

QUANTIFERON®-TB GOLD:

A DIAGNOSTIC ADVANCEMENT FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS

by **Nola Hitchick**

INTRODUCTION

Tuberculosis (TB) is a bacterial disease that unfortunately has not gone away. It is a notifiable disease in Australia. The total incidence in Australia in 2006 was 5.8 per 100 000 population¹. 85% of the total notifications of *Mycobacterium tuberculosis* infections occur amongst individuals born outside Australia, among whom the rate of notifications is 20.7 per 100 000 population. Indigenous Australians also experience a higher-than-average number of infections, with a notification rate of 6.6 per 100 000. These figures compare unfavourably with the rate of only 0.9 per 100 000 population among non-Indigenous Australian-born persons. Although these rates correctly indicate that the greatest rate per population is in the Northern Territory, TB remains a definite problem in New South Wales. In 2006, NSW reported the highest number of new cases (437) compared with any other State or Territory in Australia¹.

In Australia we are fortunate to have experienced fairly stable rates of *Mycobacterium tuberculosis* infection over the last 10 years². In addition, the treatment success rate remains approximately 95%¹, although there has been a significant world-wide emergence of extremely drug resistant TB (XDR-TB) strains.

TB IN THE WORKPLACE

The most recent report from the Communicable Diseases Intelligence in Australia states that one of the new major emerging populations with high incidence of TB infection is people who are currently or previously employed in the health care industry¹. In response to this statement, NSW Health issued a Policy Directive that refers to the Screening of health care workers, including community health care centres and divisions of General Practice⁴. The Policy Directive states that all newly recruited staff must have TB screening prior to

commencement of work. Current staff members must also have TB screening undertaken and regular repeat screening is required for staff working in medium and high-risk workplaces.

TRANSMISSION OF TB

Pulmonary TB accounts for approximately 60% of TB infections¹. It is expelled via droplets when an infected person coughs, sneezes or even speaks loudly, thus exposing numerous other people. Family, friends and workmates are at the greatest risk of infection. Once in the lungs, the bacteria may be killed by the person's immune system immediately and the exposed person is not infected. If the bacteria are not killed, they can cause a respiratory infection for a considerable amount of time in the absence of symptoms. This phase is referred to as "latent TB infection" (LTBI). Approximately 10% of people with LTBI develop active TB disease in their lifetime and may potentially infect others³.

This transmission cycle may be prevented at any of the above stages using various methods. Transmission via droplets may be prevented by the use of good infection control procedures, such as isolation of an infected patient in a negative air pressure room and the use of duck-bill masks for all clinical staff and patient visitors.

Contacts of TB-infected patients who do breathe in the droplets may be prevented from becoming infected by increasing vaccination protocols. The current TB vaccine, BCG, however is quite controversial with respect to its efficacy and adverse reactions, and it also interferes with interpretation of Tuberculin Skin Tests (Mantoux tests). As a result it is not now used widely for the general population, including most health care workers⁵.

Persons with LTBI may be prevented from developing active disease by adequate treatment, as recommended by NSW Health. The main issue with this method of prevention is adequate diagnosis of LTBI.

DIAGNOSIS OF LTBI

The Tuberculin Skin Test (TST)

The currently accepted method used for diagnosis of latent TB infection is the Tuberculin Skin Test (TST), previously known as the Mantoux Test. This method has been in use with minimal changes to the method or to the tuberculin since 1908. The test involves the intradermal injection into the forearm of a standardised amount of tuberculin, a purified protein derivative of *Mycobacterium tuberculosis* bacteria. If the test subject has previously been exposed to *Mycobacterium*, antigen presenting cells will induce T-lymphocyte activation resulting in cell recruitment and swelling at the infection site. After 3 days, the size of the induration is measured to obtain a positive or negative result. The test seems straight forward, but there are numerous factors that can affect the performance of the test, including:

- Test subjects often fail to return for the reading of TST responses.
- Inaccuracy of placement and measuring of induration – NSW Health has restricted the use of the test in an effort to better standardise its use. While this may be advantageous, it has resulted in waiting lists for TSTs among health care workers.
- Booster phenomenon – a TST cannot be repeated if the initial result was dubious or not completed, because the initial TST can boost the immune response and affect the result of a subsequent TST.
- False positives are obtained due to sensitisation with a number of related bacteria, such as BCG (vaccination) or non-tuberculous *Mycobacteria* (NTM), of which there are hundreds of species.
- False negatives may be obtained as a



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result of immuno-suppression (known or unknown).

- TST can cause adverse reactions in patients, especially those who have a strong immune response to tuberculin.

QUANTIFERON®-TB GOLD

In an effort to combat many of these problems, the QuantiFERON®-TB Gold assay (QFT) was developed in Victoria, Australia. This indirect assay measures the subject's blood interferon- γ (IFN- γ) levels in response to three specific TB antigens. Note that IFN- γ is a cytokine expressed by lymphocytes upon stimulation by antigens and antigen presenting cells. The three specific antigens used (ESAT-6, CFP-10 and TB7.7) are relatively specific in *Mycobacterium (M.) tuberculosis* and are not present in BCG, although cross reactions may occur with *M. kansasii*, *M. szulgai* and *M. marinum*. Because the antigens used in this assay are not present in BCG, previous vaccination will have no effect on the assay results.

Venepuncture is performed on the subject with 3 specialised tubes of blood collected. The tubes are pre-coated with antigens as follows:

- Nil control tube – no antigen coating. This sample is used to establish the subject's baseline levels of IFN- γ .
- Mitogen control tube – coated with a broad (non-specific) immuno-stimulant. This sample is used to ensure that the subject's immune system is capable of responding to stimulation.
- Antigen tube – coated with specific TB antigens (ESAT-6, CFP-10 and TB7.7). This sample is used to determine whether the subject has been previously infected with TB.

The blood is incubated in the collection tubes for 16 - 24 hours. The plasma component is then stored for batch testing of IFN- γ levels in the laboratory using an ELISA assay.

It is important to note that the magnitude of the IFN- γ level cannot be correlated to the stage or degree of infection, level of immune responsiveness or likelihood for progression to active disease. Therefore, only qualitative results are reported to the clinician. Three different result interpretations may be obtained:

- Negative – Indicates that the subject does not show any sign of virulent

TB and is at low risk of having been infected. False negative results may be observed during the very early stages of infection.

- Positive – Indicates that the subject carries virulent TB organisms and is at risk of future disease. This test should not be the sole or definitive basis for diagnosis of infection and there should be follow-up with further medical evaluation and diagnosis in conjunction with a chest clinic. False positive results may be obtained due to infection with *M. kansasii*, *M. szulgai* or *M. marinum*.
- Indeterminate – Indeterminate results are due to unexpected IFN- γ levels in either the Nil control tube or the Mitogen control tube. An indeterminate is still a clinically valuable result. It indicates that either the subject has excessive levels of circulating IFN- γ , or that there may be an underlying immune deficiency. Indeterminate results are always repeated by the laboratory prior to reporting to eliminate the possibility of assay error.

The sensitivity of the QFT assay is >90% as determined using culture confirmed active TB as the gold standard. The specificity is 99.2% using young, healthy, low-risk people who have all previously been vaccinated with BCG.³

There are numerous benefits associated with the QFT assay:

- The test is more specific and sensitive than TST and is not affected by BCG vaccination, thus reducing the labour and costs of follow-up on patients who may not actually require further testing or treatment.
- QFT requires a single contact with the test subject, enabling results to be obtained every time rather than being dependent on a second visit by the subject.
- The discomfort to the patient is minimised as no immune responses are induced within the subject's arm.
- The test samples can be collected routinely at collection centres by collection staff, reducing some of the load on public health units and chest clinics.
- If a clinician is unsure of QFT assay results, the test can be recollected and repeated at any time without the

interference of the boosting effect seen in repeat TSTs.

- QFT can be used for detection of latent infection of pulmonary and extra-pulmonary TB (unlike culture methods that identify only active TB).

There have been over 300 publications, reporting on studies involving over 30,000 patients published world-wide and these figures are growing weekly. There are published international guidelines regarding the routine use of this assay in most major countries, including USA, UK, Japan, Switzerland, Italy, Canada, Denmark, and many others. In Australia, NSW Health has taken a more conservative approach and are awaiting further evaluations prior to publication of formal guidelines regarding this assay. NSW Health, however, do recognise that the assay has been evaluated for use with immune-competent healthy adults and that it is no longer considered necessary to perform a TST in persons who have had a QFT assay performed⁶. Recent studies suggest that the assay may also be used in children older than 2 years of age and in immuno-suppressed individuals³.

As yet, there is no Medicare rebate for a QFT assay, unless the subject is immuno-suppressed or immunocompromised (when the item number is 69471)⁷. San Pathology charges the Healthcare facility or patient directly at the rate of \$50 for the QFT assay in addition to the collection fee.

For further information on this assay or to request a set of the special collection tubes please contact the San Pathology Microbiology Laboratory on 9487 9514, or email Nola Hitchcock at nolah@sa.org.au.

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