# Routine Hospital Use of a New Commercial Whole Blood Interferon-γ Assay for the Diagnosis of Tuberculosis Infection

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***Rationale:*** **Interferon (IFN)-γ blood tests may improve the current level of diagnostic accuracy for tuberculosis infection. The QuantiFERON-TB Gold (QFT-Gold) has been used in selected populations and shows higher specificity than the tuberculin skin test (TST).** ***Objective:*** **To evaluate the QFT-Gold test in unselected patients and assess the level of agreement with the TST.** ***Methods:*** **The test has been routinely performed on whole blood samples in our microbiology laboratory for 8 months. Demographic, clinical, and microbiological data have been collected and correlated to the QFT-Gold results.** ***Measurements and Main Results:*** **Of 318 patients tested, 68 (21.4%) gave an indeterminate (low positive mitogen control) QFT-Gold result. Indeterminate results were significantly overrepresented in patients with a negative TST (28.9% vs. 6.6% in TST-positive patients; $p < 0.0001$, $\chi^2$ test) and were more frequent in patients receiving immunosuppressive therapies than in those who were not receiving such treatments (odds ratio, 3.35; 95% confidence interval, 1.84–6.08; $p < 0.0001$). After excluding indeterminate results, the concordance between QFT-Gold and TST was significantly lower in Bacille Calmette-Guérin–vaccinated individuals (41.5%) than in nonvaccinated individuals (80.3%) ($p < 0.0001$). In 11 patients with active tuberculosis (5 culture-confirmed), QFT-Gold provided more positive results than the TST (66.7% vs. 33.3%; $p = 0.165$).** ***Conclusions:*** **The QFT-Gold test is feasible in routine hospital use for the diagnosis of tuberculosis infection. As with the TST, immunosuppression may negatively affect the test's performance, with a significant rate of indeterminate results in the most vulnerable population.**

**Keywords:** diagnosis; immunosuppression; interferon-γ; tuberculosis

Tuberculosis (TB) is a major infectious cause of mortality and morbidity, with 1.8 million deaths and 8.8 million new cases reported each year worldwide (1). Most new TB cases are diagnosed in developing countries, where poverty and human immunodeficiency virus (HIV) are the leading allies of TB (2). In industrialized countries, the HIV pandemic and increased migration from high-prevalence areas have created a new awareness of the global burden of the disease (3). The aging of the population and the increased use of immunosuppressive therapies (e.g., cancer chemotherapy and new immunomodulatory agents) highlight the need for additional strategies to maintain and improve the control of TB (4).

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Early diagnosis of infectious cases and treatment of individuals latently infected with *Mycobacterium tuberculosis*, who are at increased risk of progression to active disease, are the key strategies for reducing the incidence of TB in low-prevalence areas (4, 5). Unfortunately, the standard diagnostic tool for the diagnosis of latent TB infection (LTBI) has many well known limitations. The sensitivity of the tuberculin skin test (TST) is low in the diagnosis of active TB infection; specificity is limited by the cross-reactivity of the purified protein derivative (PPD) with the Bacille Calmette-Guérin (BCG) vaccine and with most nontuberculous mycobacteria (6). Even more importantly, the sensitivity of the TST is particularly low in the immunosuppressed patients in which the risk of progression to TB is high, thus producing significant prevalence of false-negative results among this population (5). Despite these limitations, the TST is routinely used in hospital clinical practice for the screening of LTBI, and, even though it is not recommended as a diagnostic tool for active disease (7), it often enters the diagnostic algorithm of patients who have clinical signs suggestive of active TB.

Recent studies indicate that two new interferon (IFN)-γ blood tests might have improved specificity and sensitivity over the TST for the diagnosis of LTBI (8–10), as well as a potential role in supporting the diagnosis of active TB (11–13). These new tests undoubtedly have great promise, but their application to routine clinical use has not yet been extensively evaluated. One of these tests, the QuantiFERON-TB Gold (QFT-Gold; Cellestis Ltd., Victoria, Australia), showed encouraging results in a phase 2 study in low-risk BCG-vaccinated subjects and patients with active TB (14), and in a selected population with a high risk of TB infection, such as healthy contacts with recent exposure to an individual with active smear-positive TB (9) and patients with high suspicion of active TB (13). However, it was not known whether this new test would work in an unselected population of patients independently evaluated for possible TB infection.

We report here the results of our analysis of the first 8 months in which the QFT-Gold test was routinely performed on consecutive patients in the microbiology laboratory of our hospital. The aims of our study were to evaluate (*1*) the feasibility and performance of the QFT-Gold test when used in an unselected hospital-based population, (*2*) the level of agreement between QFT-Gold and TST, and (*3*) the interpretation of discordant results in light of the final clinical diagnosis and potential confounding factors. Some of the results of this study have been previously reported in the form of an abstract (15).

## METHODS

### Study Population

Patients tested with the QFT-Gold test in the microbiology laboratory of the Policlinico Hospital of Modena between December 1, 2003, and July 30, 2004, were prospectively enrolled in the study population. The

test was performed by technical personnel after an implementation period of 6 weeks preceding the study, with specific training on a series of repeated samples with a known negative or positive result. QFT-Gold tests were prescribed by hospital physicians for inpatients or outpatients in any ward, with no influence by the investigators of the study. The results of the test were made available to the prescribing physicians, along with a note describing the characteristics of the QFT technology and the level of knowledge about it, based on available published studies. The study was approved by the local ethics committee. For all patients we collected demographic, clinical, radiologic, and microbiological data. On the request form to the laboratory, prescribing physicians reported the reason for testing (e.g., diagnosis of cancer, recent immigration to Italy from a high-prevalence country, drug addiction, recent contact of a patient with active TB, transplantation, prison inmate, treatment with immunosuppressive and/or immunomodulatory agents, renal failure, HIV infection) and clinical signs suggestive of active TB. TST results (only when available at the same time as the QFT-Gold) and BCG vaccination status (assessed by the prescribing physician through scar inspection) were also collected from hospital clinical records along with the final clinical and microbiological diagnosis.

### QuantiFERON-TB Gold

The test was performed according to the manufacturer's recommendations. Briefly, the test consisted of a negative control (nil well, i.e., whole blood without antigens or mitogen), a positive control (mitogen well, i.e., whole blood stimulated with the mitogen phytohemagglutinin [PHA]) and two sample wells, i.e., whole blood stimulated with either of the *M. tuberculosis*–specific antigens Early Secretory Antigen Target 6 (ESAT-6) or Culture Filtrate Protein 10 (CFP-10). Whole blood specimens were incubated for 20 hours (overnight) at 37°C in a humidified atmosphere. The IFN-γ level of the nil well was considered background and was subtracted from the results of the mitogen well and the antigen-stimulated wells. The result of the test was considered positive if the concentration of IFN-γ in the sample well after stimulation with ESAT-6 and/or CFP-10 was greater than or equal to 0.35 IU/ml (after subtraction of the value of the nil well), regardless of the result of the positive control (mitogen well). The result of the test was considered negative if the response to the specific antigens (after subtraction of the value of the nil well) was less than 0.35 IU/ml and if the IFN-γ level of the positive control (after subtraction of the value of the nil well) was greater than or equal to 0.5 IU/ml. The result of the test was considered indeterminate if both antigen-stimulated sample wells were negative (i.e., $< 0.35$ IU/ml after subtraction of the value of the nil well) and if the value of the positive control well was less than 0.5 IU/ml after subtraction of the value of the nil well.

### Tuberculin Skin Test

The TST was performed using 5 TU (Biocine Test PPD; Chiron, Siena, Italy) according to the Mantoux method, and after 48 to 72 hours the transverse diameter of skin induration was recorded in millimeters. As all patients were tested because of an increased risk of TB infection, the TST results were interpreted according to the level of risk, as reported in current guidelines (16).

### Statistical Analysis

Statistical analyses were performed to assess (*1*) the proportion of QTF-Gold tests with an indeterminate result and the associated risk factors, (*2*) the concordance between QFT-Gold and TST results, (*3*) the results of QFT-Gold and TST in patients with BCG vaccination, and (*4*) the rate of positive QFT-Gold and TST results in patients with a final diagnosis of active TB. The analysis of concordance between QFT-Gold and TST was performed using the Cohen κ. QFT-Gold and TST results were compared using the $\chi^2$ test. Analyses were performed using Stata version 8.0 (Stata Corporation, College Station, TX).

## RESULTS

During an 8-month period, 318 patients were tested with the QFT-Gold test. Their demographic and clinical features and the reasons for requesting the test are reported in Table 1. In more than half of cases the QFT-Gold was requested based on a clinical suspicion of active TB. One hundred sixty-nine patients (53.1%) were admitted to the hospital as inpatients, and 65 more (20.4%) were receiving immunosuppressive treatments at the time of testing. QFT-Gold was mostly requested from the wards of respiratory diseases (n = 99; 31.1%), hematology and oncology (n = 76; 23.9%), pediatrics (n = 46; 14.5%), internal medicine (n = 30; 9.4%) and infectious diseases (n = 19; 6.0%). A small number of tests were also requested from the sections of nephrology (n = 14; 4.4%), rheumatology (n = 8; 2.5%), neurology (n = 7; 2.2%), dermatology (n = 6; 1.9%), and from other wards (n = 13; 4.1%).

Of 318 QFT-Gold tests performed, 68 (21.4%) gave an indeterminate result. The distribution of these indeterminate results, stratified by risk factors for immunosuppression and the odds ratios for an indeterminate QFT-Gold result, is reported in Table 2. In the univariate analysis model, patients undergoing concomitant immunosuppressive therapies had the highest proportion of indeterminate QFT-Gold test results; compared with those not receiving immunosuppressive therapies, they had 3.35

**TABLE 1. CHARACTERISTICS OF PATIENTS INCLUDED IN THE STUDY (*n* = *318*) AND PHYSICIANS' REASONS FOR PRESCRIBING THE QFT-GOLD TEST**

| Characteristic | n (%) |
|---|---|
| Male | 163 (51.3) |
| Age, yr ± SD | 49.3 ± 24.0 |
| BCG-vaccinated | 59 (18.5) |
| Immigrant from country with endemic TB | 73 (23.0) |
| HIV infection | 7 (2.2) |
| Cancer | 72 (22.6) |
| Renal failure | 10 (3.1) |
| Reason for testing with QFT-Gold: | |
| Clinical signs suggestive of active TB | 169 (53.1) |
| Contact of patient with active TB | 55 (17.3) |
| Recent immigration from country with endemic TB | 33 (10.4) |
| Screening before immunosuppressive therapy* | 30 (9.4) |
| Clinical signs suggestive of nontuberculous mycobacterial infection | 6 (1.9) |
| Other | 25 (7.9) |

*Definition of abbreviations*: BCG = Bacille Calmette-Guérin; QFT-Gold = QuantiFERON-TB Gold; TB = tuberculosis.

* Including cancer chemotherapy, chronic systemic steroids, anti-tumor necrosis factor alpha agents, and immunosuppressive agents.

**TABLE 2. UNIVARIATE ANALYSIS OF THE INDETERMINATE QFT-GOLD RESULTS**

| Variable | QFT-Gold: Indeterminate‡ *n = 68* (%) | QFT-Gold: Determinate *n = 250* (%) | OR (95% CI) | p Value |
|---|---|---|---|---|
| Immunosuppressive therapy* | 26 (38.2) | 39 (15.6) | 3.35 (1.84-6.08) | 0.00007 |
| Diagnosis of cancer† | 8 (11.8) | 33 (13.2) | 1.07 (0.49-2.30) | 0.855 |
| Age < 3 or > 80 yr | 11 (16.2) | 26 (10.4) | 1.46 (0.68-3.12) | 0.324 |
| HIV infection | 2 (2.9) | 5 (2.0) | 2.53 (0.55-11.59) | 0.230 |
| Renal failure | 4 (5.8) | 6 (2.4) | 2.26 (0.62-8.25) | 0.214 |

Definition of abbreviations: 95% CI = 95% confidence interval; OR = odds ratio; QFT-Gold = QuantiFERON-TB Gold.

A multivariate analysis was also performed and provided similar results (immunosuppressive therapy OR 3.59, 95% CI 1.76-7.30, $p < 0.001$; diagnosis of cancer OR 1.10, 95% CI 0.53-2.26, $p = 0.797$; age < 3 or > 80 years OR 1.95, 95% CI 0.87-4.36, $p = 0.105$; HIV infection OR 2.45, 95% CI 0.45-13.24, $p = 0.298$; renal failure OR 3.12, 95% CI 0.80-12.13, $p = 0.101$).

* Patients receiving cancer chemotherapy (n = 39), systemic steroids (≥15 mg/day of prednisone for ≥1 month) (n = 31), or anti-tumor necrosis factor alpha agents (n = 3) at the time of testing with QFT-Gold; 8 patients were receiving both cancer chemotherapy and systemic steroids.

† Patients with a diagnosis of cancer who were not receiving anti-cancer treatments at the time of testing with QFT-Gold.

‡ All indeterminate QFT-Gold results except one were due to a low response to the mitogen PHA in the positive control well, according to the manufacturer's instructions.

times higher odds (95% confidence interval [CI] 1.84–6.08; $p < 0.0001$) of having an indeterminate QFT-Gold result. A documented TST result obtained around the same time as the QFT-Gold was available in 255 (80.2%) of the 318 patients. QFT-Gold produced a significantly higher proportion of indeterminate results in patients with a negative TST result (43 of 149, 28.9%), compared with the TST-positive patients (7 of 106, 6.6%) ($p < 0.0001$). Stratification of TST results according to standard cut-off values showed that the frequency of indeterminate QFT-Gold results and the size of induration in the TST results were inversely correlated ($p < 0.05$, test for trend) (Figure 1). The QFT-Gold tests were significantly more likely to be indeterminate in patients with a TST result of < 5 mm than in those with a TST result of ≥ 15 mm ($p < 0.05$) and those with a TST result of ≥ 10 and < 15 mm ($p < 0.005$).

Among the 205 patients with valid (i.e., determinate) results for both tests, the concordance between TST and QFT-Gold was 70.2%, with a κ value of 0.40 (95% CI 0.27–0.52). Forty-nine patients (23.9%) had a positive TST result and a negative QFT-Gold; of those, 30 (61.2%) were BCG vaccinated, and 1 (2.0%) received a final diagnosis of active TB. Twelve patients (5.8%) had a positive QFT-Gold result and a negative TST: one (8.3%) was BCG vaccinated and two (16.7%) had a final diagnosis of active TB. A subgroup analysis for BCG vaccination status was performed. Fifty-nine (18.6%) of the 318 patients tested were BCG vaccinated, 53 of whom (89.8%) had valid results for both tests. In this subgroup, the TST was positive in 44 (83.0%) and the QFT-Gold was positive in 15 (28.3%) ($p < 0.00001$). Therefore, the concordance between TST and QFT-Gold was lower among BCG-vaccinated individuals than in the non–BCG-vaccinated subjects (41.5% versus 80.3%). The difference between the κ value in BCG-vaccinated and non–BCG-vaccinated subjects ($\kappa = 0.09$; 95% confidence interval [CI], −0.05–0.23 vs. $\kappa = 0.56$; 95% CI, 0.40–0.71) was significant ($p < 0.0001$).

Among all patients tested with the QFT-Gold, 11 (3.5%) were diagnosed with active TB; 5 of them were culture-confirmed. QFT-Gold was positive in six (66.7%) of the nine patients with TB with a valid result, and the TST was positive in three of the nine patients with an available TST (33.3%) ($p = 0.165$; 95% CI, 0.56–28.39).

## DISCUSSION

Accurate diagnosis of asymptomatic TB infection is becoming clinically important because of the increasing frequency of risk factors associated with progression to active disease (3, 5, 16). New questions about the control of TB in resident populations are arising in Western countries, especially because of the impaired performance of the standard method of screening, the TST, in high-risk groups, such as patients undergoing immunosuppressive treatments for chronic inflammatory diseases and cancer. On the other hand, the high prevalence of BCG vaccination among immigrants from countries with endemic TB significantly reduces the usefulness of the TST because of the known cross-reactivity of PPD with the BCG vaccine, thus generating a potential cause of overuse of preventive treatments. Two new rapid commercial IFN-γ blood tests for the diagnosis of TB infection, QFT-Gold and T SPOT-TB (based on ELISPOT technology [17]), became available recently. These are *in vitro* tests with positive and negative internal controls and have already been shown to be more specific than the TST in diagnosing TB infection. The tests are not based on the cross-reactive to PPD,

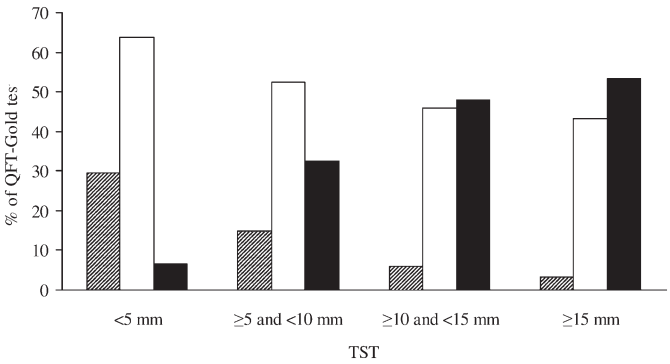


***Figure 1.*** Distribution of TST results (stratified according to the size of induration) and the QFT-Gold results in the 255 patients (80.2% of the study population) for whom results from both tests were available. *Striped bars* represent indeterminate QFT-Gold, *white bars* represent negative QFT-Gold, and *black bars* represent positive QFT-Gold. Indeterminate QFT-Gold results were significantly more frequent in patients with a TST result of less than 5 mm than in those with a TST result of greater than or equal to 15 mm ($p < 0.05$) or greater than or equal to 10 and less than 15 mm ($p < 0.005$). The frequency of indeterminate QFT-Gold results and the size of induration in TST results were inversely correlated ($p < 0.05$, test for trend).

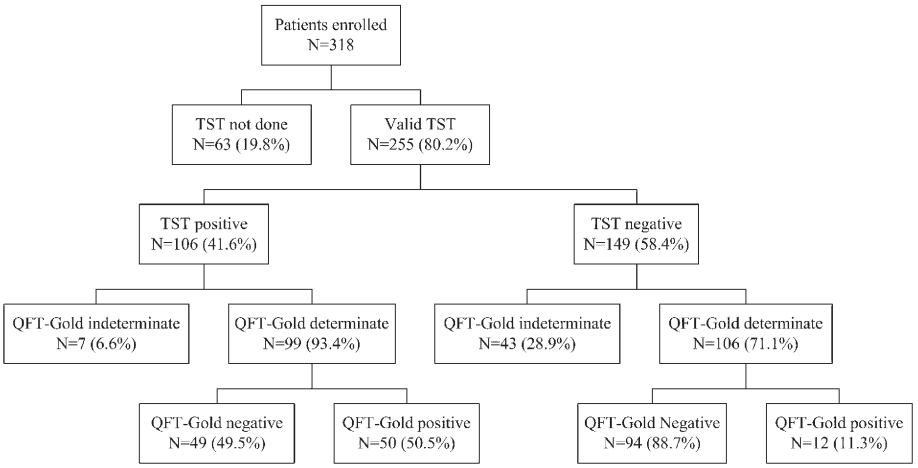


***Figure 2.*** Flow chart showing the distribution of QFT-Gold results in TST-positive and TST-negative patients. The proportion of indeterminate QFT-Gold results was significantly higher among TST-negative patients (28.9%) than among TST-positive patients (6.6%) ($p < 0.0001$). Overall κ agreement between the two tests was 0.40 (0.27–0.52). κ agreement in the BCG-vaccinated subjects was significantly lower (0.09; 95% CI, −0.05–0.23) than in the nonvaccinated population (0.56; 95% CI, 0.40–0.71) ($p < 0.0001$).

but measure the immune response to two *M. tuberculosis*–specific antigens that are absent from the BCG vaccine and most nontuberculous mycobacteria. They will significantly improve on the performance of the century-old TST in the next few years (18–20), assuming that their performance in routine clinical practice on unselected populations of patients remains good.

We report here the results of the first routine hospital-based application of one of these tests, the QFT-Gold, in a cohort of 318 consecutive unselected patients evaluated in our hospital for possible TB infection and characterized by a significant rate of immunosuppression. A high proportion—about 1 out of 5 patients—of indeterminate QFT-Gold results was found in this population. Notably, patients receiving at least one immunosuppressive drug were significantly more likely to have an indeterminate QFT-Gold result. Indeterminate QFT-Gold results were also found significantly more often in patients with a negative TST result. This finding would suggest that a significant fraction of those negative TSTs could actually be false-negative results. However, because the TST does not have an internal positive control, this hypothesis cannot be verified.

Our results support the notion that the clinical utility of the TST is limited in subgroups of immunosuppressed patients (6), that is, those at higher risk of progression from infection to active disease (5). Even if the numbers in some categories are small and the confidence intervals are consequently wide, these results suggest for the first time that the QFT-Gold, that is, an *in vitro* test, might also have limited clinical usefulness in patients undergoing immunosuppressive treatments. However, in most patients tested, the QFT-Gold produced a valid result; thus, by producing an indeterminate result in others, this IFN-γ blood test may caution physicians to question the validity of a negative TST result. In other words, because the TST does not have an internal positive control, the clinician cannot distinguish between a true negative result and a false-negative result. With *in vitro* IFN-γ blood tests, however, a proportion of false-negative test results will be scored as indeterminate, hence allowing the clinician to disregard such results. Naturally, there would still be a certain proportion of false-negative test results associated with a valid positive control, as we also observed.

A recent study in a selected population of patients with clinical signs suggestive of TB showed that the QFT-Gold had 89% sensitivity (14). Another blinded prospective study on 82 patients with a high level of clinical suspicion for active TB showed a 85% sensitivity for active disease of a QFT-RD1 test. Interestingly, about 10% of these patients had indeterminate QFT results (13). Notably, all of the 216 BCG-vaccinated individuals and more than 95% of the 152 individuals with possible LTBI who were enrolled in the QFT-Gold study by Mori and coworkers did not have any form of immunosuppression, and all tests gave a valid (i.e., determinate) result (14). In contrast, the unselected hospital population of our study included immunosuppressed patients. Furthermore, in our case many QFT-Gold tests were performed under conditions not among those listed in the guidelines released by the Food and Drug Administration for the PPD-based QFT-TB (22). Thus, our patient population was one that had not been previously evaluated as also stated in the package insert of the product (23), and consequently our results might not be generalizable to the entire population of patients who are candidates for testing with the QFT-Gold. As previously reported in other studies (13, 14, 21), QFT was not influenced by BCG vaccination status; once more, our results confirm the higher specificity of the *in vitro* methods, and support their use in BCG-vaccinated individuals being screened for LTBI. QFT-Gold appeared to give more positive results than the TST in the patients with active TB, but the difference was not statistically significant. However, the power of our study to detect a difference was low because of the small number of patients with active TB who were enrolled in our study (due to its prospective nature). A larger study with more cases of active TB would be required to determine whether QFT-Gold is significantly more sensitive than TST in a hospital-based population of patients with suspected TB disease.

In conclusion, our study provides the results of the first routine hospital application of the new IFN-γ QFT-Gold blood test for the diagnosis of TB infection. Large prospective longitudinal studies are needed to precisely identify the factors influencing the test's performance, especially in those subgroups of immunosuppressed patients who, once infected, are at increased risk of progression to active TB.

***Conflict of Interest Statement***: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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