

Fast Facts:
Quantiferon TB Plus Test
HIV Screening by BioPlex

"Quality in Laboratory Diagnosis"



Fast Facts: Quantiferon TB Plus Test

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What is the Quantiferon TB Plus Test?

The TB Plus test is an FDA-approved, fourth-generation interferon gamma release assay (IGRA). The quantiferon test is used as an indirect test for *Mycobacterium tuberculosis* infection and disease.

How do the indirect TB tests work?

During *M. tuberculosis* infection, the body generates cell-mediated immune (CMI) responses to the TB organism and its antigens. The indirect TB tests measure the body's CMI responses to exogenous TB antigens. The underlying paradigm for indirect testing is that patients with TB-sensitized lymphocytes will produce a stronger CMI response than cells that have never been exposed to the antigen.

What are the advantages of the Quantiferon test versus the TB skin test?

- Requires a single patient visit.
- Results are generally available in less than 72 hours.
- Repeat testing does not boost subsequent immune responses.
- Prior BCG (Bacille Calmette-Guérin) vaccination does not cause a false-positive IGRA test result.

TB Skin Test

The TB Skin Test (also known as the Mantoux or PPD test) is arguably the best-known procedure for assessing CMI responses to TB antigens. The TB skin test is an *in vivo* CMI test wherein 0.1 mL of a purified protein derivative (PPD) from a TB-complex organism is injected under the skin and the development of a delayed-typed hypersensitivity reaction (swelling, redness, and induration at the injection site) is visually assessed 48-72 hours later.

Quantiferon

The Quantiferon gamma release assay is an *in vitro* immune function assay performed on whole blood. In this procedure, whole blood is collected into four tubes, three of which contain stimulatory antigens. The blood is mixed with the dried antigens and the tubes are incubated to allow the live white blood cells to interact with the antigens and produce gamma interferon. At the end of the incubation period, the cells are pelleted via centrifugation and the resulting plasma is tested for the presence of gamma interferon. More information on the antigens can be found in the following pages.



Why are there two TB antigen tubes?

In contrast with the third generation Quantiferon test, the new fourth generation test has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain ESAT-6 and CFP-10, which are peptide antigens found in *Mycobacterium tuberculosis* complex organisms. These antigens elicit gamma interferon responses from TB-sensitized CD4+ T-helper lymphocytes.

The TB2 tube also contains a set of TB peptides that induce gamma interferon responses from TB-sensitized CD8+ cytotoxic T lymphocytes. Gamma interferon-producing TB-sensitized CD8+ cells have been detected in subjects with latent TB infections and with active TB. Moreover, ESAT-6 and CFP-10 specific CD8+ T lymphocytes are found more frequently in subjects with active TB disease versus latent TB, and may be associated with a recent TB exposure. TB-specific CD8+ T cells producing gamma interferon have been detected in active TB subjects with HIV co-infection and in young children with TB disease. For these reasons, the manufacturer decided to add the second TB-specific antigen tube.

What is the sensitivity of the Quantiferon test versus the TB Skin Test?

Estimates of the Quantiferon sensitivity versus TB skin testing have varied widely in published studies. The CDC guidelines for using gamma interferon assays to detect *Mycobacterium tuberculosis* (<https://www.cdc.gov/mmwr/pdf/rr/rr5905.pdf>) indicate that the sensitivity of the Quantiferon assay is similar to that of the TB skin test.

Does the Quantiferon assay have any known cross-reactivity?

Yes. The ESAT-6 and CFP-10 antigens used in the Quantiferon assay are found in *M. tuberculosis*, *M. kansasii*, *M. szulgai* and *M. marinum*. Thus, the Quantiferon assay cannot distinguish *M. tuberculosis* infections from infections with these other mycobacteria. Fortunately, the ESAT-6 and CFP-10 antigens are absent from all BCG strains and from most non-tuberculosis mycobacteria.

How do I interpret the test results?

There are five fields in the Quantiferon TB Plus report. The following table explains the significance of the results in each field.

Field Name	Usual value(s)	Interpretation
Nil	Low, usually <1.0	The Nil result is a measure of the background level of gamma interferon in the specimen. Elevated levels of interferon gamma may occur due to the presence of heterophile antibodies or intrinsic gamma interferon secretion in the patient.
Mitogen - Nil	High, usually >10.0	This is a measure of the cell's ability to produce gamma interferon in response to non-specific mitogen stimulation. Low values can occur due to sample handling errors that adversely impact lymphocyte activity/viability, low lymphocyte counts, and other factors that decrease cell mediated immune responses.
TB Ag1 - Nil	< 3.5 IU/mL	CD4+ gamma interferon response to ESAT-6 and CFP-10 antigens. Values ≥ 3.5 are positive. The magnitude of the measured gamma interferon level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease. All screen positive patients are retested in duplicate prior to reporting.
TB Ag2 - Nil	< 3.5 IU/mL	Gamma interferon response in CD4+ and CD8+ cells. Values ≥ 3.5 are positive. The magnitude of the measured gamma interferon level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease. All screen positive patients are retested in duplicate prior to reporting.
Quantiferon TB Plus	POSITIVE Negative Indeterminate	POSITIVE result suggests that <i>M. tuberculosis</i> complex infection is likely. Negative result suggests that <i>M. tuberculosis</i> complex infection is not likely. However, a negative result does not rule out infection, particularly in patients with impaired immune function or patients suspected to have <i>M. tuberculosis</i> disease. Indeterminate - (see explanation next page)

What is an indeterminate result?

An indeterminate result means that we cannot determine whether the specimen is positive or negative. The individual values were nonsensical. Please consider sending another specimen for evaluation.

Indeterminate results are uncommon and may be caused by a number of factors. Improper specimen collection and handling are

the most common reasons for an indeterminate result. Delays (>6 hours) between blood collection and incubation at 37°C, refrigeration of the filled blood collection tubes before incubation, inefficient mixing of the blood and the antigens during collection, and over-incubation of the filled tubes can adversely affect the gamma interferon production. The laboratory monitors indeterminate result rates as part of its overall quality assurance program.

Other Test Limitations

- The magnitude of the measured gamma interferon level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease.
- Results from Quantiferon-Plus testing must be used in conjunction with each individual's epidemiological history, current medical status, and other diagnostic evaluations.
- The **predictive value of a positive Quantiferon-Plus result** in diagnosing *M. tuberculosis* infection depends on the probability of infection, which is assessed by historical, epidemiological, diagnostic, and other findings.
- A diagnosis of latent TB infection requires that tuberculosis disease must be excluded by medical evaluation including an assessment of current medical and diagnostic tests for disease as indicated.
- The performance of the Quantiferon-Plus test has not been extensively evaluated **with specimens from the following groups of individuals:**
 - ✓ Individuals who have impaired or altered immune functions, such as those who have HIV infection or AIDS.
 - ✓ Transplant patients managed with immunosuppressive treatment or others who receive immunosuppressive drugs (e.g., corticosteroids, methotrexate, or azathioprine).
 - ✓ Those who have other clinical conditions, such as diabetes, silicosis, chronic renal failure, and hematological disorders (e.g., leukemia and lymphomas).
 - ✓ Patients with other specific malignancies (e.g., carcinoma of the head or neck and lung).
 - ✓ Individuals younger than age 17 years.
 - ✓ Pregnant women.

Still have questions?

Contact Warde Medical Laboratory
at (800) 876-6522.

HIV Screening by BioPlex

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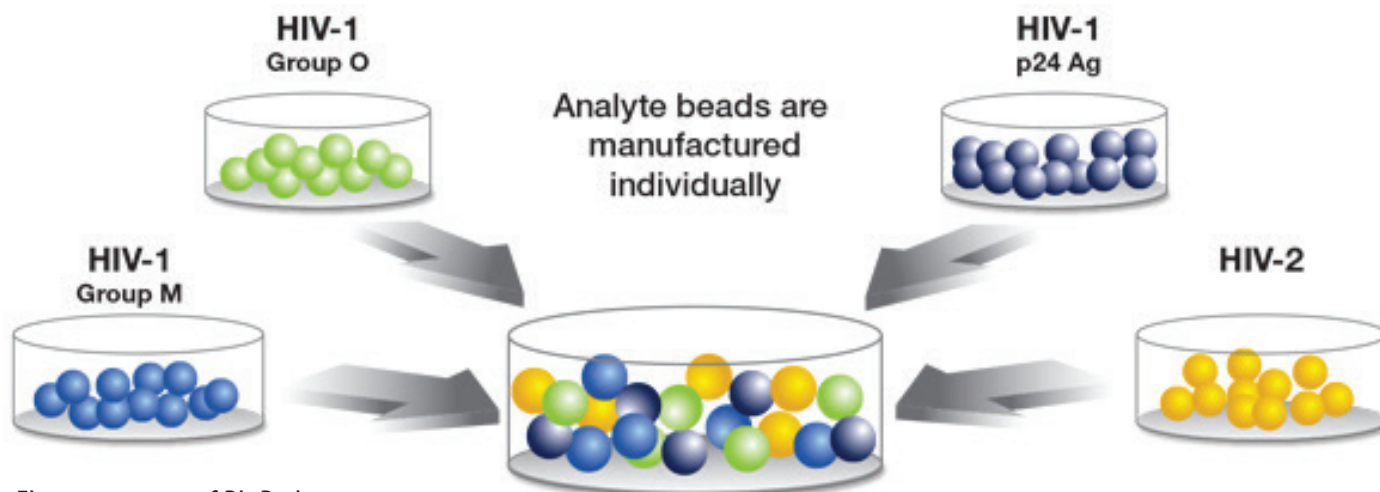


Figure courtesy of BioRad

This summer, Warde Medical Laboratory will transition to a new method for initial screening for infection with human immunodeficiency virus (HIV). Currently, the Laboratory uses a 4th generation HIV screen-- so-called because, in addition to assessing for the presence of antibodies to HIV-1 and HIV-2 (as was the case for 3rd generation screens), it also assesses for the presence of the HIV-1 p24 antigen, enabling initial detection in the early phases of infection, potentially weeks before the development of anti-HIV antibodies.

The new platform, sometimes referred to as "5th generation,"¹ also assesses for p24 antigen in addition to HIV-1 and HIV-2 antibodies, but allows for the separate determination of each of these components in a single reaction vessel.

The new method is manufactured by BioRad under the BioPlex brand, and is based in Luminex bead technology. This method uses microscopic beads coated with different ligands

specific to the detection of each particular analyte. This allows for separate detection of antibodies to HIV-1 (groups M and O), antibodies to HIV-2, and detection of the HIV-1 p24 antigen at the time of initial screen (see Figure). In a process analogous to flow cytometry, beads are passed through a pair of lasers after initial incubation, where bound antibody (or bound antigen) is detected using a fluorochrome tag.

Since the current CDC algorithm for diagnosis of HIV infection still requires a supplemental method for initial reactive screens, we will still be following up initial antibody-positives with the BioRad Geenius HIV 1/2 differentiation assay. As such, users likely will not see major changes in test resulting and formatting.

Overall, this multiplex approach to HIV screening has similar sensitivity and

specificity to existing 4th generation platforms,^{2, 3} but offers some advantages over previous methods. Some users may see an improvement in turnaround time due to greater random access capability for required repeats of initial positives, and due to the potential for more rapid auto-release of negative results. Also, the separate identification of “antigen-only” positives might abrogate the need for supplemental antibody testing in some cases.

Initially, the BioPlex will be used only for HIV screening, but our acquisition of this platform could allow for the adaptation of many different immunoassay-based serologic studies, potentially increasing efficiency in laboratory operations beyond HIV screening.

References

1. Salmona M, Delarue S, Delauigere C, Simon F, Maylin S: *Clinical evaluation of BioPlex 2200 HIV Ag-Ab, an automated screening method providing discrete detection of HIV-1 p24 antigen, HIV-1 antibody, and HIV-2 antibody.* J Clin Microbiol 2014; 52(1):103-107.
2. Eshleman SH, Piwowar-Manning E, Sivay MV, Debevec B, et al: *Performance of the BioPlex 2200 HIV Ag-Ab assay for identifying acute HIV infection.* J Clin Virol 2018; 99-100:67-70.
3. Qiu X, Sokoll L, Yip P, Elliott DJ, et al: *Comparative evaluation of three FDA-approved HIV Ag/Ab combination tests using a genetically diverse HIV panel and diagnostic specimens.* J Clin Virol 2017; 92:62-68.