Fourth generation QuantiFERON-TB Gold-Plus: What is the evidence?

Arena Shafeque¹, Jacob Bigio²,³, Catherine A. Hogan¹,⁴, Madhukar Pai³,⁵, Niaz Banaei¹,⁴,⁶

¹ Department of Pathology, Stanford University School of Medicine, Stanford, CA
² Research Institute of the McGill University Health Centre, Montreal, QC, Canada
³ McGill International TB Centre, Montreal, QC, Canada
⁴ Clinical Microbiology Laboratory, Stanford Health Care, CA, USA
⁵ Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada
⁶ Division of Infectious Diseases & Geographic Medicine, Stanford University School of Medicine, Stanford, CA

Running title: Evidence for QuantiFERON-TB Gold-Plus

Correspondence
Niaz Banaei, MD
Rm. 1602, 3375 Hillview Ave, Palo Alto, CA 94304
Phone: 650-736-8052 Fax 650-725-5671
E-mail: nbanaei@stanford.edu
Abstract

QuantiFERON-TB Gold Plus (QFT-Plus) is the latest generation of interferon-gamma release assays (IGRAs) to receive approval from the US FDA, replacing its predecessor QuantiFERON-TB Gold In-Tube (QFT-GIT). The novelty of QFT-Plus is that it elicits a response from CD8 T-cells in addition to CD4 T-cells, thus collecting a broader response from T-cell subsets compared with QFT-GIT. It was developed with the aim to improve detection of M. tuberculosis infection (LTBI), especially among recently exposed, immunocompromised hosts and young children. In this mini review, we summarize the performance of QFT-Plus compared with QFT-GIT among active TB patients (a surrogate for LTBI), high-risk populations, and low-risk individuals based on recent publications. Studies comparing QFT-Plus to QFT-GIT currently do not support superior performance of QFT-Plus in individuals with active TB and LTBI. The difference in sensitivity between QFT-Plus and QFT-GIT in active TB patients was not significant in nearly all studies and ranged from -4.0 to 2.0%. Among high-risk groups, the agreement between QFT-Plus and QFT-GIT was 89.9 to 96.0% (kappa 0.80 to 0.91). The specificity in the low-risk population was slightly lower in QFT-Plus than QFT-GIT with a difference ranging from -7.4 to 0%. Further studies are needed to accurately evaluate the sensitivity of QFT-Plus in immunocompromised hosts and children. In addition, further evidence is required to validate a modified interpretation of QFT-Plus for the identification of false-positive results in low-risk healthcare workers.
Introduction
Up to one quarter of the global population is estimated to be infected with *Mycobacterium tuberculosis* (Mtb) (1), 5-10% of whom will progress to active tuberculosis (TB) during their lifetime (https://www.who.int/publications-detail/who-consolidated-guidelines-on-tuberculosis-module-1-prevention-tuberculosis-preventive-treatment). To achieve the End TB Strategy target of a 90% reduction in TB incidence rate by 2035, the World Health Organization (WHO) recommends the testing and preventive treatment of latent TB infection (LTBI) in high-risk groups (https://www.who.int/publications-detail/who-consolidated-guidelines-on-tuberculosis-module-1-prevention-tuberculosis-preventive-treatment). These groups include people living with HIV, household contacts of people with active TB and patients initiating immunotherapy, receiving dialysis or preparing for transplant (https://www.who.int/publications-detail/who-consolidated-guidelines-on-tuberculosis-module-1-prevention-tuberculosis-preventive-treatment). Widespread LTBI testing is required to achieve this target goal.

Current testing options for LTBI include the conventional tuberculin skin test (TST) and more recently introduced interferon-gamma (IFN-γ) release assays (IGRAs). IGRAs are in vitro blood tests which measure IFN-γ release by antigen-specific T-cells in response to stimulation by Mtb antigens. Advantages and limitations of IGRAs have been covered in prior reviews (2, 3). Unlike the TST, IGRAs do not cross react with Bacille Calmette–Guérin (BCG) vaccine and nontuberculous mycobacteria with the exception of *M. kansasii, M. szulgai* and *M. marinum* (https://www.quantiferon.com/us/wp-
IGRAs share some of the limitations of the TST. Neither can reliably distinguish LTBI from active TB, both have reduced sensitivity in immunocompromised patients, and neither has an adequate positive predictive value for progression to active TB (2). In addition, IGRAs have shown lower specificity and more variability than TST in low-risk subjects especially low-risk North American healthcare workers (2).

The most widely used IGRAs are the QuantiFERON (Qiagen, Venlo, Netherlands) and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom). The latest IGRA to receive FDA approval is the fourth generation QuantiFERON-TB Gold Plus (QFT-Plus) assay, a replacement for the QuantiFERON-TB Gold In-Tube (QFT-GIT). This review will focus solely on QFT-Plus.

QFT-Plus is an enzyme-linked immunosorbent assay (ELISA)-based whole blood test which measures the IFN-γ response of T-cells to the ESAT-6 and CFP-10 peptide antigens. The measured response is in international units (IU) per milliliter (mL). Unlike QFT-GIT, it does not contain TB7.7 antigen and the formulation of antigen varies between QFT-Plus and QFT-GIT such that antigen is sprayed in QFT-Plus vs. resin coated in QFT-GIT (https://www.quantiferon.com/us/wp-content/uploads/sites/13/2020/01/L1095849-R06-QFT-Plus-ELISA-IFU.pdf). The QFT-Plus assay consists of four tubes, rather than the three tubes of QFT-GIT: a negative control (nil) tube which measures background IFN-γ response, a positive control (mitogen) tube, which measures antigen-independent T-cell response, the TB1 antigen...
tube, which contains ESAT-6 and CFP-10 peptide antigens to primarily detect the CD4 T-cell response, and the TB2 antigen tube, which contains additional shorter peptides from ESAT-6 and CFP-10 to detect both CD4 and CD8 T-cell responses. The TB1 antigen tube is essentially the same as the QFT-GIT TB antigen tube with the exception of TB7.7 antigen missing from the former. As shown in Table 1, results of the QFT-Plus assay, like QFT-GIT, are reported qualitatively as positive, negative or indeterminate.

The modification of QFT-GIT to additionally detect a CD8 T-cell response was included to broaden the immune response to Mtb antigen in hope of improving assay sensitivity for detection of Mtb infection, especially among recent contacts, immunocompromised hosts and young children. (https://www.quantiferon.com/us/wp-content/uploads/sites/13/2020/01/L1095849-R06-QFT-Plus-ELISA-IFU.pdf). Prior studies have shown greater frequency of antigen-specific CD8 T-cells, producing IFN-γ and other cytokines, in active TB compared to LTBI (4-6) and among recent contacts of TB patients compared to TB patients and healthy controls (7). Increased mycobacterial bacillary load has also been reported to produce a greater CD8 T-cell response (6).

There is evidence that active TB in children can be distinguished from TB exposure by the magnitude of the CD8 T-cell response, especially in those under 5 years of age (8). In HIV-infected individuals, monofunctional CD8 T-cell responses to Mtb antigens were observed, even with low CD4 cell count (9-11). However, alongside these potential benefits, the QFT-Plus assay requires an additional blood collection tube and extra ELISA well so its adoption over QFT-GIT decreases testing throughput and increases
the per-test cost in most settings. Thus, it is crucial that modifications made to QFT-Plus improve its clinical performance and justify the added costs of labor and reagents.

In this mini review, we summarize the emerging literature on performance of QFT-Plus compared with QFT-GIT among patients with active TB (a surrogate for LTBI), high-risk patients, and low-risk individuals. PubMed electronic database was searched until December 2019. We focused on cross-sectional studies with head-to-head comparisons to obtain an accurate assessment of QFT-Plus compared with QFT-GIT since performance characteristics of QFT-GIT are well-studied and summarized in several meta-analyses.

**Sensitivity in active TB patients**

Several investigators have conducted head-to-head studies comparing the sensitivity of QFT-Plus to QFT-GIT in patients with active TB (Table 2). Both microbiological and clinical reference standards were used. Except for one pediatric study discussed below, all other studies were conducted in adult patients and had very low representation of HIV coinfection and immunocompromising conditions.

Collectively, these studies show nearly identical sensitivities between QFT-Plus (range, 85% to 100%) and QFT-GIT (range, 85% to 100%). As shown in Table 2, the difference in sensitivity ranged from -4.0 to 2.0%.
Quantitatively, IFN-γ response in QFT-GIT (TB Ag-Nil) was shown to be significantly higher than QFT-Plus for either TB1 or TB2 antigen tube minus Nil (Table 2). This finding is most likely due to reformulation of the antigens in QFT-Plus (sprayed in QFT-Plus vs. resin coated in QFT-GIT). However, removal of TB 7.7 antigen from QFT-Plus could also account for a lower response in TB1 and TB2 compared with QFT-GIT.

Furthermore, in several studies, higher positivity rate and higher median IFN-γ level was reported with TB2 tube compared with TB1 (Table 2) (12-14), which is likely due to stimulation of both CD8 and CD4 T-cells in TB2. Response to TB2 antigen alone in absence of TB1 response has also been reported (15-17).

Sensitivity of QFT-Plus compared to QFT-GIT in patients coinfected with HIV and TB remains poorly characterized. A study conducted in Zambia showed 85% sensitivity with QFT-Plus among culture-positive, active TB patients who were HIV positive (n=68) (17). While the study did not include a head-to-head comparison with QFT-GIT, the authors argued that QFT-Plus has higher sensitivity than QFT-GIT in HIV coinfected patients given that 63% sensitivity with QFT-GIT was observed in an earlier study in the same setting (18). Similar to QFT-GIT, this study also showed that the positivity rate decreases in HIV infected patients with decreasing CD4 T-cell count (17). Thus, although QFT-Plus may appear to have enhanced sensitivity compared with QFT-GIT in HIV-positive TB patients, head-to-head comparison of QFT-Plus to QFT-GIT with adjustment for CD4 T-cell count is needed to accurately demonstrate higher sensitivity of QFT-Plus in this population.
A single study compared the sensitivity of QFT-Plus to QFT-GIT in children with TB. This small study conducted in Eswatini showed identical sensitivity between QFT-Plus and QFT-GIT among children with active TB based on microbiological and clinical reference standards (Table 2) (19).

**Detection of latent infection**

Performance of QFT-Plus compared to QFT-GIT for diagnosis of LTBI has been assessed in high-risk populations including close contacts of active TB cases, immigrants from high risk countries, immunocompromised individuals such as HIV infected, individuals having received a solid organ or hematopoietic stem cell transplant, patients on immunotherapy, children <5 years, and institutionalized individuals (https://www.cdc.gov/tb/topic/basics/risk.htm). As shown in Table 3, except for one study, all other studies have demonstrated significant agreement between the two tests (≥93.7%). Kappa values overall ranged from 0.80 to 0.91. Lack of discordance between QFT-Plus and QFT-GIT indicates that QFT-Plus has a comparable sensitivity to QFT-GIT for detection of LTBI (Table 3). Most discordant results were due to TB response close to assay cut-off in the range of 0.2-0.7 IU/ml (20-23). In the only pediatric study among 46 children with household Mtib exposure, agreement between the QFT-Plus and QFT-GIT was 96% and the positivity rate was identical (19). One study reported ≥10% higher positivity rate with QFT-Plus compared with QFT-GIT, however, the positivity rate with TB1 and TB2 were identical (25). Given that TB1 contains the same antigens as those in QFT-GIT except for the exclusion of TB7.7, this suggests that higher positivity observed with QFT-Plus over QFT-GIT may have been due to antigen
The difference in IFN-γ response between TB2 and TB1 in QFT-Plus has been used by some investigators as a surrogate for CD8 T-cell response (20, 21, 25). A difference (TB2-TB1) >0.6 IU/ml was considered as the threshold for CD8 T-cell response. Using this approach, some studies have shown an association between CD8 T-cell response and exposure intensity, proximity to index case and proximity to time of infection (21, 25). However, these findings have not been reproducible in other studies (20). This may be in part explained by the fact that TB1 antigens also elicit a CD8 T-cell response through class 1 MHC antigen presentation (13). Further studies are needed to show whether TB2-TB1 difference can be used as an accurate measure of CD8 T-cell response.

Specificity in low-risk populations

Several studies have compared the specificity of QFT-Plus to QFT-GIT in low-risk populations. This group includes healthy adults with no or low risk factors for TB exposure and healthcare workers in low TB incidence settings. The risk was assessed by TB questionnaires obtained before study enrollment. Specificity was estimated by measuring the percent negativity for QFT-Plus and QFT-GIT. Overall, these studies show comparable specificity between QFT-Plus and QFT-GIT (Table 4). The specificity of QFT-Plus is slightly lower than QFT-GIT in some studies, but the difference is not...
statistically significant, and no clear pattern has emerged in these studies. One study showed that the specificity of QFT-Plus is not affected by infection by *M. avium* complex and *M. abscessus* group, the two most common NTM (26).

QFT-Plus and QFT-GIT were qualitatively and quantitatively highly concordant in low-risk HCWs (Table 4). Positivity rate in 626 HCWs with no risk factors for LTBI was 3.0% for QFT-Plus using the manufacturer’s interpretation compared with 2.1% for QFT-GIT. CDC recently withdrew the recommendation for serial TB screening with IGRA in low-risk HCWs due to high conversion and reversion rates, and higher false-positive rates compared with TST (27, 28). Moon and colleagues have proposed a conservative interpretation of QFT-Plus, based on positivity of both TB1 and TB2 vs. manufacturer’s interpretation where either tube can be positive, to increase assay specificity in low-risk HCWs (29). Application of this approach led to a reduction in the positivity rate in no-risk HCWs from 3.0% to 1.0%. Follow-up testing of eleven HCWs with discordant results between TB1 and TB2 in QFT-Plus showed reversion to negative results in ten cases with no progression to active TB in any of the participants. If confirmed in other studies, the conservative interpretation of QFT-Plus in low-risk populations may represent a viable approach to identifying false-positive results in low-risk individuals without the need for repeat testing.

**Sources of variability**

Sources of variability impacting IGRA are classified into pre-analytical, analytical, postanalytical, manufacturing and immunological (3). Although sources of variability
were largely investigated and described for the QFT-GIT, these might apply to QFT-Plus as well. Further research and modeling are needed to investigate and quantify the variability introduced from known sources due to addition of the second antigen tube in QFT-Plus. Agarwal and colleagues have recently identified a previously unrecognized source of variability for QFT-Plus due to the method of blood collection (30). Blood was collected directly in QFT-Plus tubes (plus-direct) and also in a separate blood collection tube from where blood was transferred to the QFT-Plus tubes (plus-transfer). Positive rate for plus-direct was 12% compared with 17% for plus-transfer method. Agreement between plus-direct and plus-transfer was 85% (kappa 0.37, p<0.001). This finding supports variability in QFT-Plus and highlights the need for consistent blood collection methods in individuals undergoing serial testing.

Predictive value of QFT-Plus
No study has yet been published on the predictive value of positive QFT-Plus on progression from latent infection to active TB. Two studies evaluating the prognostic performance of QFT-Plus- The Correlate of Risk Targeted Intervention Study in HIV uninfected (CORTIS-01) (https://clinicaltrials.gov/ct2/show/NCT02735590) and HIV infected (CORTIS-HR) (https://zivahub.uct.ac.za/articles/CORTIS-HR_Statistical_Analysis_Plan/11792079) have recently been completed in South Africa. Findings from these trials are currently being analyzed and should be published soon.

Conclusion
Although QFT-Plus was launched with the promise of improved performance over QFT-GIT through the addition of CD8 T-cell response, studies directly comparing QFT-Plus with QFT-GIT in TB patients, high-risk groups, and low-risk population have not revealed any significant improvement in its performance. Further research in immunocompromised individuals and children is needed to determine the performance of QFT-Plus in these groups.

Unanswered questions

Although studies described in this mini review have advanced our understanding on the performance of QFT-Plus, there are a number of questions that remain unanswered. The following represent areas in need of further research to complete our understanding of QFT-Plus.

1. **Sensitivity in HIV coinfected individuals.** Head-to-head comparison of QFT-Plus to QFT-GIT with adjustment for CD4 count is needed in patients with active TB and HIV to assess whether QFT-Plus has a higher sensitivity in the HIV-coinfected individuals.

2. **Sensitivity in children.** Head-to-head comparison of QFT-Plus to QFT-GIT with sufficient number of children with TB disease is needed to accurately assess QFT-Plus sensitivity in this group.

3. **Predictive value.** Head-to-head comparison of QFT-Plus to QFT-GIT is needed to determine the predictive value of QFT-Plus for progression to active TB. Studies are...
also needed to determine whether the CD8 T-cell response derived from QFT-Plus can accurately identify patients with recent and high intensity exposure who are at a greater risk of progressing to active TB.

4. **Conservative interpretation.** Further studies are needed to validate the conservative interpretation of QFT-Plus in low-risk populations and to define quantitative cut-offs that enhance its accuracy.

5. **Reproducibility.** Further research is needed to assess reproducibility of QFT-Plus and investigate sources of variabilities introduced with the addition of second tube and reformulation of peptide antigens.

**References**


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47:1587-1590.

Goletti D. 2017. Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold
In-tube assays in subjects with or without tuberculosis. Tuberculosis 106:38-43.


Table 1. Result interpretation of QFT-Plus and QFT-GIT

<table>
<thead>
<tr>
<th>Result</th>
<th>QFT-Plus</th>
<th>QFT-GIT</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Nil ≤8.0; and TB1 and/or TB2 minus Nil ≥0.35 and ≥25% of Nil</td>
<td>Nil ≤8.0; and TB Antigen minus Nil ≥0.35 and ≥25% of Nil</td>
<td><em>M. tuberculosis</em> infection likely</td>
</tr>
<tr>
<td>Negative</td>
<td>Nil ≤8.0, Mitogen minus Nil ≥0.5; and TB1 and TB2 minus Nil &lt;0.35 or ≥0.35 and &lt;25% of Nil</td>
<td>Nil ≤8.0, Mitogen minus Nil ≥0.5; and TB Antigen minus Nil &lt;0.35 or ≥0.35 and &lt;25% of Nil</td>
<td><em>M. tuberculosis</em> infection is not likely</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Nil &gt;8.0; or Nil ≤8.0 and TB1 and TB2 minus Nil &lt;0.35 or ≥0.35 and &lt;25% of Nil and Mitogen minus Nil &lt;0.5</td>
<td>Nil &gt;8.0; or Nil ≤8.0 and TB Antigen minus Nil &lt;0.35 or ≥0.35 and &lt;25% of Nil and Mitogen minus Nil &lt;0.5</td>
<td>Likelihood of <em>M. tuberculosis</em> cannot be determined</td>
</tr>
</tbody>
</table>
Table 2. Head-to-head comparison of sensitivity between QFT-Plus and QFT-GIT in patients with active TB disease

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Country</th>
<th>Sample size</th>
<th>No. (%) of IC hosts</th>
<th>Adult/Pediatric (Median age)</th>
<th>Sensitivity</th>
<th>Median or Mean IFN-γ (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(31)</td>
<td>Germany</td>
<td>24 (MRS)</td>
<td>4 (7.0)</td>
<td>Adult (NA)</td>
<td>95.8%</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33 (CRS)</td>
<td></td>
<td></td>
<td>84.8%</td>
<td>3.70</td>
</tr>
<tr>
<td>(12)</td>
<td>USA and Japan</td>
<td>164 (MRS)</td>
<td>4 (2.4)</td>
<td>Adult (71)</td>
<td>93.0%</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94.3%</td>
<td>3.56</td>
</tr>
<tr>
<td>(13)</td>
<td>Italy</td>
<td>27 (23 MRS, 4 CRS)</td>
<td>0 (0.0)</td>
<td>Adult (38)</td>
<td>85.0%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>89.0%</td>
<td>NA</td>
</tr>
<tr>
<td>(15)</td>
<td>Italy</td>
<td>69 (49 MRS, 20 CRS)</td>
<td>0 (0.0)</td>
<td>Adult (35)</td>
<td>90.0%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>88.0%</td>
<td>NA</td>
</tr>
<tr>
<td>(32)</td>
<td>Japan</td>
<td>162 (MRS)</td>
<td>9 (5.5)</td>
<td>Adult (59)</td>
<td>91.1%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90.7%</td>
<td>NA</td>
</tr>
<tr>
<td>(16)</td>
<td>South Korea</td>
<td>33 (16 MRS, 17 CRS)</td>
<td>0 (0.0)</td>
<td>Both (17)</td>
<td>93.9%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>93.9%</td>
<td>NA</td>
</tr>
<tr>
<td>(19)</td>
<td>Eswatini</td>
<td>5 MRS</td>
<td>5 (41.7)</td>
<td>Pediatric (NA)</td>
<td>80.0%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80.0%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 CRS</td>
<td></td>
<td></td>
<td>14.0%</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: MRS = Mycobacterium tuberculosis, CRS = Coccidioides immitis, CI = Confidence interval.
MRS, microbiological reference standard which includes patients with positive culture, nucleic acid test or histopathological findings consistent with active TB, both pulmonary or extrapulmonary; CRS, clinical reference standard which includes patients with clinical and radiological symptoms and signs consistent with active TB in the absence of bacteriological confirmation by culture, nucleic acid test or histopathology after excluding other diseases;

IC, immunocompromised; CI, confidence interval; IFN-γ, interferon-gamma;

IU, international units; mL, milliliter; No., number; NA, not available
### Table 3. Agreement between QFT-Plus and QFT-GIT among high-risk groups

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Country</th>
<th>Sample size (% IC)</th>
<th>Adult/Pediatric (Median age)</th>
<th>Test indications</th>
<th>Test positivity proportion</th>
<th>Agreement (Kappa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(25)</td>
<td>Italy</td>
<td>119 (9.2)</td>
<td>Adult (38)</td>
<td>TB contacts with TST conversion</td>
<td>57.1%*</td>
<td>89.9% (0.80)</td>
</tr>
<tr>
<td>(20)</td>
<td>USA</td>
<td>508 (4.0)</td>
<td>Both (32)</td>
<td>TB contacts; immigrants from high burden countries; HIV+</td>
<td>23.0%</td>
<td>94.0% (0.81)</td>
</tr>
<tr>
<td>(21)</td>
<td>Netherlands and Belgium</td>
<td>1031 (17.0)</td>
<td>Adult (44**)</td>
<td>Pre-immunotherapy; TB contacts; TB exclusion; routine screening</td>
<td>14.5%</td>
<td>95.0% (0.83)</td>
</tr>
<tr>
<td>(24)</td>
<td>Japan</td>
<td>412 (NA)</td>
<td>Adult (44)</td>
<td>TB contacts</td>
<td>7.5%</td>
<td>NA (0.82)</td>
</tr>
<tr>
<td>(33)</td>
<td>Germany</td>
<td>134 (NA)</td>
<td>Adult (25**)</td>
<td>Immigrants from high-risk countries</td>
<td>8.2%</td>
<td>NA (0.85)</td>
</tr>
<tr>
<td>(22)</td>
<td>China</td>
<td>616 (NA)</td>
<td>Adult (47)</td>
<td>At-risk health care workers</td>
<td>31.2%</td>
<td>94.8% (0.87)</td>
</tr>
<tr>
<td>(23)</td>
<td>Taiwan</td>
<td>229 (NA)</td>
<td>Adult (80)</td>
<td>Individuals in long term care facility</td>
<td>32.3%</td>
<td>93.9% (0.86)</td>
</tr>
<tr>
<td>(34)***</td>
<td>South Korea</td>
<td>169 (100.0)</td>
<td>Adult (54)</td>
<td>Pre-organ transplant</td>
<td>37.9%</td>
<td>93.7% (0.86)</td>
</tr>
<tr>
<td></td>
<td>105 (100.0)</td>
<td>Adult (53)</td>
<td>Pre-stem cell transplant</td>
<td>17.1%</td>
<td>15.2%</td>
<td>1.9% (-8.1 to 11.9)</td>
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<td>----------------</td>
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</tr>
<tr>
<td>43 (100.0)</td>
<td>Both (45)</td>
<td>Pre-immunotherapy</td>
<td>23.3%</td>
<td>20.9%</td>
<td>2.4% (-15.1 to 19.9)</td>
<td></td>
</tr>
<tr>
<td>(19) Eswatini</td>
<td>46 (2.0)</td>
<td>Children &lt;15 years (NA)</td>
<td>TB contacts</td>
<td>32.6%</td>
<td>32.6%</td>
<td>0.0% (-19.2 to 19.2)</td>
</tr>
</tbody>
</table>

IC, immunocompromised; CI, confidence interval; Kappa, kappa coefficient;

*No difference in positivity rate was observed between TB1 and TB2

**Mean age was provided

***Overall positivity rate for QFT-Plus 27.8% and QFT-GIT 29.0%
Table 4. Comparison of specificity between QFT-Plus and QFT-GIT among low-risk populations

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Country</th>
<th>Sample size</th>
<th>Specificity</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(26)</td>
<td>USA</td>
<td>262 non-HCW including 51 NTM patients</td>
<td>98.1%</td>
<td>98.9%</td>
</tr>
<tr>
<td>(31)</td>
<td>Germany</td>
<td>77 low-risk HCW</td>
<td>87.0%</td>
<td>89.6%</td>
</tr>
<tr>
<td>(15)</td>
<td>Italy</td>
<td>19 non-HCW</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>(16)</td>
<td>South Korea</td>
<td>27 non-HCW</td>
<td>92.6%</td>
<td>100%</td>
</tr>
<tr>
<td>(32)</td>
<td>Japan</td>
<td>212 non-HCW</td>
<td>97.0%</td>
<td>98.6%</td>
</tr>
<tr>
<td>(35)</td>
<td>USA</td>
<td>626 no-risk HCW</td>
<td>97.0%</td>
<td>97.9%</td>
</tr>
</tbody>
</table>

HCW, healthcare worker; NTM, nontuberculous mycobacteria; CI, confidence interval