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Interferon-gamma release assays (IGRAs) in Japan:

QuantiFERON®-TB and T-SPOT®.*TB*

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Japan has advanced tuberculosis (TB) control measures in place and actively screens at-risk populations for both active and latent TB infection (LTBI). Historically, Japan has used the tuberculin skin test for LTBI screening, but due to the heavy use of BCG vaccination this test suffers from poor specificity in Japan. The advent of interferon-gamma release assays (IGRAs), which are unaffected by BCG vaccination, has led to significant improvements in the way LTBI is diagnosed.

There are two different IGRAs approved for use in Japan: QuantiFERON-TB was first approved in 2005 and the In-Tube version (QFT) in 2009 and: T-SPOT.*TB* (T-Spot) in 2012. While these two tests are commonly referred to as IGRAs, it is important to note that they have significant differences in the way IFN- γ is measured, the assay principles, interpretation, costs and labour requirements and clinical performance.

QFT employs an ELISA to measures the amount of IFN- γ released by lymphocytes in a 1mL whole blood culture, whereas T-Spot uses and ELISpot assay to measure the number of cells producing IFN- γ in a purified lymphocyte culture. Differences in results may occur if only a few cells are producing a large amount of IFN- γ (potentially QFT positive but T-Spot negative) or alternatively many cells are producing only small amounts of IFN- γ (potentially T-Spot positive but QFT negative).

The cut-off for QFT is 0.35 IU/mL of IFN- γ in response to the TB-specific peptides. This cut-off is employed universally with only two variations: In Japan a "grey zone" between 0.1 and 0.35 IU/mL is used for people at high risk of infection with the goal of enhancing sensitivity and; in the USA a "grey zone" od 0.35 to 1.1 IU/mL is recommended when serially testing low risk persons (only), with those falling into this range followed up at a later time point.

T-Spot has different cut-offs recommended in different parts of the world. In Europe the cut-off for positivity is 6 spots or more in response to the TB-specific peptides. In Japan and the USA the cut-off is 8 spots or more for positivity and results with 5, 6 or 7 spots are deemed borderline and requiring retesting. Unfortunately, much of the published data on the performance of T-Spot has been generated using the 6 spot cut-off only and test performance estimates are generally based on these publications. Very few studies have been published using the US and Japanese cut-offs, but we would expect lower sensitivity with improved specificity along with around 5% of people requiring retesting due to a borderline result.

A further difference with T-Spot is that the test can be run using lymphocytes isolated from blood less than 6 hours old or, with the use of a reagent termed T-Cell Extend®, the test can be run with lymphocytes isolated from blood up to 32 hours old. However, we should not assume that these two variations of the same test will provide the same answers. Data from the T-Spot US Package Insert, if carefully analysed, suggests that use of the Extend reagent results in a greater than 12% loss of sensitivity and 1.6% in specificity, along with a 3.2% increase in Borderline results. Thus it may be important from a clinical perspective to know by which method the T-Spot test was performed.

QFT has the benefit of employing whole blood, thereby minimizing the amount of laboratory labour required to perform the test and thus reducing costs. T-Spot requires purification, washing and counting of lymphocytes, procedures that are labour intensive. T-Spot also requires the use of asceptic techniques, sterile culture media and a CO_2 incubator, whereas QFT requires equipment common to most testing laboratories.

QFT results are interpreted on the basis of ELISA optical densities, measures by an automated ELISA plate reader. T-Spot, however, requires the manual counting of spots using magnification. This process is subjective and studies have demonstrated that different people can obtain different results from the same assay (Franken *et al. Clin Vaccine Immunol 2009*).

Comparison of test performance for the two IGRAS is probably best performed using data from independent meta-analyses, rather than published values from the two manufacturers. However, even using such studies is inexact as few studies have reported head to head studies in the same people and for T-Spot, most published studies use the 6 spot cut-off rather than that approved in Japan. Overall, meta-analyses suggest that sensitivity for active TB is around 83% (80 to 84.5%) for QFT and 85% (81 to 88.5%) for T-Spot. Specificity is universally higher for QFT (99.4%), compared with up to 98% for T-Spot. Both tests perform significantly better than the TST.

In conclusion, IGRAs have provided much improved tools for detecting LTBI and thus enhanced TB control. However, the two approved IGRAs differ in many ways from each other and for T-Spot, results may also differ depending on which method is used to perform the test. In deciding which IGRA to use, all of the above points merit consideration.