Comparative study between using QuantiFERON and tuberculin skin test in diagnosis of *Mycobacterium tuberculosis* infection

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**KEYWORDS**
*Mycobacterium tuberculosis*; Gamma interferon; QuantiFERON-TB Gold In-Tube (QFT-Gold IT); BCG vaccination; Tuberculin skin test; Ziehl–Neelsen stain

**Abstract**  
*Aim*: Study degree of sensitivity and specificity of IFN-γ as QuantiFERON-TB Gold In-Tube assay in diagnosis of tuberculosis instead of tuberculin test.

**Subjects and methods**: Forty patients were included in this study subdivided into two groups. First included 20 patients with sputum positive for tuberculosis, while the second included 20 patients suspected to catch TB infection, guided by: clinical and radiological examinations with smear negative for TB. Control group included 10 clinical and radiological healthy controls previously vaccinated by BCG with positive tuberculin test. All subjects were submitted for full clinical history physical examination routine laboratory tests, plain chest X-ray, sputum study for acid fast Bacilli by Ziehl–Neelsen stain, tuberculin test using Mantoux technique, sputum culture on Lowenstein-Jensen Medium and QuantiFERON-TB Gold In-Tube (QFT-Gold IT) assay.

**Results**: QFT-Gold IT test sensitivity = 100% specificity = 100% predictive value positive = 100% which means that 100% of the disease positive patients gave positive QFT-Gold IT test and predictive value negative = 100% which means that 100% of the disease negative patients...
gave negative QFT-Gold IT test. Agreement between tuberculin test and QFT-Gold IT test was good agreement where the χ² was 0.65 (CI = 0.39–0.91). Study revealed positive correlation between QuantiFERON level and severity of infection in sputum, which was statistically significant, where χ² was 0.92 and P-value was <0.05.

Conclusion: QFT-Gold IT, has excellent sensitivity and specificity unaffected by BCG vaccination. Tuberculin test specificity is high in non-BCG-vaccinated populations but low and variable in BCG vaccinated populations.

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Introduction

One-third of the world’s population is believed to be latently infected with Mycobacterium tuberculosis, the etiological agent of tuberculosis (TB), supported by the human immunodeficiency virus type I (HIV-1) pandemic and emerging multidrug resistance, underlines the need for new control measures and strategies to make a specific diagnosis and prevent transmission [17] although the tuberculin skin test (TST) is the method of choice for detecting latent M. tuberculosis infection (LTBI), it cannot be considered a gold standard because of the number of false-positive and negative reactions, and the variability of their interpretation [2].

New in vitro tests based on an ability to detect the gamma interferon (IFN-γ) released by activated T lymphocytes have recently been proposed [30]. These assays use antigens specific for M. tuberculosis, such as early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are two low-molecular-mass secretory proteins encoded by genes located within region of difference I (RD 1) of the M. tuberculosis genome [8,36]. All of the vaccine strains of BCG and most of nontuberculous mycobacteria (NTM) are not prevalent in this region, with the exception of Mycobacterium kansasi, Mycobacterium marinum, and Mycobacterium szulgai [24]. These proteins and the synthetic overlapping peptides corresponding to the full length of each elicit a strong T-cell response in animal models of TB [38] and human with active TB infection [34,39] or LTBI [35].

The QuantiFERON-TB assay (Cellestis Limited, Carnegie, Victoria, Australia) and the T-SPOT TB assay (Oxford Immunotec, Oxford, United Kingdom) are two commercial IFN-γ assays, and a number of in-house assays have also been assessed [3,30].

Aim of the work

The aim of this work was to study the degree of sensitivity and specificity of using IFN-γ as QuantiFERON-TB Gold In-Tube (QFT-Gold IT) assay (Cellestis Limited, Carnegie, Victoria, Australia) in diagnosis of infected population with M. tuberculosis instead of the tuberculin skin test (TST), as one of the new diagnostic methods also not affected by BCG vaccination so that it can be possible to create new control measures and strategies to make a specific diagnosis and prevent transmission.

Patients, materials and methods

Forty patients attending to Benha University hospital outpatient clinics were included in this study. Full clinical evaluation was undertaken including a full clinical and family history, physical examination, and routine laboratory tests.

Patients were subdivided into two groups. The first group included 20 patients with sputum positive for M. tuberculosis (TB) infection, while the second group included 20 patients suspected to catch TB infection, guided by: clinical examinations (symptoms and signs) and radiological studies with smear negative for TB. Control group included 10 age and sex matched clinical and radiological healthy controls previously vaccinated by BCG with positive tuberculin test.

The patients and controls were subjected for full clinical evaluation including a full clinical and family history, physical examination, and routine laboratory tests, plain chest X-ray (P.A. and lateral view), sputum study for acid fast Bacilli by Ziehl–Neelsen stain, tuberculin skin test (TST) using Mantoux technique, sputum culture on conventional Lowenstein-Jensen medium. The ready to use media for the primary isolation and identification of mycobacteria particularly M. tuberculosis was obtained from DIFCO Laboratories named the Bacto-Lowenstein medium.

QuantiFERON-TB Gold In-Tube (QFT-Gold IT) assay (Cellestis Limited, Carnegie, Victoria, Australia) was done as follows: a total 3 ml of blood was collected from each subject, with 1 ml directly collected into each of 3 colored tubes in the order of gray (negative control, “nil”), red (test tube), and purple (positive control; mitogen-coated) tubes. The test tube is a specifically designed blood collection tube coated with Mtb-specific antigens (ESAT-6, CFP-10, and a portion of TB antigen) and the synthetic overlapping peptides corresponding to the full length of each elicit a strong T-cell response.

The patients and controls were subjected for full clinical evaluation including a full clinical and family history, physical examination, and routine laboratory tests, plain chest X-ray (P.A. and lateral view), sputum study for acid fast Bacilli by Ziehl–Neelsen stain, tuberculin skin test (TST) using Mantoux technique, sputum culture on conventional Lowenstein-Jensen medium. The ready to use media for the primary isolation and identification of mycobacteria particularly M. tuberculosis was obtained from DIFCO Laboratories named the Bacto-Lowenstein medium.

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Comparison of quantitative variables between the study groups was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is < 5. Odds ratio (OR) was calculated as a measure of association for polymorphism and allelic presence between cases and control groups. A probability value ($P$ value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Regarding clinical variables among studied groups, 9 patients (45%) of group I had chronic cough, 3 patients (15%) had hemoptysis and 12 patients (60%) had toxemia. As for group II: 5 patients (25%) had chronic cough, 1 patient (5%) had hemoptysis and 3 patients (15%) had toxemia. Comparison between group I and group II according to symptoms was statistically insignificant as shown in as $P$-value was >0.05. According to signs: 13 patients (65%) of group I had crepitations and 5 patients (25%) had signs of toxemia. As for group II: 5 patients (25%) had crepitations and 1 patient (5%) had signs of toxemia. Comparison between group I and group II according to signs was statistically insignificant as shown in (Table 1) as $P$-value was > 0.05.

Discussion

The QuantiFERON-TB Gold assay is an enzyme-linked immunosorbent assay (ELISA) that measures the production of interferon gamma (IFN-$\gamma$) by T-cells after sensitization with $M. tuberculosis$ antigens using whole blood [6,26,18] (Fig. 1,Tables 2–6 and 8).

This work aimed to study the degree of sensitivity and specificity of using IFN-$\gamma$ as QuantiFERON-TB Gold In-Tube (QFT-Gold IT) assay (Cellestis Limited, Carnegie, Victoria, Australia) in diagnosis of infected population with $M. tuberculosis$ instead of the tuberculin skin test (TST), as one of the new diagnostic methods so that it can be possible to create new control measures and strategies to make a specific diagnosis and prevent transmission.

The age in group I was 28.4 ± 4.7 years, in group II was 27.3 ± 5.1 years, and in control was 28.9 ± 4.6 years. It was noticed that all groups were age matched and there were no statistical differences between them.

This study revealed that, the results of tuberculin test and Z.N. in diseased groups was:

In group I: 16 patients were positive by tuberculin test and all of them were positive by Z.N., and 4 patients were negative by tuberculin test, while they were positive by Z.N.

In group II: 12 patients were positive by tuberculin test and all of them were negative by Z.N., and 8 patients were negative by tuberculin test, and all of them were negative by Z.N.

In group I: male patients were 15 (30%) while female patients were 5 (10%), in group II: male patients were 13 (26%) while female patients were 7 (14%) and in control: male patients were 8 (16%) while female patients were 2 (4%). It was noticed that all groups were sex matched and there were no statistical differences between them.

Evaluation of tuberculin test as a diagnostic test in relation to culture showed that: tuberculin test sensitivity = 94.7%, specificity = 80%, positive predictive value (PPV) = 90% which means that 90% of the disease positive patients gave positive tuberculin test and negative predictive value (NPV) = 66.7% which means that 66.7% of the disease negative patients gave negative tuberculin test.

In our results, there was a positive correlation between Quantiferon level and severity of infection in sputum, which was statistically significant, where ‘$r$’ was 0.92 and $P$-value was <0.05.

Evaluation of QFT-Gold IT test as a diagnostic test for TB in relation to culture showed that: QFT-Gold IT test sensitivity = 100%, specificity = 100%, positive predictive value (PPV) = 100% which means that 100% of the disease positive patients gave positive QFT-Gold IT test and negative predictive value (NPV) = 100% which means that 100% of the
Agreement between tuberculin test and QFT-Gold IT test was a good agreement, where the 'κ' was 0.65 (CI = 0.39–0.91). Approximate to our results: in a US study, QuantiFERON-TB Gold IT and TST results were available for 391 volunteers. None were BCG vaccinated. QuantiFERON-TB Gold IT Specificity was 99.2% while that of TST was 98.5%. A second specificity study was performed with QuantiFERON-TB Gold IT in low risk individuals in Japan, approximately 90% who had received BCG vaccination, it was 98.4% ([33], Cellestis). Also TB-suspects from Australia and Japan who were subsequently confirmed to have *M. tuberculosis* infection by culture were tested to evaluate sensitivity of QuantiFERON-TB Gold IT. Overall sensitivity of QuantiFERON-TB Gold IT for active TB disease was 89% ([33], Cellestis).

In addition to the multicenter study, two other published studies have demonstrated moderate concordance between TST and QFT [37,32]. One of the first reports that assessed the liberation of IFN-γ after exposure to tuberculin was performed in Australia and showed a specificity of 98% and a sensitivity of 90% [37]. The other study evaluated QuantiFERON-TB (QIFN), in 455 individuals from three groups: group I, 237 immigrants from high-risk countries; group II, 127 health care workers undergoing Mantoux testing; group III, 91 patients being investigated for possible active tuberculosis. The QIFN results were compared either to those of the Mantoux test. For group I, the agreement between QIFN and Mantoux results was 89% for Mantoux-negative and 64% for Mantoux-positive individuals. For group II, the agreement was 81% for Mantoux-negative and 67% for Mantoux-positive individuals. For group III, agreement was 81% for Mantoux-negative and 86% for Mantoux-positive patients.

### Table 3  Comparison between different groups according to residence.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Control</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>14</td>
<td>28</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Rural</td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

### Table 4  Results of Quantiferon levels in different groups.

<table>
<thead>
<tr>
<th>Quantiferon levels in different tubes (IU/ml)</th>
<th>Group I</th>
<th>Group II</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>10.85</td>
<td>9.9</td>
<td>0.65</td>
</tr>
<tr>
<td>Mitogen</td>
<td>4.64</td>
<td>4.23</td>
<td>2.34</td>
</tr>
<tr>
<td>Nil</td>
<td>0.16</td>
<td>0.22</td>
<td>0.14</td>
</tr>
</tbody>
</table>

### Table 5  Correlation between Quantiferon and degree of Z.N. positivity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r (Correlation coefficient)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity of infection in sputum</td>
<td>0.92</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>(smear positivity)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 6  Evaluation of QFT-Gold IT test as a diagnostic test for TB in relation to culture in diseased groups.

<table>
<thead>
<tr>
<th>Culture</th>
<th>QFT-Gold IT</th>
<th>QFT-Gold IT Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>–ve</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

### Table 7  Correlation between Quantiferon and diameter of cavity in X-ray.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r (Correlation coefficient)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavitations in X-ray</td>
<td>0.83</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>(diameter of cavity)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Similar to our results, in an Indian study of 726 healthcare workers: there was setting of very high TB rates. There was 40% QFT-Gold IT positive and 41% TST positive. QFT-Gold IT showed high concordance with TST, no effect of BCG on either test. Both tests related to risk factors of age and period of work in healthcare [29].

Also, in a study of 105 hospitalized children in whom TB was suspected, QFT-Gold IT and TST was done. 10.5% QFT-Gold IT was positive and 9.5% TST was positive. Agreement between tests was 95.2% overall and 100% in non-BCG vaccinated [16].

On the other hand, in a study of 309 German contacts: 51% were BCG vaccinated and 27% foreign born. 70% of BCG vaccinates and 18% of non-vaccinated were TST positive, whereas 9% and 11% were QFT-Gold IT positive, respectively. QFT-Gold IT was associated with TB risk. TST was only associated with BCG vaccination [15].

Similar to our results, QFT results are less subject to reader bias and error compared with TST. In a CDC-sponsored multicenter trial, QFT and TST results were moderately concordant (overall kappa value = 0.60). The level of concordance was adversely affected by prior bacille Calmette–Guerin (BCG) vaccination, immune reactivity to nontuberculous mycobacteria (NTM), and a prior positive TST [25]. However, one of the five sites involved in the CDC study reported less agreement [7].

In addition to the multicenter study, two other published studies have demonstrated moderate concordance between TST and QFT [37,32]. One of the first reports that assessed the liberation of IFN-γ after exposure to tuberculin was performed in Australia and showed a specificity of 98% and a sensitivity of 90% [37]. The other study evaluated QuantiFERON-TB (QIFN), in 455 individuals from three groups: group I, 237 immigrants from high-risk countries; group II, 127 health care workers undergoing Mantoux testing; group III, 91 patients being investigated for possible active tuberculosis. The QIFN results were compared either to those of the Mantoux test. For group I, the agreement between QIFN and Mantoux results was 89% for Mantoux-negative and 64% for Mantoux-positive individuals. For group II, the agreement was 81% for Mantoux-negative and 67% for Mantoux-positive individuals. For group III, agreement was 81% for Mantoux-negative and 86% for Mantoux-positive patients.
For patients being evaluated for active tuberculosis, the performance of the Mantoux test was not statistically different from that of the QIFN assay. In patients with active tuberculosis, the assay had a sensitivity of 77%, not significantly higher for extrapulmonary than pulmonary cases (83% versus 74%). QIFN sensitivity was not significantly different for smear-negative or smear-positive cases (80% versus 71%). The QIFN assay is a potential replacement for the Mantoux test. The acceptability of these performance values and those of similar evaluations could determine the place this test will have in detecting evidence of mycobacterial infection [32].

Also, a recently published study demonstrated that a positive IGRA result is predictive of future active TB risk. Moreover, IGRA was at least as sensitive and was more specific compared to traditional TST. In this study of immunocompetent recently exposed close contacts of active TB cases, the progression rate to active disease among untreated TST positives was significantly greater than for untreated QFT positive individuals (14.6% versus 2.3%). Although the numbers were small, all of the close contacts who went onto develop active TB were QFT positive, but only 83% were TST positive [14].

As noted above, prior BCG vaccination can produce false positive TST results. In a study of military personnel returning from missions, about one-half of the positive TSTs were falsely positive [12]. A more recent study of military returning from missions, reported evidence suggesting false positive TST results are common and that QFT testing could guide more targeted treatment and alleviate unnecessary anti-tuberculosis treatment [19].

According to the FDA approved package insert QuantiFERON-TB Gold In-Tube has a consistent specificity of >99% in low risk individuals and a sensitivity as high as 92% in individuals with active disease, depending on setting and extent of disease. The specificity in two studies of a few hundred people is 96–98% in a health immunized population ([33], Cellestis). In a previous study, QuantiFERON-TB test was used to detect infection in contacts in a tuberculosis outbreak at a Danish high school. The majority of the contacts were BCG-unvaccinated, which allowed a direct comparison of the skin test and the novel blood test in individuals whose skin test was not confounded by vaccination. An excellent agreement between the two tests was found (94%, x value 0.866), and in contrast, the novel blood test was not influenced by the vaccination status of the subjects tested [10].

In countries with a higher prevalence of non-tubercular mycobacterial infections, the correlation between the tests may decline; for example, a report from Korea described a poor correlation between the test in healthy volunteers with and without risk factors for tuberculosis exposure (κ = 0.08–0.17). Kang and colleagues found 44 out of 54 (81.5%) patients with active TB were QFT positive, whereas the TST detected 42 (77.8%). The TST was clearly corrupted by BCG [21].

Among 785 study participants, inters assay agreement between TST and Interferon-γ assays was 89.6% (κ = 0.59) [4].

A study that compared the TST with the QFT-G, carried a sensitivity of 42.2% and a specificity of 97.9%. The QFT-G agreed with a positive skin test result for 86.5% [5].

In 129 patients with culture-proven M. tuberculosis disease the sensitivity of Interferon-Gamma test was 81% compared to 89% for TST (P = 0.06). When positive responses in both TST and Interferon-Gamma test were combined, 96% of TB patients were detected. Interferon-Gamma test may prove an alternative to skin testing for detecting M. tuberculosis infection [11].

Results of 30 elderly patients with active TB, 27% had a positive TST result and 77% had a positive QFT-2G result. Of 100 younger patients with active TB, 70% had a positive TST result and 87% had a positive QFT-2G result. Although there was no significant difference between the two patient groups in the positive rate of QFT-2G test (P = 0.185), there was a significant difference in the rates of positive TST result between elderly and younger patients (P = 0.012). The study confirmed that the QFT-2G test might be a more useful method of diagnosing TB infection than the TST test for elderly patients [22].

Data for 207 children with valid tuberculin skin test and QuantiFERON-TB Gold In-Tube test results were analyzed. There was an excellent correlation between negative tuberculin skin test results and negative QuantiFERON-TB Gold In-Tube test results; however, only 23% of children with positive tuberculin skin test results had positive QuantiFERON-TB Gold In-Tube test results. The study confirmed that the QuantiFERON-TB Gold In-Tube test is a specific test for M. tuberculosis exposure in children, with performance characteristics similar to those for adults residing in regions with low levels of endemic disease [23].

Another study compared the effectiveness of the QuantiFERON-TB Gold (QFT) assay with the Mantoux tuberculin skin test to detect M. tuberculosis infection in 29 children during a school outbreak of tuberculosis. Of the 21 children with M. tuberculosis infection, 11 had a radiograph suggestive of the infection. The QFT assay was positive in all 21 of the children, and the Mantoux test was negative at first testing in 2 children. The findings demonstrated that the QFT test is extremely useful in accurately identifying infected and uninfected children, permitting rapid intervention [27].

In a study of 938 enrollees from Kenya, 909 had a tuberculin skin test reading, and QuantiFERON-TB assay results and were included in further analysis. Overall, 553 (61%) had a positive tuberculin skin test (TST) and 512 (56%) had a positive QuantiFERON-TB assay. There was substantial agreement between TST and QuantiFERON-TB assay with a kappa of 0.77 (p > 0.001). A BCG scar was visible on 537 (59%) of the 909. In these 537 individuals, 387 (72%) had a positive TST and 339 (63%) a positive QuantiFERON-TB assay again with a kappa of 0.77 (p > 0.001). In this setting, both methods were more reactive in BCG vaccinated individuals [1].

On the contrary to our study, 207 Japanese healthcare students recruited at Okayama University received both the TST and the QFT to assess the level of agreement between these two tests. The agreement between the QFT and the TST results was poor, with positive result rates of 1.4% vs. 27.5%, respectively. As a baseline screening test for low-risk students at their course entry, QFT yielded quite discordant results, compared with the TST, probably because of the low specificity of the TST results in the BCG-vaccinated population [20].

A recent Review article incorporated newly reported evidence from 20 studies into an updated meta-analysis on the sensitivity and specificity of IGRA, including studies that evaluated QuantiFERON-TB Gold, QuantiFERON-TB Gold In-Tube (both from Cellestis, Victoria, Australia), and T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom) or...
its precommercial ELISpot version, when data on the commercial version were lacking. The pooled sensitivity was 78% (95% CI, 73–82%) for QuantiFERON-TB Gold, 70% (CI, 63–78%) for QuantiFERON-TB Gold In-Tube, and 90% (CI, 86–93%) for T-SPOT.TB. The pooled specificity for both QuantiFERON tests was 99% among non-BCG-vaccinated participants (CI, 98–100%) and 96% (CI, 94–98%) among BCG-vaccinated participants. The pooled specificity of T-SPOT.TB (including its precommercial ELISpot version) was 93% (CI, 86–100%). Tuberculin skin test results were heterogeneous, but specificity in non-BCG-vaccinated participants was consistently high (97% [CI, 95–99%]). The study concluded that the IGRAs, especially QuantiFERON-TB Gold and Quantiferon-TB Gold In-Tube, have excellent specificity that is unaffected by BCG vaccination. Tuberculin skin test specificity is high in non-BCG-vaccinated populations but low and variable in BCG-vaccinated populations. Sensitivity of IGRAs and TST is not consistent across tests and populations, but T-SPOT.TB appears to be more sensitive than both Quantiferon tests and TST [31].

ELISA-IGRA seems to be highly specific for active tuberculosis, as defined by the number of patients without tuberculosis who have negative ELISA-IGRA. In 4 studies that addressed this issue, the specificity of ELISA-IGRA especially QFT ranged between 97% and 100% [9,10,28,34]. Mori and colleagues, found the sensitivity of QFT test to be 89%, with QFT ranged between 97% and 100%[9,10,28,34]. Mori and addressed this issue, the specificity of ELISA-IGRA especially QFT ranged between 97% and 100%. Specificity is high in non-BCG-vaccinated populations but low and variable in BCG-vaccinated populations. Sensitivity of IGRAs and TST is not consistent across tests and populations, but T-SPOT.TB appears to be more sensitive than both Quantiferon tests and TST [31].

There was also a positive correlation between Quantiferon level and cavitations in X-ray, which was statistically significant, where r² was 0.83 and P-value was < 0.05 (Table 7). Similarly, In the 16th European Congress of Clinical Microbiology and Infectious Diseases, results showed a generally good agreement between the clinical findings and the Quantiferon-TB test results [13].

Conclusion

QFT-Gold IT has excellent sensitivity and specificity that is unaffected by BCG vaccination or other variables. TST specificity is high in non-BCG-vaccinated populations but low and variable in BCG-vaccinated populations. There is a good agreement between the clinical findings and the QuantiFERON-TB test results.

References


