QFT®
QuantiFERON-TB Gold

Clinical Guide

Clinical experience with QuantiFERON®-TB Gold

HIV / AIDS

Cellestis Clinical Guide series 2011

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This clinical guide is intended to provide healthcare professionals with an overview of key clinical information and benefits of using QuantiFERON-TB Gold® (QFT®) for tuberculosis (TB) testing in individuals living with human immunodeficiency virus (HIV).

Common Questions

Why does one need to screen for latent TB in HIV positive individuals?

Conversion from latent TB infection to active TB disease can occur as a result of the immune system being significantly compromised. HIV infection is the biggest known risk factor for reactivation of latent TB infection. Individuals co-infected with HIV and *M. tuberculosis* have a 50 to 200-fold increased risk of reactivation of latent to active TB.[1] The risk of active TB is also increased among HIV-infected individuals receiving antiretroviral therapy.[2]

What are the diagnostic options available for latent TB infection?

Previously, the only tool available for identifying latent TB infection was the tuberculin skin test (TST), or Mantoux. The TST is an *in vivo* test measuring immune responses to tuberculin PPD, which is made up of a multitude of bacterial proteins. Most of these proteins are present in the TB vaccine, Bacille Calmette-Guérin (BCG), and shared with many environmental mycobacteria.

A new paradigm in diagnosing TB infection has emerged in recent years: interferon-gamma release assays (IGRAs). IGRAs are *in vitro* blood tests that detect the interferon-gamma (IFN-γ) response to TB infection.

The most clinically tested and proven IGRA is the QuantiFERON-TB Gold In-Tube test (QFT), with over 500 peer-reviewed published clinical papers.

What are the limitations of the TST in individuals with HIV?

A variety of factors, other than latent TB infection, are known to induce a positive TST result. These include BCG vaccination, exposure to non-tuberculous mycobacteria, the inherent inability of the test to distinguish a current infection from past resolved infections, the inherent boosting effects of prior testing, and the subjectivity and variability (intra- and inter-observer) when reading the test results. False-positive results often lead to unnecessary treatment for TB infection with possible detrimental side effects.[3]

What is the dependency of QFT test performance on the CD4⁺ T-cell count?

A large number of studies have analyzed QFT performance in individuals living with HIV. Some studies have shown that the sensitivity of QFT in HIV co-infected individuals is impaired compared with HIV-negative patients with TB, although to a lesser degree than the TST. However, the clinical reality of anergy should not be a surprise and needs to be appropriately managed.

The “real” or practical performance of QFT relates to the IFN-γ response to the TB-specific antigens. Published data from TB/HIV co-infected persons in developing countries, suggests reactivity to mitogen (positive control) lessens with decreasing CD4⁺ count. It follows that reactivity to the TB antigens will also likely decrease in HIV-infected people, leading to a corresponding compromise in test sensitivity. Sensitivity estimates for QFT are significantly lower in those with low CD4⁺ cell count (< 200/µL) and also in those with advanced TB disease. In contrast, QFT sensitivity estimates are higher in those with CD4⁺ cell count > 200/µL approximating sensitivity seen in non-HIV-infected populations.[3]

A significant benefit of QFT over the TST is that it incorporates a positive control. Indeterminate results (failure of the positive control) are more prevalent in HIV-infected people with CD4⁺ cell count < 100/µL than those with higher counts. However, in people with CD4⁺ cell count > 200/µL, available data suggests that QFT’s performance is commensurate with that in immunocompetent populations. This finding is not surprising as it is IFN-γ produced by CD4⁺ T-cells that are predominantly being detected in the QFT mitogen (positive control) tube. In other words, QFT relies on functional CD4⁺ cells, and its performance might be negatively influenced by low and impaired CD4⁺ cell counts in HIV-infected individuals.[3] Unlike the TST, QFT’s positive control provides an indicator of this immune function.

An indeterminate QFT result in an HIV-infected person with a low CD4⁺ cell count provides valuable information. It suggests possible anergy and does not indicate a failed test.[3]

What are the negative predictive value (NPV) and positive predictive value (PPV) of QFT in the HIV population?

Both NPV and PPV are heavily dependent on the prevalence of infection in the population being tested. In most developed countries, the prevalence of *M. tuberculosis* (MTB) infection is relatively low. The largest study in a low-endemic region is by Aichelburg et al., who explored the PPV of QFT longitudinally in 830 HIV-positive individuals. Of the 37 who were QFT-positive, three (8.1%) developed active TB during the follow-up period of 19 months. This rate of progression translates to a PPV of 91.9%. None of the 793 QFT-negative individuals progressed to active TB, indicating an excellent NPV, in this case, an NPV of 100%. One important observation of the Aichelburg et al. data is the relative scarcity of study individuals with very low (<200) $CD4^+$ cell counts. This may indicate an inherent study bias. There is a high likelihood that individuals with such low $CD4^+$ cell counts, and infected with MTB, would have already progressed to active disease. As such, these individuals would be selected out of this study and, therefore, the results.[3,4]

How dependent are NPV and PPV on the $CD4^+$ cell count?

NPV decreases as sensitivity decreases, so in a HIV-infected population with low $CD4^+$ cell count, we would expect the NPV to be reduced. This means that a negative result cannot be used to exclude TB infection in a person with low $CD4^+$ cell count (or indeed in any person at risk). This is because QFT relies on functional $CD4^+$ cells and its performance might be negatively influenced by low and impaired $CD4^+$ cell count, as is the case for the TST. Clinicians should take into account all available evidence and history in making their diagnosis. Similarly, for those with low $CD4^+$ cell counts, PPV estimates would also be reduced due to lower sensitivity

Does one have to investigate active TB based upon a positive QFT test?

A positive QFT result indicates the person tested is likely infected with *M. tuberculosis*. The individual with a positive QFT result should then be evaluated for active TB symptoms and treated accordingly, for active or latent TB based on the outcome of the clinical evaluation.

What does the literature say about QFT use in the HIV population?

Numerous studies have investigated the use of QFT (In-Tube version) in HIV-positive populations. The characteristics of these studies are outlined in the Table according to low- and high-income countries. Of note is that QFT has been shown to have equal or higher sensitivity when compared with the TST.[3]

Perhaps the biggest disadvantage of the TST is that it lacks an internal control to distinguish false negative results due to anergy from true negative results. On a pragmatic front, obtaining a TST result can take up to 72 hours, and a return visit is required for the TST reading.[3]

> "An indeterminate QFT result in an HIV-infected person with a low $CD4^+$ cell count provides valuable information. It suggests possible anergy and does not indicate a failed test." [3]

QFT is approved by the US FDA

QFT is approved by FDA as an *in vitro* diagnostic aid for detection of *Mycobacterium tuberculosis* infection. It uses a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7(p4) proteins to stimulate cells in heparinized whole blood. Detection of IFN-γ by ELISA is used to identify *in vitro* responses to these peptide antigens that are associated with *M. tuberculosis* infection. FDA approval notes that QFT is an indirect test for M. tuberculosis infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations. QFT Package Inserts, available in up to 25 different languages, can be found at **www.cellestis.com.**

Summary of Published Studies

High-Income Countries

Details	Key Findings
First Author: Aichelburg **Year (ref):** 2009 (4) **Country:** Austria **Patient population:** Potential LTBI, Active TB **n:** 822, 8 **CD4 count, Median (QR):** 393 (264-566)	The QFT assay yielded positive or indeterminate results in 44 (5.3%) and 47 (5.7%) of the 830 patients, respectively. A positive QFT result occurred at significantly higher frequencies among patients from high-prevalence countries than among patients from low-prevalence countries ($p<0.001$). In patients with indeterminate QFT results, both median actual and nadir $CD4^+$ counts were significantly lower than in patients with interpretable QFT results ($p<0.001$). During the follow-up period, progression to active tuberculosis occurred exclusively in patients with a positive QFT result, at a rate of 8.1% (3 of 37 patients; $p<0.001$). Collectively, the sensitivity of the QFT assay for active tuberculosis was 90.9%.
First Author: Brock **Year (ref):** 2006 (5) **Country:** Denmark **Patient population:** Potential LTBI **n:** 590 **CD4 count, Median (QR):** 523	27/590 (4.6%) of the individuals were QFT positive, indicating the presence of latent TB infection. Among QFT-positive patients, 78% had risk factors such as long-term residency in a TB high endemic area, known TB exposure or previous TB disease. The prevalence of latent TB infection in these groups was 13%, 16% and 19% respectively. There was a strong correlation between low $CD4^+$ cell count and a low mitogen response ($p<0.001$) and more patients with low $CD4^+$ cell count had indeterminate results.
First Author: Luetkemeyer **Year (ref):** 2007 (6) **Country:** United States **Patient population:** Potential LTBI **n:** 294 **CD4 count, Median (QR):** 363 (214-581)	Of 294 participants, 70% returned for an evaluable TST. Concordance between QFT and TST was 89.3% (kappa= 0.37, $p=0.007$). However, in subjects with positive test results by either TST or QFT, only 28% had positive test results by both modalities. TST-positive/QFT-negative discordant results were found in 5.1% of subjects and TST-negative/QFT-positive discordance in 5.6%. Indeterminate QFT results occurred in 5.1%, all due to a failure to respond to the phytohemagglutinin-positive control. Subjects with a CD4+ count of <100 cells/µL had a relative risk of an indeterminate result of 4.24 compared with those with ≥100 cells/µL.
First Author: Richeldi **Year (ref):** 2009 (7) **Country:** Italy **Patient population:** Potential LTBI **n:** 116 **CD4 count, Median (QR):** 439	Overall, the TST provided fewer positive results (10.9%) than T-Spot®.*TB* (18.4%; $p<0.001$) and QFT (15.1%; $p=0.033$). Indeterminate blood test results due to low positive control values were significantly more frequent with QFT (7.2%) than with T-Spot®.*TB* (0.6%; $p<0.001$).
First Author: Santin **Year (ref):** 2010 (8) **Country:** Spain **Patient population:** Potential LTBI **n:** 135 **CD4 count, Median (QR):** 300 (156-522)	The prevalence of latent TB was 6.7% by the TST and 9.6% by QFT ($p=0.3$) in HIV-seropositive subjects, and 34.8% by the TST and 21.5% by QFT ($p=0.02$) among controls. TST reactivity declined sharply as $CD4^+$ cells fell. A less pronounced fall occurred with QFT. No cases of tuberculosis occurred during follow-up (0.26 per 100 person-years).Simultaneous testing with the TST and QFT early in the course of HIV infection might minimize the risk of tuberculosis in these patients.
First Author: Sauzullo **Year (ref):** 2010 (9) **Country:** Italy **Patient population:** Potential LTBI, Active TB **n:** 163, 44 **CD4 count, Median (QR):** 219 (4-995)	In HIV-infected patients, the level of agreement between the TST and QFT tests was 68% and QFT sensitivity was 66%. The proportion of indeterminate QFT results was 33.4%, which correlated with $CD4^+$ count <200 cells/µL ($p<0.0001$). When excluding the indeterminate results, the QFT sensitivity increased to 86.6%.
First Author: Talati **Year (ref):** 2009 (10) **Country:** United States **Patient population:** Potential LTBI **n:** 336 **CD4 count, Median (QR):** 334 (0-1380)	Overall, 8.0% of patients had at least one positive diagnostic test for latent TB: 2.1% had a positive TST, 2.7% a positive QFT, and 4.2% a positive T-Spot®.*TB*. Agreement between the three diagnostic tests was poor. An indeterminate test result occurred among 1.8% of QFT and 14% of T-Spot tests. In multivariate analysis, patients with a $CD4^+$ count of ≤200 cells/µL were significantly more likely to have an indeterminate result.

Low- and Middle-Income Countries

Details	Key Findings
First Author: Aabye **Year (ref):** 2009 (11) **Country:** Tanzania **Patient population:** Active TB **n:** 68 **CD4 count, Median (QR):** 272 (172–478)	Sensitivity of the QFT test was higher in HIV-negative (75/93) than in HIV-positive (44/68) patients (81% vs. 65%, p=0.02) and increased with $CD4^+$ cell count in HIV-positive patients (test for trend p=0.03). Twenty-three (23) patients (14%) had an indeterminate result and this proportion decreased with increasing $CD4^+$ cell count in HIV-positive patients (test for trend p=0.03). Sensitivity when excluding indeterminate results was 86% (95% CI: 81–92%) and did not differ between HIV-negative and HIV–positive patients (88 vs. 83%, p=0.39).
First Author: Balcells **Year (ref):** 2008 (12) **Country:** Chile **Patient population:** Potential LTBI **n:** 116 **CD4 count, Median (QR):** 368 (264-482)	The TST (5mm cut-off) and QFT results were positive in 10.9% and 14.8% of individuals, respectively, with moderate agreement between both tests (kappa = 0.59). A history of both known TB exposure and past TB were associated with a positive QFT result. Only past TB was significantly associated with a positive TST result. Among the subjects with TST <5mm, 8.2% were positive by QFT test. These individuals had a lower mean $CD4^+$ cell count than those detected positive by both tests (328 cells/µL and 560 cells/µL, respectively, p=0.03). QFT appears less affected by more advanced immunosuppression.
First Author: Kabeer **Year (ref):** 2009 (13) **Country:** India **Patient population:** Active TB **n:** 105 **CD4 count, Median (QR):** 116 (48-209)	Of 105 tested, QFT was positive in 65%, negative in 18% and indeterminate in 17% of patients. The sensitivity of QFT remained similar in pulmonary TB and extra-pulmonary TB patients. The QFT positivity was not affected by low $CD4^+$ count, but it often gave indeterminate results especially in individuals with $CD4^+$ count <200 cells/µL. Among the 105 patients, 31% showed ≥5mm induration for TST. All the TST-positive individuals were also QFT-positive . The sensitivity of TST was decreased when $CD4^+$ cell count declined. QFT yielded fewer false-negative results than the TST, even in individuals with low $CD4^+$ count.
First Author: Leidl **Year (ref):** 2010 (14) **Country:** Uganda **Patient population:** Potential LTBI, Active TB **n:** 109, 19 **CD4 count, Median (QR):** 283, 182	TST results were not available for 20/109 (18.3%) individuals from the group of HIV-infected persons without active TB, as individuals did not return for test reading. In patients with active TB, no TST was performed. Only 12.5% of persons with <100 CD4+ cells/µL exhibited a positive TST reaction, and the diameter of TST induration was significantly correlated to numbers of circulating $CD4^+$ cells. The HIV-infected individuals without active TB were stratified according to $CD4^+$ cell counts of >250 cells/µL, 100-250 cells/µL, and <100 cells/µL. Frequencies of positive results for QFT test declined significantly from 77.3 % to 66.7 % and 10 % (p<0.0001) respectively, while frequencies of positive test results for T-Spot®.*TB* test were 50%, 57.6% and 70%, respectively.
First Author: Markova **Year (ref):** 2009 (15) **Country:** Bulgaria **Patient population:** Potential LTBI, Active TB **n:** 77, 13 **CD4 count, Median (QR):** N/A	In the 13 subjects with culture-confirmed active TB, 12 (92%) were QFT positive compared with 8 (62%) T-Spot positive. Of the 77 subjects without culture-confirmed active TB, 30% were positive, 64% negative, and 6% indeterminate by QFT, compared with 29%, 62% and 9% respectively by T-Spot®.*TB*. The QFT and T-Spot®.*TB* tests had similar high sensitivity.
First Author: Raby **Year (ref):** 2008 (16) **Country:** Zambia **Patient population:** Active TB **n:** 59 **CD4 count, Median (QR):** 212 (109-332)	Marked decrease in sensitivity was observed in HIV-positive patients with 37/59 (63%) being QFT-positive compared to 31/37 (84%) HIV-negative patients [chi^2, p=0.033]. Low $CD4^+$ count was associated with increases in both indeterminate and false-negative results. The TST test was impaired in HIV-positive subjects with only 26/47 (55%) having a positive TST compared to 25/31 (81%) in the HIV-negative group (chi^2, p=0.021). A $CD4^+$ count of <200 cells/µL was clearly associated with negative TST (p<0.001). Although there was little difference in the overall sensitivities, agreement between TST and QFT was poor.
First Author: Tsiouris **Year (ref):** 2006 (17) **Country:** South Africa **Patient population:** Active TB **n:** 26 **CD4 count, Median (QR):** N/A	In a subgroup analysis of patients with known HIV status, the sensitivity of QFT was higher in HIV-infected patients than in HIV-negative patients (81% versus 73%). However, of the 41 patients with known HIV status (26 HIV-infected and 15 HIV-negative), five had indeterminate results, all of whom were HIV-infected (19% versus 0%, respectively; p=0.139). If these five indeterminate results were considered negative, then the sensitivity of QFT in HIV- infected individuals with active TB would be 65%.

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References

1. Nunn P, Williams B, Floyd K, Dye C, Elzinga G, Raviglione M. Tuberculosis control in the era of HIV. *Nat Rev Immunol 2005; 5:819-26.*
2. Girardi E, Sabin CA, d'Arminio Monforte A, Hogg B, Phillips AN, Gill MJ, Dabis F, Reiss P, Kirk O, Bernasconi E, Grabar S, Justice A, Staszewski S, Fätkenheuer G, Sterne JA. Incidence of tuberculosis among HIV-infected patients receiving highly active antiretroviral therapy in Europe and North America. *Clin Infect Dis 2005; 41:1772-82.*
3. Aichelburg MC, Rieger A, Breitenecker F, Pfistershammer K, Tittes J, Eltz S, Aichelburg AC, Sting G, Makristathis A, Kohrgruber N. Detection and Prediction of Active Tuberculosis Disease by a Whole-Blood Interferon-γ Release Assay in HIV-1–Infected Individuals. *Clin Infect Dis 2009; 48: 954-962.*
4. Luetkemeyer AF, Charlebois ED, Flores LL, Bangsberg DR, Deeks SG, Martin JN, Havlir DV. Comparison of an interferon-gamma release assay with tuberculin skin testing in HIV-infected individuals. *Am J Respir Crit Care Med. 2007 Apr 1;175(7):737-42.*
5. Richeldi L, Losi M, D'Amico R, Luppi M, Ferrari A, Mussini C, Codeluppi M, Cocchi S, Prati F, Paci V, Meacci M, Meccugni B, Rumpianesi F, Roversi P, Cerri S, Luppi F, Ferrara G, Latorre I, Gerunda GE, Torelli G, Esposito R, Fabbri LM. Performance of tests for latent tuberculosis in different groups of immunocompromised patients. *Chest. 2009 Jul;136(1):198-204.*
6. Santin M, Casas S, Saumoy M, Andreu A, Moure R, Alcaide F, Ferrer E, Podzamczer D. Detection of latent tuberculosis by the tuberculin skin test and a whole-blood interferon-γ release assay, and the development of active tuberculosis in HIV-seropositive persons. *Diagn Microbiol Infect Dis. 2011 Jan;69(1):59-65.*
7. Sauzullo I, Mengoni F, Scrivo R, Valesini G, Potenza C, Skroza N, Marocco R, Lichtner M, Vullo V, Mastroianni CM. Evaluation of QuantiFERON-TB Gold In-Tube in human immunodeficiency virus infection and in patient candidates for anti-tumour necrosis factor-alpha treatment. *Int J Tuberc Lung Dis. 2010 Jul;14(7):834-40.*
8. Talati NJ, Seybold U, Humphrey B, Aina A, Tapia J, Weinfurter P, Albalak R, Blumberg HM. Poor concordance between interferon-gamma release assays and tuberculin skin tests in diagnosis of latent tuberculosis infection among HIV-infected individuals. *BMC Infect Dis. 2009 Feb 10;9:15.*
9. Aabye MG, Ravn P, PrayGod G, Jeremiah K, Mugomela A, Jepsen M, Faurholt D, Range N, Friis H, Changalucha J, Andersen AB. The impact of HIV infection and CD4 cell count on the performance of an interferon gamma release assay in patients with pulmonary tuberculosis. *PLoS One. 2009;4(1):e4220.*
10. Balcells ME, Pérez CM, Chanqueo L, Lasso M, Villanueva M, Espinoza M, Villarroel L, García P. A comparative study of two different methods for the detection of latent tuberculosis in HIV-positive individuals in Chile. *Int J Infect Dis. 2008 Nov;12(6):645-52.*
11. Syed Ahamed Kabeer B, Sikhamani R, Swaminathan S, Perumal V, Paramasivam P, Raja A. Role of interferon gamma release assay in active TB diagnosis among HIV infected individuals. *PLoS One. 2009 May 28;4(5):e5718.*
12. Leidl L, Mayanja-Kizza H, Sotgiu G, Baseke J, Ernst M, Hirsch C, Goletti D, Toossi Z, Lange C. Relationship of immunodiagnostic assays for tuberculosis and numbers of circulating CD4+ T-cells in HIV infection. *Eur Respir J. 2010 Mar;35(3):619-26.*
13. Raby E, Moyo M, Devendra A, Banda J, De Haas P, Ayles H, Godfrey-Faussett P. The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay in Zambian adults with active tuberculosis. *PLoS One. 2008 Jun 18;3(6):e2489.*
14. Tsiouris SJ, Coetzee D, Toro PL, Austin J, Stein Z, El-Sadr W. Sensitivity analysis and potential uses of a novel gamma interferon release assay for diagnosis of tuberculosis. *J Clin Microbiol. 2006 Aug;44(8):2844-50.*
15. Hoffmann M & Ravn P. The use of interferon-gamma release assays in HIV- positive individuals *Eur Infect Dis 2010; 4: 23-29.*
16. Brock I, Ruhwald M, Lundgren B, Westh H, Mathiesen L R, Ravn P. Latent Tuberculosis in HIV positive, diagnosed by the M. Tuberculosis Specific Interferon-γ test. *Respir Res 2006; 7: 56-64*
17. Markova R, Todorova Y, Drenska Y, Elenkov I, Yankova M, Stefanova D. Usefulness of interferon- gamma release assays in the diagnosis of tuberculosis infection in HIV- infected patients in Bulgaria. *Biotechnol 2009; 23: 1103-1008.*

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