Optimising pre-analytical performance of interferon-γ release assays for TB exposure

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Interferon-γ release assays (IGRAs) have been developed in the last decade to screen for latent *Mycobacterium tuberculosis* (TB) infection. They are aimed at, and have been optimised for use with, contacts of people with active pulmonary TB. These contacts may themselves develop active tuberculosis, but more often they can become infected without developing any symptoms (latent infection). Prior to the introduction of IGRAs, screening for latent infection relied on the tuberculin skin test, requiring intradermal injection of purified protein derivative (derived from TB) and a repeat visit a few days later.

National Institute for Health and Clinical Excellence guidelines recommend the use of an IGRA in selected contacts of active TB patients.[1] Of the two commercially available IGRA assays, we chose to implement QuantiFERON-TB Gold (Cellestis Europe, Darmstadt, Germany), as it proved suitable for scaling up testing in an outbreak of TB during our initial assessment (data not shown). The assay measures the release of interferon-γ in whole blood in response to stimulation by the mycobacterial peptides ESAT-6, TB7.7 and CFP-10. The samples are incubated as soon as possible after receipt and then the whole blood is centrifuged and the plasma collected for interferon-γ measurement.

The QuantiFERON-TB Gold assay incorporates a positive and negative control. The positive control tube contains a mitogen (phytohaemagglutinin-P), which stimulates T lymphocytes in a non-antigen-specific manner. A low signal is obtained if technical problems or biological factors prevent secretion of interferon-γ by T lymphocytes, and this is reported as an indeterminate result. One cause of a low signal is failure to incubate the sample within 16 hours of venepuncture. An indeterminate result usually requires the patient to have a repeat blood test, which causes treatment delay and unnecessary distress.

Here, we describe our experience of using this assay over a four-year period between 2005 and 2009, and report factors influencing performance of the assay as estimated by the indeterminate rate. The rate of indeterminate results in our laboratory depended on a number of variables, and through the identification and targeting of these variables we aim to improve the quality of our service delivery.

Data were collected for 2276 consecutive samples, and the overall rate of indeterminate results was 5.9%. While the majority (60.3%) were sent to assess close contacts for latent TB infection, a substantial proportion (24.4%) was sent by clinicians attempting to make a diagnosis of active TB. We found that the indeterminate rate was higher when the diagnosis of active TB was sought, in comparison to individuals tested for either contact tracing or because they had recently arrived to the UK from a country where TB prevalence is high (Fig. 1). In Figure 1, the histogram labelled 'for immune suppression' includes patients with rheumatological disease or inflammatory bowel disease who are being assessed for latent TB prior to starting biological therapies that are know to reactivate TB. Many of these patients will be receiving corticosteroids or other immunosuppressive drugs.

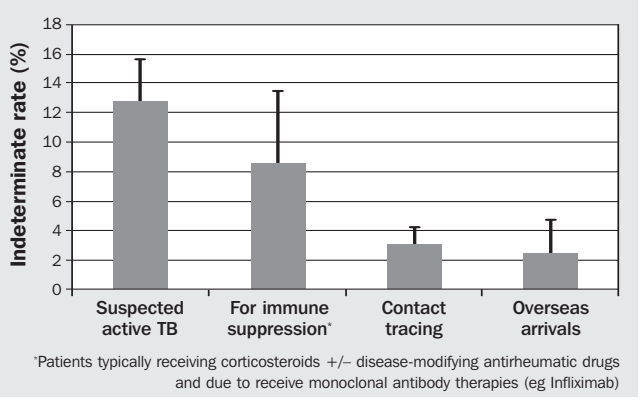

Fig. 1. A comparison of diagnostic group versus indeterminate rate. The x-axis shows the reason the test was requested. The y-axis shows the proportion with indeterminate results. Error bars indicate 95% confidence intervals.

Our laboratory provides a regional service to institutions across north-west England. Some send samples to the regional laboratory for incubation, while others incubate locally, centrifuge and send plasma samples for interferon-γ testing. Delays in transportation, or transportation under inappropriate conditions, can affect the viability of samples. When distant laboratories simply relay samples (without processing), the indeterminate rates are significantly higher (6.4% versus 3.3%, $P=0.005$ [χ^2], Fig. 2). However, when distant laboratories incubate and centrifuge samples locally, indeterminate rates are not significantly different from those obtained by the testing centre (2.0% versus 3.3%, $P=0.28$ [χ^2]).

The assay is sensitive to the volume of blood collected. Our data indicate that as the magnitude of the overfilling error increases the rate of indeterminate results also rises (Fig. 3).

Analysis of our data confirmed previous findings that indeterminate rates are higher among patients receiving immunosuppressive therapy (11.5% versus 3.7%, $P<0.001$

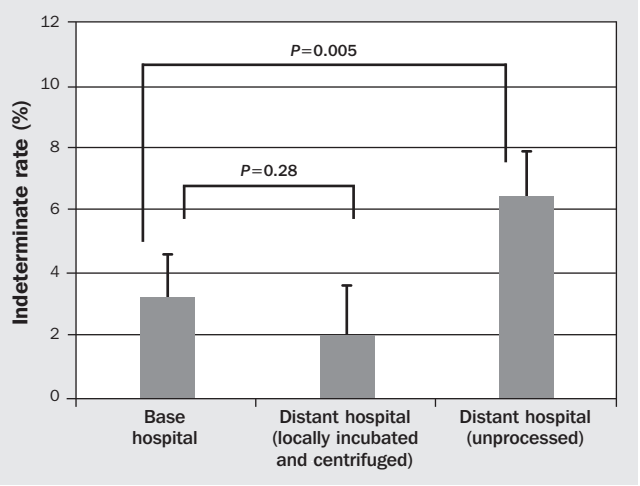

Fig. 2. Indeterminate rate caused by sample-handling errors, collection errors or incubation location.

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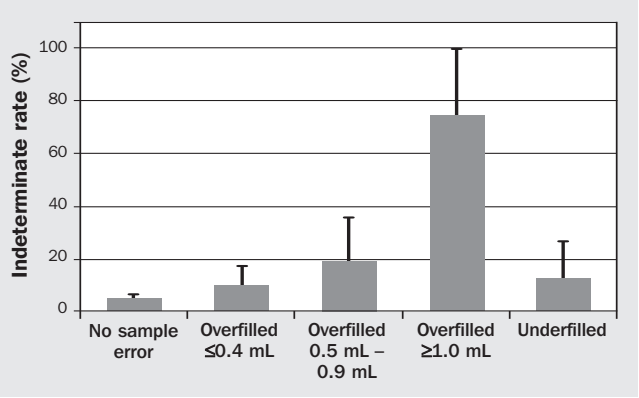

Fig. 3. Indeterminate rate caused by filling errors.

[Fisher's Exact test]) or with human immunodeficiency virus (HIV) infection (21.0% versus 3.7%, $P<0.001$ [Fisher's Exact test]).[2,3] In HIV-infected people, indeterminate rates were higher in those with a CD4 count <200 cells/μL, although these results did not achieve statistical significance (23.1% versus 10.5%, $P=0.35$ [Fisher's Exact test]). Previous studies have shown that, in HIV infection, lower CD4 counts are associated with a higher rate of indeterminate results.[4,5]

Our data confirm[6] that the QuantiFERON-TB Gold assay can have a very low indeterminate rate (2–3%). Simple measures can be taken to ensure the test performs optimally. We recommend that laboratories offering IGRA testing engage with users to ensure they understand the limitations of testing in those with possible symptomatic TB infection or when immunocompromised.

Samples should be collected correctly. Laboratories remote from the testing centre may obtain improved results if they incubate and centrifuge their samples prior to sending separated plasma. □

References

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