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Sullivan Nicolaides PATHOLOGY
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Update: Assessing *Mycobacterium tuberculosis* infection with QuantiFERON-TB Gold Plus (QFT-Plus)

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Interferon Gamma (IFN-γ) release assays have been in use for a number of years as a measure of exposure to *Mycobacterium tuberculosis*.

Advantages of the Interferon Gamma (IFN-γ) release assays (IGRAs) over the Tuberculin Skin Test (TST) include:

- they are not affected by the Bacille-Calmette Guérin (BCG) vaccination;
- there is less likelihood of false positive results associated with exposure to the majority of other non-tuberculosis mycobacterias (NTMs) with the exception of *M. marinum*, *M. kansasii*, and *M. szulgai*;
- only a single blood test is required compared to the TST where a second visit is needed to read the reaction;
- TST readings can be subjective and the results must be interpreted carefully;
- the Mantoux reagent is often in short supply.

Like the TST, IGRAs alone cannot distinguish active TB disease from latent infection. They are intended for use in conjunction with risk assessment, radiography and other clinical and diagnostic evaluations.

Over the years, evidence for the role of IGRAs as a replacement for TSTs has grown, and in 2017 the National Tuberculosis Advisory Committee (NTAC) updated its recommendations.[1] It concluded that IGRAs and TSTs have similar (but poor) ability to identify patients with latent tuberculosis infection (LTBI) or at risk of developing active TB disease. The improved specificity of IGRAs, however, is likely to reduce the number of patients requiring preventive therapy for presumed LTBI. Either a TST or an IGRA for the investigation of LTBI in most circumstances is now recommended. The guidelines state that this assay has no place in the initial investigation of active TB disease in adults. Active and latent infection cannot be distinguished and the test may be falsely negative in active tuberculosis. However, a positive test, particularly in non-pulmonary active TB, may support the diagnosis.

QuantiFERON-TB Gold Plus

In April 2016, Sullivan Nicolaides Pathology introduced the QuantiFERON-TB Gold Plus (QFT-Plus), an improved version of the QuantiFERON-TB Gold. This assay, like its predecessor, uses a positive control tube – containing mitogen – and a negative control tube. However, the new QFT-Plus uses two TB antigen tubes. TB1 tube contains pooled synthetic antigens, the early secretory protein 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) from the M. tuberculosis-specific region of difference 1 (RD1), which are designed to elicit a cell-mediated immunity (CMI) response from CD4+ T-helper lymphocytes. The TB2 tube contains an additional set of peptides to detect a CMI response from CD8+ cytotoxic lymphocytes. After the four tubes are inoculated with the patient's blood and incubated for 16–24 hours, the plasma is separated and the concentration of IFN-γ which is released from the lymphocytes is measured by ELISA.

Since the introduction of QFT-Plus, Sullivan Nicolaides Pathology has performed more than 21,000 tests with a positivity rate of 6.8%. The population tested has widely differing prevalence of TB, ranging from very low (e.g. tertiary entrance screening of young Australians) to high (e.g. Papua New Guinean nationals). For 80.6% of the positive QFT-Plus interpretations, positive results were present in both the TB1 and TB2 antigen tubes; 6.4% were positive for only the TB1 antigen; and 13.0% were positive for only theTB2 antigen.

The indeterminate rate was low at 0.95% and was mainly due to an inadequate mitogen response 97% of the time. The test population included immunocompromised patients and those on immunosuppressive therapy. A small percentage (3%) of tests was indeterminate because of very high background IFN-γ response in the negative control tube. This very low indeterminate rate is much improved compared to the previous QFT-Gold assay which, on occasions, approached 7-10%. The new QFT-Plus assay has proven more robust as both assays are subject to not only analytical but also preanalytical factors, including faulty kit manufacturing, kit transport temperatures, blood volume inoculation, tube shaking, and delayed tube incubation. Where an indeterminate result cannot be resolved with repeat testing or a TST, the patient's LTBI status needs to then be determined from TB exposure history and other results.

The practice of serial testing healthcare workers (HCWs) is contentious. High rates of reversions and conversions can occur, especially when the initial TB antigen results are close to the cut-off (0.35 IU/mL). The manufacturer does not recommend a 'grey zone', but the literature suggests that IFN-γ results of 0.25-1.0 IU/mL should be interpreted with caution.[2] Depending on the clinical circumstances of the HCW, repeating the IGRA test is one option if the initial result falls within a pre-determined grey zone. However, the overall interpretation and management should be based on the composite of clinical, laboratory and radiological evidence. An alternative approach is to consider QFT–Plus positive only if both the TB1 and TB2 antigen tubes are greater than 0.35 IU/mL, particularly for HCWs with no known risk factors for TB working in a low-TB-incidence setting.[3]

References

1. Position statement on interferon-γ release assays for the detection of latent tuberculosis infection. Ivan Bastian, Chris Coulter and the National Tuberculosis Advisory Committee (NTAC) CDI Vol 41 No 4 2017 E322 – E336.
2. Daley CL et al. A summary of meeting proceedings on addressing variability around the cut point in serial interferon-γ release assay testing. Infect Control Hosp Epidemiol 2013; 34: 625-30.
3. Moon HW et al. Evaluation of QuantiFERON-TB Gold-Plus in health care workers in a low incidence setting. J Clin Microbiol 2017; 55:1650–1657.

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Update: *Helicobacter pylori;* its new superbug status demands a paradigm shift in therapy

In February 2017, *Helicobacter pylori,* along with other notable pathogens was listed by the WHO as a high priority organism posing the greatest threat to human health. There was a call for urgent attention to be given to the development of new antibiotics and treatments because of significant emerging antibiotic resistance.

At least three new international guidelines have called for a paradigm shift in therapy, and have raised concerns about the first-line use of empiric triple therapy, namely a proton pump inhibitor + amoxicillin + clarithromycin (PAC), as no longer being able to achieve reliable cure rates.

Data from Sullivan Nicolaides Pathology confirms that resistance is a major issue in treatment failures. While not advocating that cultures and susceptibility testing are performed prior to treatment of all *H. pylori* infections, these data reaffirm that a lack of information can be a major barrier to effective and reliable therapy.

Pathologist-in-charge of Sullivan Nicolaides Pathology's Department of Microbiology, Dr Jenny Robson, has prepared a doctor's bulletin that includes a list of key points to help the clinician when making decisions. Having examined the new international guidelines, she provides advice on who to test, who to treat, types of therapy, and what to do in the event of treatment failure.

For a copy of the bulletin please contact your Medical Liaison Manager
P 1300 767 284 **E** education@snp.com.au

Cervical Screening Test: using the correct term is essential for correct testing and Medicare rebate

Under the revised National Cervical Screening Program, new terms have been specified that must be used when requesting tests.

It is important to use these new terms to ensure the laboratory performs the appropriate tests and that the tests meet eligibility conditions for a Medicare rebate. Using old, superseded terminology may result in delays to testing and the Medicare rebate being declined.

Practice management systems such as Medical Director and Best Practice have recently added the new terms. It is advisable to use the drop-down list supplied and to clear the superseded terms from your favourites. It is also essential to include specific and detailed clinical notes when requesting.

✓ Correct Terms	x Incorrect Terms
CST routine (HPV)	Pap smear
Co-test (HPV+LBC)	Cervical smear
HPV test	Cervical cytology
LBC test	Pap cervix
Vaginal co-test (HPV+LBC)	Pap CX
Vaginal HPV test Vaginal LBC test	ThinPrep® (inc. ThinPrep® pap test, ThinPrep® pap test cx)
Vaginal self-collected HPV test	HPV & Reflex LBC

Medicare rebates for BRCA1, BRCA2 and PALB2 gene testing

Testing of the most common genes responsible for familial breast or ovarian cancer is now rebated; however, certain conditions apply.

To be eligible for a Medicare rebate, the test must only be ordered by a medical specialist or consultant physician. Pre-test counselling with written consent must be completed. Requesting via our dedicated form is preferred; however, requests will also be accepted on the normal request form.

Testing can be requested under the following MBS items:

- Item 73296 – a patient with breast or ovarian cancer and relevant family history
- Item 73295 – a patient with advanced ovarian cancer
- Item 73297 – a patient who does not have cancer but has family history.

For patients not meeting MBS requirements, testing is available providing they are willing to cover the full cost of the test themselves.

For a copy of the comprehensive doctor's bulletin giving details on BRCA and PALB2 testing, Medicare rebates and the conditions applying, please contact your Medical Liaison Manager **P** 1300 767 284 **E** education@snp.com.au