



# QuantiFERON®-TB gold in-tube is not Useful for Diagnosing Active Tuberculosis in HIV/AIDS Patients with Severe Immunodeficiency: Results from Brazil

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## Abstract

**Purpose:** To assess interferon gamma release assay (IGRA) and tuberculin skin test (TST) performances in the diagnosis of active tuberculosis (TB) in adults with HIV/AIDS with different degrees of immunodeficiency.

**Methods:** Cross-sectional study conducted with 90 HIV/TB-coinfected adults, São Paulo, Brazil. TB diagnosis was established based on the presence of positive sputum smear, culture, or anatomic-pathological examination. The participants responded to a questionnaire and were submitted to physical examination, chest x-ray (CRX), serum CD4+ and CD8+ T cell count, TST, and IGRA (QuantiFERON®-TB Gold In Tube, Cellestis, Carnegie, Australia).

**Results:** Characteristics of 90 HIV/TB-coinfected individuals: male (60.0%), white (54.4%), single (57.3%), and average age 39 ( $\pm 10.8$ ) years with pulmonary TB (45.6%) and average CD4+ T-cell count (198.92 cells/mm<sup>3</sup>). TST was positive in 25.56%, and IGRA was positive in 65.56%. IGRA performance was better when compared to TST ( $p < 0.001$ ) and was able to diagnose TB with 93.75% probability when CD4+  $\geq 187$  cells/mm<sup>3</sup>; TST showed similar efficacy when CD4+  $\geq 500$  cells/mm<sup>3</sup>.

**Conclusion:** IGRA exhibited better performance for TB disease diagnosis in HIV-infected individuals with severe immunodeficiency when compared to TST. Nevertheless, both tests may exhibit false-negative results in this type of population. Despite the fact that IGRA has better performance than TST in the diagnosis of active TB in patients with HIV/AIDS, the practical utilization of the method seems to be limited and should be considered only for patients with CD4+  $\geq 187$  cells/mm<sup>3</sup>.

**Keywords:** QuantiFERON®-TB Gold In-Tube; Purified Protein Derivative (PPD); *Mycobacterium tuberculosis*; HIV; Number of CD4

## Introduction

Tuberculosis (TB) is a severe public health concern in Brazil and worldwide [1]. TB is the main cause of death in individuals with acquired immunodeficiency syndrome (AIDS), as well as one of the most frequent opportunistic infections [2,3]. The criteria for establishing the diagnosis of TB do not differ between individuals infected or not by the human immunodeficiency virus (HIV); however, in patients with immunodeficiency, the isolation of *Mycobacterium tuberculosis* is particularly relevant to rule out a large number of differential diagnoses. In this context, novel diagnostic tests are warranted [2–4].

Until 2001, the tuberculin skin test (TST) was the only available test to assess individuals with latent TB infection (LTBI). This test is also used in the diagnosis of active TB, particularly for the extrapulmonary forms of the disease. In addition, assays involving the detection of interferon-gamma (IFN- $\gamma$ ) have been increasingly used in recent years as IFN- $\gamma$  plays a significant role in the T cell-mediated immune response to *M. tuberculosis* [4]. The interferon-gamma release assay (IGRA) is a test based on the secretion of IFN- $\gamma$  by circulating T cells following *ex vivo* stimulation with specific antigens. IGRA has shown promising results for the diagnosis of LTBI as well as active TB [4–6]; however, its role in patients coinfected with TB and HIV has not yet been thoroughly established [3,5,7,8].

In regard to LTBI, the main advantage of IGRA is its higher specificity in comparison with TST. In addition, IGRA requires only a single visit from tested individuals [4–10]. However, in regard to active TB, 10 to 25% of patients do not exhibit positive reactions to TST or

IGRA, mostly corresponding to individuals with immunodeficiency, recent infection, or young children [11].

The aim of the present study was to assess the performance of TST and IGRA in the diagnosis of active TB in adults with HIV/AIDS with different degrees of immunodeficiency.

In Brazil, the IGRA is not yet available for routine use in the health care system and the test approved for use is the QuantiFERON®-TB gold in-tube. The cost was also considered for the choice of this method, besides the ease of performing the test.

## Study Population and Methods

This cross-sectional study included 90 individuals from both genders, who were older than 18 years and were treated at three healthcare Institutions in the city of São Paulo: Instituto de Infectologia Emílio Ribas – (IIER), Instituto Clemente Ferreira – ICF), and

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**Received** August 18, 2014; **Accepted** October 20, 2014; **Published** October 23, 2014

**Citation:** Klautau GB, Bammann RH, Ferreira NVS, Afione JB, Burattini MN, et al. (2014) QuantiFERON®-TB gold in-tube is not Useful for Diagnosing Active Tuberculosis in HIV/AIDS Patients with Severe Immunodeficiency: Results from Brazil. J Trop Dis 2: 150. doi: [10.4172/2329-891X.1000150](https://doi.org/10.4172/2329-891X.1000150)

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Irmandade da Santa Casa de Misericórdia de São Paulo (ISCMSp). This study was approved by the respective institutional research ethics committees. The participants were selected from March 2012 to April 2013. To be included in the study, all subjects had to exhibit infection by HIV and diagnosis of active TB; the patients were either treatment-naïve or had started treatment less than 15 days earlier. Diagnosis of pulmonary or extrapulmonary TB was based on a positive sputum smear and/or culture or anatomic-pathological results compatible with TB according to the standards formulated by the Brazilian Health Ministry [12]. After receiving an explanation of the aim and procedures of the present study and signing an informed consent form, the participants completed a questionnaire to collect clinical and epidemiological information and were then subjected to the following tests: simple chest x-ray (CXR), serum CD4+ and CD8+ T cell counts, HIV viral load (to assess the immune status relative to the HIV infection), TST, and IGRA - QuantiFERON-TB Gold In Tube (QFT-GIT), Cellestis, Carnegie, Australia.

TST was performed using the Mantoux technique [13]. Disposable plastic syringes and 27G needles were used to administer 0.1 ml of purified protein derivative (PPD-RT 23) equivalent to 2 tuberculin units (2TU) per intradermal route on the middle third of the anterior surface of the left forearm. Reading was performed 48-96 hours later by identifying the extension of induration by palpation and measuring its maximum transverse diameter in millimeters using rulers provided by the National Health Foundation. Positive reactions were classified as PPD5 when the diameter of induration was equal to or greater than 5 mm and as PPD10 when it was equal to or greater than 10 mm. All TSTs were applied by professionals previously trained for the purpose of standardization to ensure a minimum intra- and inter-examiner agreement of 80% and to minimize variations associated with application or measurement. IGRA was performed according to the manufacturer's instructions (QuantiFERON®-TB Gold In-Tube, Cellestis, Carnegie, Australia). Blood samples of 3 ml were collected by trained technicians and placed in a set of three test tubes labeled with numbers corresponding to the volunteers' individual codes. These tubes included a negative control (Nil) tube, a tube pre-coated with TB-specific antigens (ESAT-6, CFP-10, TB7, 7 {p4}), and a tube pre-coated with mitogen (phytohemagglutinin, positive control). The tubes were placed in a Styrofoam container, which was sealed, labeled as containing high-risk biological material, and sent to the reference laboratory. After collection and homogenization, the tubes were incubated at 37°C for 16-24 hours. Then, they were centrifuged, and IFN-γ was assessed by means of ELISA.

The production of IFN-γ by T cells was assessed in each volunteer's sample after stimulation with *M. tuberculosis*-specific antigens, inert antigens (Nil control), or mitogen (positive control). The ELISA raw results were analyzed using QuantiFERON - TB Gold IT specific software, which assesses the test quality, generates a standard curve (log(e) of the mean OD (y-axis) against log(e) of the IFN-γ concentration of the standards in IU/mL (x-axis)), and provides the results of each individual sample. The test final results are rated as positive (TB antigen minus Nil equal to or greater than 0.35 IU/ml) or negative.

## Results

In the total sample comprising 90 individuals with HIV and active TB, 60.0% were male, 54.4% were white, 57.3% were single, and the average age was 39 (± 10.8) years. In compliance with the Brazilian immunization program, most volunteers (88.9%) had been given BCG vaccination in childhood.

Only 12 volunteers (13.3%) reported recent contact with smear-positive TB cases, of which 10% represented domiciliary exposure. A total of 25 participants (27.8%) reported a history of TB, and only two of these patients had not completed full treatment.

The following TB-compatible clinical signs and symptoms were identified: fever (83.3%), night fever (56.7%), cough (66.7%), weight loss greater than 10% of the usual body weight (85.6%), sweats (60%), and hemoptysis (11.1%). The clinical forms of TB identified in the present case series included pulmonary (45.6%), extrapulmonary (25.6%), disseminated (21.1%) and combined (7.8%) (Table 1).

Lymph node TB was the most common extrapulmonary form detected (69.6%). *M. tuberculosis* was most frequently isolated from the sputum (55.8%), followed by the lymph nodes (17.4%), blood (10.5%), lymph nodes and sputum (5.8%), bronchoalveolar lavage fluid (2.3%), cerebrospinal fluid (5.8%), breasts (1.2%), and bowels (1.2%). Anatomic-pathological examination was performed in 43 volunteers, and the results were compatible with TB in 22 cases (51.2%).

The average number of CD4+ T cells was 198.92 cells/mm<sup>3</sup>, and that for CD8+ T cells was 529.58 cells/mm<sup>3</sup>, corresponding to an average CD4/CD8 ratio of 0.36%. The average HIV viral load log<sup>10</sup> was 4.41.

Only 23 volunteers (25.56%) tested positive to TST with the cutoff point of 5 mm, while only 15 volunteers (16.67%) tested positive to TST with the cutoff point of 10 mm. IGRA was positive in 59 participants (65.56%).

In the present sample of individuals coinfecting by HIV and TB, there was a low average CD4+ T cell count (198.92/mm<sup>3</sup>) and low rates of positive responses to TST and IGRA. However, the performance of IGRA was better compared to TST (p<0.01).

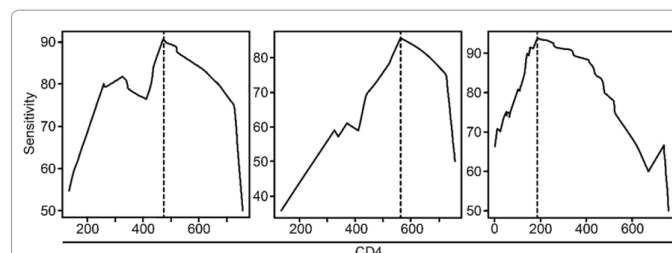
To improve the performance of TST and IGRA, taking into consideration the degree of immunosuppression of the present sample of coinfecting (HIV/TB) individuals, we sought to correlate various cutoff points for the CD4+ T cell count with positive responses to TB diagnostic tests (IGRA, PPD5, and PPD10) (Figure 1).

Our analysis showed that IGRA could diagnose TB with a

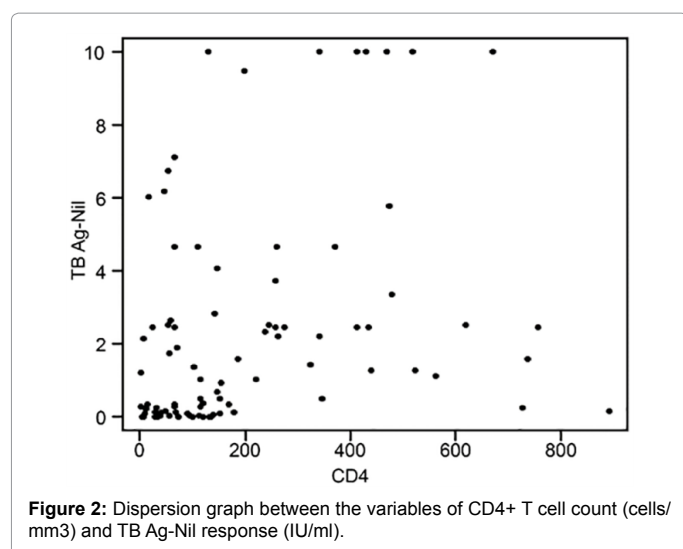
Variable	Group	Frequency (n)	Percentage (%)
Clinical Forms	Pulmonary	41	45,56
	Extrapulmonary	23	25,56
	Disseminated	19	21,11
	Combination of forms*	7	7,78

\*Combination of forms: pulmonary + ganglionic or pulmonary + pleural.

**Table 1:** Distribution of frequencies and of the respective percentages of clinical forms of TB in 90 HIV/TB coinfecting individuals.



**Figure 1:** Cutoff points for the CD4+ T cell count to maximize (probability of 93.75) the diagnostic performance of PPD5 (left), PPD10 (center), and IGRA (right). The vertical line indicates the cutoff point of the CD4+ T cell count that maximized the diagnostic performance of the tests.



probability of 93.75% in individuals with CD4+ cell counts  $\geq 187$  cells/mm<sup>3</sup>. However, TST (both PPD5 and PPD10) achieved a similar efficacy in cases with CD4+ cell counts of approximately  $\geq 500$ /mm<sup>3</sup>.

Analysis of the correlation between CD4+ T cell counts and TB Ag-Nil responses (IGRA) demonstrated a greater concentration of data in the area corresponding to the lowest values for both variables, as shown in Figure 2.

The correlation between these two variables, although not strong, proved to be significant (correlation coefficient of 0.447;  $p < 0.001$ ) using Spearman's test.

## Discussion

The classical signs and symptoms of TB were a frequent finding in the present sample of individuals with HIV infection, thus indicating the significance of this infection in this type of patient [14,15].

Although pulmonary TB was the most frequently identified form in the present sample, it is worth noticing that the rates of extrapulmonary and disseminated TB were higher than those previously reported in the literature for individuals not infected by HIV [16-20]. This is probably due to the fact that the population investigated in the present study was mostly composed of individuals with severe immunodeficiency, which favors the occurrence of extrapulmonary and disseminated TB [21,22].

The specificity of TST is low because PPD includes antigens shared by several species of mycobacteria, such as environmental forms *M. tuberculosis*, and *M. bovis* (BCG) [23,24].

In previous studies, the diagnostic specificity of IGRA and TST in immunocompetent individuals vaccinated with BCG was reported to be 98.1% and 68.1%, respectively. However, the sensitivity of IGRA is difficult to establish [25,26], as this result has been shown to vary from 64 to 87% in previous studies on TB disease [11,27].

We believe that BCG vaccination did not influence the results of the present study because this vaccine is applied at birth in Brazil and because all volunteers were older than 18 years of age and more than 10 years had elapsed since vaccination in all cases. According to some studies, BCG vaccination may influence the response to TST within the first two years after its application in adult populations [28]. A study conducted in Ethiopia with healthy students using TST and QuantiFERON-TB Gold IT for diagnosis of LTBI did not find any influence of BCG vaccination on the TST results [29].

Another issue is whether TST affects the results of QuantiFERON-TB Gold IT; studies suggest that especially when IGRA is performed after TST (from seven days up to three months), the interferon- $\gamma$  levels may increase, particularly in individuals who tested positive to TST [30-32].

In the present study, TST resulted in a positive response in only (25.56%) of the volunteers with active TB compared to 65.56% when using IGRA. We believe that the low sensitivity of both methods was due to the severe immunodeficiency exhibited by the investigated sample (average CD4+ T cell count of 198.92 cells/mm<sup>3</sup>). This situation notwithstanding, the performance of IGRA was superior in comparison to TST ( $p < 0.001$ ).

Due to the difficulties associated with interpreting TST results in patients with HIV/AIDS and various degrees of immunodeficiency, IGRA may represent a relevant contribution to the diagnosis of LTBI and TB disease in this type of patients, especially in cases with (false) negative results by TST [33]. Factors that could potentially influence the IGRA results in individuals with active TB, leading to indeterminate or false-negative results, include old age, low lymphocyte count, malnutrition (inhibition of the specific immune response) [34], HLA genotype, high C-reactive protein (CRP) values, and coinfection by HIV [35-37].

Previous studies have shown that both TST and IGRA can exhibit false-negative results in individuals with severe immunodeficiency. However, the performance of IGRA seems superior compared to TST, and this test may therefore be considered an auxiliary tool for the diagnosis of active TB in individuals with AIDS.

The cutoff point of TB Ag-Nil suggested by the test manufacturer for the diagnosis of LTBI is 0.35 IU/ml. However, this value may not be accurate in immunodeficient individuals with LTBI or active TB. In this regard, it is worth noting that IGRA has been recently approved for the differential diagnosis of active TB.

In patients with LTBI or active TB, peripheral blood lymphocytes are capable of recognizing TB antigens and producing cytokines (IFN- $\gamma$ ). Accordingly, QuantiFERON-TB Gold IT measures the T cell production of IFN- $\gamma$  following stimulation with peptide antigens from mycobacterial proteins (ESAT 6, CFP 10, and TB 7, 7 (p4)). Therefore, responsiveness to IGRA depends on the number and function of T cells, which were both severely affected in the participants in the present study. These results suggest that different cutoff points should be established for the differential diagnosis of active TB in immunodeficient individuals with low CD4+ T cell counts. In fact, the ideal approach would be to assess these responses in clinical studies conducted at areas with high and low TB prevalence rates [36,37]. In the present study, the TB Ag-Nil values were lower among individuals with the lowest CD4+ T cell counts, and these subjects were liable to present false-negative results when this count was less than 187 cells/mm<sup>3</sup>. Because TST exhibited false-negative results with CD4+ T cell counts lower than 473 cells/mm<sup>3</sup>, the performance of IGRA was comparatively better in the investigated population. However, further studies are needed to establish the precise contribution of IGRA to the diagnosis of TB disease in individuals with HIV infection.

## Conclusions

IGRA exhibited better performance for TB disease diagnosis in HIV-infected individuals with severe immunodeficiency when compared to TST. Nevertheless, both tests may exhibit false-negative results in this type of population. Despite the fact that IGRA has better performance than TST in the diagnosis of active TB in patients with HIV/AIDS, the

practical utilization of the method seems to be limited and should be considered only for patients with CD4+ T  $\geq$  187 cells/mm<sup>3</sup>.

## Acknowledgments

### Sources of funding

The authors thank the São Paulo Research Foundation (Fundação Amparo à Pesquisa do Estado de São Paulo - FAPESP) Process no. 2011/05805-1 (Regular Research Project) and the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq) Process no. 141705/2011-6 for the financial support. FAPESP and CNPq have an interest in the publication of the research. FAPESP, financial support. CNPq, scholarship for doctoral researcher Giselle Klautau.

The authors thank the Immunology Laboratory of Prof. Dr. Reinaldo Solomão - UNIFESP for technical support.

## Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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