold standard". A small number of studies report sensitivities of up to 80% with specificities of (94-100%), with better correlation with TB exposure and less cross-reactivity with BCG strains and NTM than the TST. However, indeterminate results, such as in immunosuppressed individuals, the severely-ill and elderly, have been noted and the performance of IGRAs in these patient population warrants further study to determine whether the tests provide clinically useful information. The reactivation of TB in the setting of severe immunocompromise eg. use of tumour necrosis factor inhibitor therapy, also requires consideration. Many clinicians recommend screening for LTBI prior to initiation of such therapies. IGRAs offer significant advantages over the TST, especially in BCG-vaccinated, and in persons born in TB-endemic countries.

3. Investigation of recent exposure

Since IGRAs correlate well with exposure to TB infection and have given uniformly negative results in unexposed control individuals, they are an attractive alternative to the TST for contact tracing purposes.

In the absence of a standard method for LTBI detection, most studies have utilised an exposure gradient to the infectious source case in contact investigations or, active TB as surrogate markers for LTBI. Using the exposure gradient approach, the T-SPOT.TB assay, was found to have superior correlation compared with the TST in point-source contact tracing in low incidence settings. Where newly diagnosed active TB has been used as the surrogate, meta-analyses have shown a pooled sensitivity of 76%, 88% and 70% for the QFT-IT, T-SPOT.TB and TST, respectively. More recently, both IGRAs were compared with the TST in a prospective study of 601 immunocompetent contacts of TB patients, revealing a significantly higher predictive value for development of active TB following exposure as measured by IGRAs. The progression rate for those who were IGRA-positive was almost 15% compared with 2.3% for the TST. Although the TST identified 243 contacts as candidates for chemoprophylaxis (66 contacts identified by the IGRA), it failed to identify 1 of 6 contacts who developed active TB.

The US Centers for Disease Control and Prevention (CDC) currently recommends that IGRAs may be used in all circumstances in which the TST has been used, including in contact tracing investigations, evaluation of TB exposure in recent immigrants and for employment screening and surveillance programs in hospitals. However, the British Royal College of Physicians caution that IGRAs should be considered for diagnosing LTBI only when necessary to confirm positive TST results or when the TST may not be reliable.

4. Monitoring of treatment response of TB or LTBI

It would be useful to be able to rely on a simple test as a marker of cure for TB. Results of studies (all using T-SPOT.TB) assessing the effect of TB treatment on IFN- γ response have, however, been conflicting – at least 2, but not all, studies showed that the response to ESAT-6 decreased with TB treatment.

There are also no means to ascertain the success (or otherwise) of LTBI treatment, or to predict those who have a higher risk of progression to active TB. Few studies have addressed the effect of LTBI treatment on host IFN- γ responses. The only published study reports a significant but incomplete reduction in T-cell response using the T-SPOT.TB assay (reversion to negative results in 37.6% patients); however, there was a statistically significant change in the response only to CFA-10 and not to ESAT-6. There is no information on the impact of TB chemoprophylaxis on the results of the QFT-IT assay.

Conclusion

IGRAs are useful adjuncts to culture and molecular-based laboratory tests for diagnosing TB disease and LTBI, for screening patients prior to immunosuppression who may benefit from chemoprophylaxis against TB and to assess recent TB exposure. In these settings, both the TSPOT.TB and QFT-IT assay provide at least the same sensitivity as the TST test and offers superior specificity. At the CIDMLS, the QFT-IT assay has been used since 2005 for these purposes. The QFT-IT costs AUD 29 per test. The TSPOT.TB test is marketed in the UK and Europe and Asia for similar indications but not in Australia. This assay is associated with a cost of AUD 54 per test.

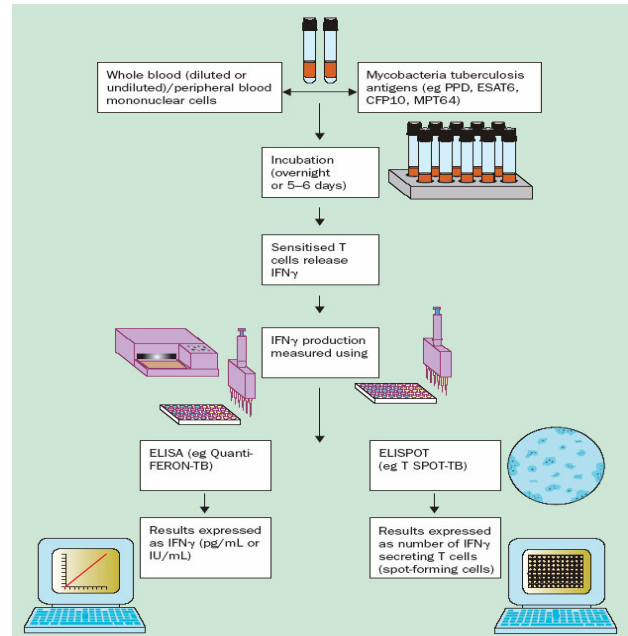


Figure 1. Principles and steps of the QFT-IT or T-SPOT.TB assay. Pai et al, Lancet Infectious Diseases 2004;4: 761-76

Further reading:

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