

# PEARLS OF LABORATORY MEDICINE

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**TITLE: Interferon Gamma Release Assays (IGRAs)**

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**Slide 1:**

Hello, my name is Anna Plourde. I am a Fellow in Medical Microbiology at the University of Chicago Medical Center and NorthShore University HealthSystem. Welcome to this Pearl of Laboratory Medicine on Interferon Gamma Release Assays, or IGRAs.

**Slide 2:**

Interferon gamma release assays are a type of test designed to detect tuberculosis, a disease caused by the organism *Mycobacterium tuberculosis*. Tuberculosis is the leading cause of infectious disease mortality globally, causing an estimated 1.4 million deaths worldwide in 2018. TB comes in two forms: Latent TB infection, which is asymptomatic and not contagious, and active TB disease, which is symptomatic and contagious. Symptoms may include a cough lasting weeks, chest pain, and hemoptysis, as well as a variety of constitutional symptoms. TB is typically spread from person to person when someone with active TB coughs, generating aerosols which can then infect another person who inhales those aerosols. So if latent TB infection is not contagious, why should we care about it? Well, latent TB infection can progress to the active (i.e. contagious) form of TB in a subset of people, especially those with a weakened immune system. Therefore, detecting and treating latent TB as well as active TB is critical for TB control efforts.

## **Slide 3:**

There are two types of tests designed to screen for TB infection, the tuberculin skin test (or TST) and IGRAs. Both are indirect tests for *Mycobacterium tuberculosis*, meaning that they do not directly detect the organism but rather the body's immune response to mycobacterial protein antigens. Before I go further, I should mention that neither of these tests are capable of distinguishing between latent TB and active TB. There is unfortunately no gold standard diagnostic assay that one can use to definitively diagnose latent TB. Rather, latent TB is a clinical diagnosis, made only after active TB has been excluded using a variety of other information, including patient history, signs/symptoms, chest imaging, and so forth.

## **Slide 4:**

Let's briefly talk about the first type of test available for TB screening - the TST, which many of us are likely familiar with. Before 2001, this was the only commercially available immunologic test for TB infection in the United States. In the US, it is performed by the Mantoux method, in which 0.1 mL of tuberculin (also known as purified protein derivative or PPD) is injected intracutaneously into the volar surface of the forearm, and the area examined 48-72 hours later for induration. If this area is large enough, it is interpreted as a positive screen for TB. Although a relatively simple test, the TST has several limitations. First, false positive results may occur among people who were vaccinated with *Bacille Calmette-Guerin* (BCG), or who are infected with non-tuberculous mycobacteria, because PPD contains antigens that are also in BCG and certain nontuberculous mycobacteria. Second, patients must return to a healthcare provider for test interpretation, which can be difficult for certain patient groups. Third, it has been shown that variability exists in test readers' measurement of induration.

## **Slide 5:**

Interferon gamma release assays, the second category of screening tests for TB infection, were designed at least in part to address these limitations of TST. They are in vitro blood tests that are now recommended by the Centers for Disease Control and Prevention as an aid in diagnosing infection with *M. tuberculosis*. The first FDA-approved IGRAs, the QuantiFERON TB test, came to market in 2001. Three generations of this QuantiFERON assay followed, each replacing the last. The currently available QuantiFERON assay, called the QuantiFERON Gold

Plus, is the 4th generation of this line. The other type of IGRA available in the United States, the T-SPOT.TB assay, was FDA-approved in 2008. These two types of IGRAs are also available in Canada, Europe, and many other countries.

### **Slide 6:**

IGRA development became possible after one of the key pathways in the body's immune response to TB infection, depicted here, was elucidated. Briefly, during infection with TB, antigen presenting cells (or APCs) encounter mycobacterial organisms (shown here in purple). The APCs then engulf and process the mycobacteria. Next they present these chewed-up bits of mycobacteria in the form of antigens to CD4+ and CD8+ T cells. Finally, the activated T cells then release the cytokine interferon-gamma, depicted as these little orange triangles. IGRAs are designed to elicit and then detect this interferon gamma, an indirect marker of TB exposure.

### **Slide 7:**

Both the QuantiFERON Gold Plus assay and the T-SPOT.TB assay, are based on the same biology just described. Their basic steps, listed here, are very similar. After blood collection from the patient, blood is mixed with antigens derived from *Mycobacterium tuberculosis* complex, namely early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens are designed to stimulate IFN-gamma release from T-cells previously exposed to TB, and are more specific for *M. tuberculosis* than PPD because they are not shared with any BCG vaccine strains or most species of NTM. After exposure of the T cells to these antigens, the amount of IFN-gamma released in patient samples is then measured and compared with that from positive and negative controls in order to formulate a result.

### **Slide 8:**

As mentioned, the principle on which the QuantiFERON and T-SPOT.TB assays are based is the same, but there are some differences in the procedure. Let's take a more detailed look at each, starting with the QuantiFERON assay. Blood is first drawn from the patient into four QuantiFERON-specific tubes. Two of these tubes, referred to as TB1 and TB2, and shown here with green and yellow tops, respectively, contain the peptide antigens ESAT-6 and CFP10, designed to stimulate CD4+ T cells. The TB1 tube also contains an additional set of "short" peptides which are designed to stimulate CD8+ T cells. The set of four tubes also includes one gray-topped negative control tube, as well a purple-topped positive control tube. Immediately after blood collection, all four tubes are shaken 10 times to mix the blood with the antigens. The

blood incubates in these tubes to allow interferon gamma stimulation and release to occur. The tubes are then centrifuged. An aliquot of plasma is taken from each tube and placed into a 96-well plate, with each aliquot placed into its own well. In the following steps, an enzyme-linked immunosorbent assay (ELISA) is performed. Capture antibodies which capture interferon-gamma line the bottom of each well. Enzyme-linked conjugate antibodies which can stick to IFN-gamma are also added to each well. The plate is incubated, allowing any IFN-gamma present to bind to the antibodies. After washing, enzyme substrate is added to each well, which is converted to a colored product by the enzymes linked to the conjugate antibody. This colored product is measured as optical density (OD) of each well, which can be translated into an amount of IFN-gamma present.

### **Slide 9:**

Let's now look at how TSPOT.TB works in comparison. The process again starts with blood collection, but here there are no special tubes. The blood is drawn into a regular lithium heparin tube. The tube is centrifuged and the layer of peripheral blood mononuclear cells (which includes T cells) is transferred into a conical tube. The cells are washed and counted, and based on the cell count, a suspension of PBMCs is prepared at a specific concentration. These cells are added to a 96-well plate containing capture antibody. ESAT-6 and CFP10 antigens (depicted here in green and blue) are added to the PBMCs to stimulate IFN-gamma release. A negative control and positive control (shown here in white and purple, respectively) are also added. After incubation and washing, enzyme-linked conjugate antibody and substrate are added. The substrate is cleaved by enzyme bound to the conjugate to form a dark blue spot of insoluble precipitate at the site of the reaction. After allowing the plate to dry, these dark blue spots are counted and recorded.

### **Slide 10:**

Let's talk about what types of results are possible with IGRAs. After comparison with positive and negative controls, and based on an algorithmic approach, the assay measurements are then translated to result categories for each patient -- positive, negative, or an "uncertain" category, termed indeterminate for QuantiFERON and borderline for T-SPOT. If the IFN-gamma response is significantly above the negative control value above a certain threshold, the patient is considered positive. If the IFN-gamma response is below a certain threshold and the

negative control is also negative, the test is negative. The “uncertain” categories encompass results that are not decidedly positive or negative. So what should be done clinically with this information? Well, IGRA results always need to be interpreted in clinical context, as false positive and negative results can always occur. Generally, patients with a positive IGRA should be further evaluated for active TB – which may include taking a detailed history of TB exposure, evaluation of signs/symptoms of TB, a chest radiograph, and/or sputum culture. Patients in whom active TB is ruled out should be considered for treatment of LTBI. Among patients with a negative IGRA, TB is less likely than in those with a positive IGRA. However, false negative results can occur for a variety of reasons including poor immune function, and technical-operational variability. Therefore, treatment for LTBI despite a negative IGRA may be considered on an individual basis. Finally, patients whose result falls into the “uncertain” category should have their IGRA repeated. If the repeat IGRA result is also uncertain, patients should be screened for TB by another method (e.g. the TST).

## **Slide 11:**

So how well does IGRA perform? Studies which have examined two measures of IGRA performance - sensitivity and specificity - have shown that IGRA generally performs well in most people for detection of TB – 80-90%+ sensitive, and 95%+ specific. However, it is important to keep in mind that these studies and their estimates are imperfect. Because there is no gold standard for diagnosing LTBI, studies have relied on surrogates for LTBI infection. For instance, studies of IGRA sensitivity for LTBI are usually conducted among patients with culture-confirmed active TB, a best approximation of a gold standard, but biased, because LTBI and active TB are inherently different entities. Patients with active TB are more likely to have immunologic abnormalities that place them at greater risk for having developed the disease in the first place, and since IGRA is an immunologic-based test it may not perform as well in these study participants. Therefore, it is difficult to accurately say how sensitive IGRAs actually are for LTBI. Sensitivity studies conducted among patients with immunosuppression and young children suggest that IGRA sensitivity is lower in these groups, and can be as low as 50-60%, though results vary. Specificity studies are generally conducted among people at low risk for TB, but without a gold standard, it is impossible to say that these study participants truly do not have LTBI. Therefore, specificity estimates are also imperfect. Despite these limitations, the data suggest that IGRAs perform fairly well in non-immunosuppressed people over 2 years old.

## **Slide 12:**

As mentioned, IGRAs were developed in order to overcome some of the limitations of TST. Advantages they offer over TST include lack of cross-reactivity with BCG and most non-tuberculous mycobacteria, relatively rapid turn-around time, no need for the patient to return to a healthcare facility for test interpretation, and lack of a boosting effect. With the TST, PPD is injected in order to elicit a localized skin reaction – this has been shown to boost or stimulate the patient's immune reaction to subsequent TSTs through immunologic recall. In contrast, because IGRAs are conducted in vitro, undergoing an IGRA has no effect on a patient's immune response or on subsequent IGRAs. IGRAs also have limitations to consider. Key limitations include pre-analytic sources of variability, including under- or overfilling of blood tubes, too vigorously shaking the collection tubes after blood draw, and delays in incubation time, all of which can affect the accuracy of the result; the lowered sensitivity of the IGRA in certain patient groups; (as with TST) IGRA's inability to distinguish between LTBI and active TB; and IGRA's cross-reactivity with certain non-tuberculous mycobacteria, including *M. marinum*, *M. szulgai*, *M. flavescens*, and *M. kansasii*.

## **Slide 13:**

If clinicians call looking for guidance on how best to screen their patient for latent TB, CDC's website on IGRA testing is always a great resource, offering a number of guidelines on whom to test for TB, when, and by what method. Key guidelines are listed here. IGRAs (and TSTs) should be used as aids in diagnosing *M. tuberculosis* infection, and can be used for surveillance or to identify persons likely to benefit from treatment, including contacts of active TB patients, people from areas w/ high incidence of TB, immunosuppressed patients, children < 5 years old, et cetera. On the other hand, IGRAs (and TSTs) should NOT be used for testing persons with low risk for infection and progression to active TB if infected. In addition, they should also not be used to monitor anti-tuberculosis therapy response, as no correlation of IGRA results with clinical or microbiologic response to anti-TB therapy has been demonstrated.

## **Slide 14:**

CDC also offers advice on whether IGRA versus TST is preferred for particular patient groups, based on the characteristics of these assays. IGRAs are preferred over TSTs for groups with historically low rates of return for a TST interpretation, such as homeless persons and drug users. IGRAs are also preferred for persons who have received BCG due to their lack of cross-reactivity with BCG. On the other hand, TSTs are preferred for children < 5 years old due to IGRAs' relatively low sensitivity in this age group. Either test is acceptable for recent contacts of active TB patients, as well as for periodic screening of persons at risk for occupational exposure such as healthcare workers. Finally, there are situations in which both tests may be considered, as when the initially ordered test (IGRA or TST) is negative, but there is high suspicion for active TB, or the risk for infection and/or progression is increased. Ordering both tests could also be considered when the initial test is positive, but the risk for infection and/or progression is thought to be low.

### **Slide 15:**

In summary, interferon gamma release assays (IGRAs) are a diagnostic aid for TB infection. They work by detecting IFN-gamma release from T cells in response to TB-derived antigens. Sensitivity and specificity are generally high but can be lower among some patient groups, so it is important to interpret results in their clinical context as well as to keep in mind the assay's strengths and limitations. And you can always refer to CDC's helpful guidelines for IGRA use; a link is provided here.

### **Slide 16: References**

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## **Slide 17: Disclosures**

## **Slide 18: Thank You from [www.TraineeCouncil.org](http://www.TraineeCouncil.org)**

Thank you for listening to this Pearl of Laboratory Medicine on Interferon Gamma Release Assays!