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Evaluation of STANDARD E TB-Feron ELISA for the diagnosis of latent tuberculosis infection in healthcare workers

Running title: Evaluation of the STANDARD E TB-Feron ELISA kit

Authors:

Oh Joo Kweon[a], Yong Kwan Lim[a], Hye Ryoun Kim[a], Tae-Hyoung Kim[b], and Mi-Kyung Lee[a]#

Affiliations:

[a]Department of Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Republic of Korea

[b]Department of Urology, Chung-Ang University College of Medicine, Seoul, Republic of Korea

#Address correspondence to Mi-Kyung Lee, M.D, Ph.D., cpworld@cau.ac.kr

Abstract

Laboratory diagnosis of latent tuberculosis infection (LTBI) is mainly performed with interferon-gamma release assays (IGRAs). We compared the performance of a new ELISA-based IGRA, STANDARD E TB-Feron ELISA (TBF; SD Biosensor, Gyeonggi-do, Republic of Korea), and a widely used assay, QuantiFERON-TB Gold In-Tube (QFT-GIT; Qiagen, Hilden, Germany), in a population of 425 healthcare workers (HCWs). All HCWs were screened with both assays per the manufacturers' protocols and in a "cross-manner," where tube sets from one assay were used with the alternative ELISA. Results were compared both qualitatively and quantitatively. TBF and QFT-GIT identified 11.3% (48/425) and 12.9% (55/425) positive samples, respectively. TBF demonstrated 81.6% positive and 97.4% negative percentage agreement with QFT-GIT, with a Cohen's kappa value of 0.78 (strong agreement). Discordant results were detected in 20 subjects (4.3%): 13 samples (65.0%) were TBF(-)/QFT-GIT(+), 6 (30.0%) were TBF(+)/QFT-GIT(-), and one provided TBF/QFT-GIT indeterminate/negative results. We observed a statistical significant degree of correlation between interferon-gamma reactivity between both assays (r_s=0.551, P<0.01) and between standard assays and cross-manner tests (r_s ranging from 0.449 to 0.816, P<0.01 for all combinations). Cross-manner tests also revealed that the ELISA kit of TBF provided higher TB antigen and nil values than ELISA of QFT-GIT under the same conditions (P<0.01), although these differences disappeared in the TB antigen minus nil value. TBF showed a comparable and acceptable clinical performance in detecting LTBI comparing to QFT-GIT. TBF represents a useful alternative tool of ELISA-based IGRA, especially in large-scale LTBI screening in HCWs.

Key words: interferon-gamma release assay, latent tuberculosis infection, STANDARD E

45 TB-Feron ELISA kit, QuantiFERON-TB Gold In-Tube

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Introduction

Latent tuberculosis infection (LTBI) is characterized by infection with *Mycobacterium tuberculosis* without evidence of active tuberculosis (TB) disease, including the absence of clinical signs or symptoms and a normal chest radiograph (1). In 2018, the World Health Organization (WHO) reported that approximately 1.7 billion people, 23% of the world's population, were estimated to have LTBI (2), and in a lifetime, approximately 8–10% of LTBI reactivates into active TB, with clinical manifestations and spread-potential (1).

The risk of progression to active TB can be significantly reduced by preventive medication (3), and thus, the identification of a person with LTBI, leading to adequate therapy, is a fundamental component of TB elimination, not only for individual clinical benefit, but also for public prevention of TB spread and for TB elimination (1, 4). The Republic of Korea has an intermediate *M. tuberculosis* burden, and the government operates a massive LTBI screening program in high risk populations for TB transmission, such as healthcare workers (HCWs) and military service personnel (5).

Interferon gamma (IFN-γ) release assays (IGRAs) are widely used laboratory alternatives to the tuberculin skin test (TST) (6). In TST, the purified protein derivate (PPD) is used as the antigen, and the cellular immunity to PPD antigens can reflect exposure to bacillus Calmette-Guérin (BCG) vaccine strains or non-tuberculous mycobacteria (6). IGRAs are an *ex vivo* blood test, measuring the T-cell response after overnight stimulation with antigens specific for *M. tuberculosis* complex, such as early secreted antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP-10), and TB7.7 (7). These antigens are more specific for *M. tuberculosis* than PPD and are not produced by BCG strains, and thus, IGRA results are not

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68 impacted by prior BCG vaccination (8). In countries with high TB prevalence, the majority of
69 the population receive the BCG vaccine; therefore, IGRAs are a more useful LTBI diagnostic
70 tool in these clinical settings (6, 8).

71 The QuantiFERON-TB Gold In-Tube (QFT-GIT; QIAGEN, Germantown, MD) is one of the
72 most widely used IGRAs, and is an enzyme-linked immunosorbent assay (ELISA)-based,
73 whole-blood test that uses three antigens (ESAT-6, CEP-10, and TB7.7 peptides) to
74 predominantly stimulate $CD4^+$ T cells in an in-tube format (9). Three tubes are used in the
75 assay: a TB antigen tube, a positive control tube (mitogen), and a negative control tube (nil),
76 and quantitative values from each tube are used to generate a qualitative result.

77 Recently, a new ELISA-based IGRA, STANDARD E TB-Feron ELISA (TBF; SD Biosensor,
78 Gyeonggi-do, Republic of Korea), was approved by the Ministry of Food and Drug Safety of
79 Korea. The principles of this assay including antigens for stimulating T cells are almost same
80 to those of QFT-GIT(10, 11). One distinctive differences between the TBF and QFT-GIT was
81 the structure of TB-specific antigens; antigens in TBF were recombinant whole proteins of
82 ESAT-6, CEP-10, and TB7.7 (11), but those of QFT-GIT were TB-specific synthetic peptide
83 antigens (10). In addition, the incubation temperature and time of the incubation procedure in
84 ELISA were differ, and TBF offered advantages in terms of cost-effectiveness compared to
85 QFT-GIT.

86 The aim of this study was to compare the analytical performance of STANDARD E TB-
87 Feron with that of QFT-GIT for detection of LTBI in HCWs. In addition, because both assays
88 consist of two stages, blood incubation and ELISA-based IFN-γ measurement, we tested the
89 two IGRAs in “cross-manner”, where tubes from one assay were combined with the ELISA
90 from the other, to investigate the performance of each stage separately.

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Materials and Methods

Study Population

The evaluation was performed from August 2018 to May 2019 at the Department of Laboratory Medicine, Chung-Ang University Hospital, Seoul, Republic of Korea. In the Chung-Ang University Hospital, HCWs are screened for LTBI or TB infection with IGRA and chest X-ray annually. Once a HCW shows positive results, the particular HCW is no more screened for LTBI and is usually subjected to a prophylactic treatment. Within this framework, a total of 425 HCWs were involved in this study, and they were examined by chest X-ray and two IGRAs, the TBF and the QFT-GIT. The subjects who were suspected of having active TB based on chest X-ray were excluded. None of the subjects was undergoing treatment for TB or had a past medical history of LTBI, positive results from IGRAs, or active TB.

STANDARD E TB-Feron ELISA

TBF test procedures consisted of two stages, where the first stage included blood incubation from each tube, and the second stage involved plasma IFN-γ analysis by ELISA. For analysis, 1 mL of whole blood was collected into each of the following three tubes: TB antigen, nil, and mitogen. Immediately after, the tubes were gently shaken to coat the entire inner surface with blood. Then, the tubes were transferred to a 37 ℃ incubator, within 4 h after collection. After 16-h incubation, the tubes were centrifuged for 15 min at 2500 ×*g*, and plasma were separated. Then, the ELISA kit was used to quantify IFN-γ in the separated plasma from each tube. The ELISA of TBF required 60-min incubation at a 37 ℃ for accurate reactions. The

results were interpreted as positive when the IFN-γ value of the TB antigen tube (TB antigen value) minus that of the nil tube (nil value) was ≥ 0.35 IU/mL and at least 25% of the nil value. Where the nil value was > 8.0 IU/mL or the value of the mitogen tube (mitogen value) minus the nil value was < 0.50 IU/mL, results were considered indeterminate. Any other results were determined to be negative.

QuantiFERON-TB Gold In-Tube

The QFT-GIT assay procedure was almost analogous to that of TBF, with the exception that the incubation step during ELISA was conducted at room temperature for 120 minutes, whereas the ELISA of TBF required incubation at 37 °C for 60 minutes. Interpretation criteria were identical to those for TBF, as described above.

“Cross-manner” IGRAs

Plasma were additionally analyzed using the tubes from one assay and the ELISA from the other (hereafter, referred to as “cross-manner”), where the QFT-GIT ELISA was used to measure IFN-γ from TBF tubes (hereafter, TBF tubes/QFT ELISA) and the ELISA of TBF was used to measure IFN-γ from QFT-GIT tubes (hereafter, QFT tubes/TBF ELISA). Cross-manner tests were conducted at the same time as standard IGRAs.

Statistical analysis

To determine the qualitative concordance between TBF and QFT-GIT assays, the positive

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percentage agreement (PPA), negative percentage agreement (NPA), and Cohen's κ value were assessed using 3-by-3 crosstab analysis. The κ values were interpreted according to the criteria proposed by Landis and Koch (12) as follows: "bad" for values of 0.01–0.20, "fair" for 0.21–0.40, "moderate" for 0.41–0.60, "strong" for 0.61–0.80, and "almost perfect" for 0.81–1.00. Confidence intervals (95%) were also calculated for PPA, NPA, and κ values.

The normality of quantitative IGRA values was assessed using the Kolmogorov-Smirnov test. IFN-γ values, including those from cross-manner tests, were compared using an independent student's t-test or one-way analysis of variance, where normally distributed, and the Mann-Whitney U test or the Kruskal-Wallis test where distribution was not normal. Spearman's rank correlation test was performed and rho values (r_s) were calculated to investigate the correlation between the non-parametric values from each IGRA. The r_s values were interpreted as follows: "very high correlation" for values 0.90–1.00 (absolute values), "high correlation" for 0.70–0.90, "moderate correlation" for 0.50–0.70, "low correlation" for 0.30–0.50, and "negligible correlation" for 0.00–0.30 (13). In addition, Bland and Altman plots were drawn to evaluate the agreement among two IGRAs and cross-manner tests. Statistical analyses were performed using SPSS software, version 19 (IBM, Armonk, NY, USA), or MedCalc V.18.9.1 (MedCalc Software, Ostend, Belgium), and where $P \leq 0.05$, observations were considered statistically significant.

Ethical Approval

The protocol was approved by the Institutional Review Board (IRB) of Chung-Ang University Hospital (Seoul, Korea; approval no. 1873-001-334), and informed consent was

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obtained from the study subjects according to the IRB's policy. Study subjects with positive IGRA were managed in accordance with the guidelines of Korea Centers for Disease Control and Prevention (5), based on the QFT-GIT results.

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Results

Subject characteristics

Total 425 HCWs were involved in this study. The subjects were between 21 and 63 years old, with a median age of 31, and 80.5% subjects were female. The professions of subjects were nurse (49.9%, 212/425), medical technician (25.9%, 110/425), clinicians (8.9%, 38/425), clerk (7.3%, 31/425), or other professions (8.0%, 34/425), such as hospital cleaner. There were 20 subjects who showed discordant results between two standard IGRAs. Median age of the subjects with discordant results was 32 years (range 23 to 56 years), and 85% (17/20) of them were females. Moreover, 35% (7/20) were nurses, 20% (4/20) were medical technicians, 20% (4/20) were clerks, 20% (4/20) had other professions, and 5% (1/20) was a clinician.

Qualitative comparison of QuantiFERON-TB Gold In-Tube and STANDARD E TB-Feron assays

TBF showed 11.3% of positivity that was slightly lower value than that of QFT-GIT, 12.9%. The absolute agreement between QFT-GIT and TBF qualitative results was 95.3% (405/425), with a κ value of 0.78, which is interpreted as a “strong” agreement. PPA and NPA were 81.6% and 97.4%, respectively. A total of 4.7% (20/425) of samples showed discordant results between the two IGRAs. Details of qualitative comparison tests are shown in Table 1.

The 20 discordant results are summarized in Fig. 1. Among discordant results, 13 (65%) were TBF(-)/QFT-GIT(+), where the median IFN-γ value was 0.24 IU/mL (min-max, 0.09 - 0.34 IU/mL) using TBF and 0.78 IU/mL (0.37 - 1.89 IU/mL) using QFT-GIT. Six samples (30.0%) were TBF(+)/QFT-GIT(-), where median TBF and QFT-GIT IFN-γ values were 0.43 IU/mL

(0.35 - 0.63 IU/mL) and 0.16 IU/mL (0.0 - 0.31 IU/mL), respectively. One sample (5.0%) was interpreted as indeterminate in the TBF assay, where the mitogen minus nil value was 0.43 IU/mL < 0.5 IU/mL, and negative in the QFT-GIT.

Quantitative comparison of QuantiFERON-TB Gold In-Tube and STANDARD E TB-Feron assays

Spearman's rank correlation test of quantitative IGRA results revealed statistically significant correlation between both assays in "TB antigen" and "TB antigen minus nil" values. For IFN-γ reactivity in TB antigen tubes, r_s was 0.551 (P<0.01), and the value for "TB antigen minus nil" value was 0.461 (P<0.01); they were interpreted as the "moderate" correlation. For all combinations of comparisons between results of IGRAs including cross-manner tests r_s ranged from 0.449-0.816 (P<0.01). Details of the correlation analyses are listed in supplementary table S1.

Bland and Altman plot between two IGRAs for the TB antigen minus nil antigen values is illustrated in Fig. 2A. It was revealed that 96.2% (409/425) of values were within two standard deviation (2SD), and among the 20 discordant results, only one sample showed differences higher than 2SD.

Cross-manner IGRAs

Qualitative comparison of the two standard IGRAs and the two cross-manner tests, TBF tubes/QFT ELISA and QFT tubes/TBF ELISA, are listed in Table 2. κ values ranged from 0.72 (TBF and QFT-GIT tubes/TBF ELISA, P<0.01) to 0.88 (QFT-GIT to QFT tubes/TBF

ELISA, $P<0.01$). Noticeably, higher κ values were observed when comparing a standard IGRA to a cross-manner test using the same tubes as that IGRA. The proportions of positive results for TBF tubes/QFT ELISA and QFT tubes/TBF ELISA were 8.9% and 14.8%, respectively. Two cases of indeterminate results were recorded for QFT tubes/TBF ELISA.

Among the 405 samples which showed concordant results between both IGRAs, 19 (4.7%) showed discordant results in one, or both, of the cross-manner tests. Of the 42 samples positive in both IGRAs, 10 (23.8%) gave discordant results in cross-manner tests, of which the majority (90%, 9/10) was found with TBF tubes/QFT ELISA. Of the negative samples, 9 (2.4%) gave positive or indeterminate results with QFT tubes/TBF ELISA. Samples which showed discordant results between the two standard IGRAs provided a variety of results in the cross-manner studies. Of the 13 TBF(-)/QFT-GIT(+) samples, the majority (10/13, 76.9%) were negative with TBF tubes/QFT ELISA and positive with QFT tubes/TBF ELISA. A schematic of the IFN-γ values (TB antigen minus nil values) from all assays including cross-manner tests for the samples with discordant result between the two standard IGRAs is illustrated in Fig. 3 (except for the one TBF-indeterminate sample), and qualitative result distribution for the standard IGRAs and the cross-manner tests is shown in supplementary table S2.

Quantitative comparisons are summarized in Table 3; three distinct findings were observed. First, TBF TB antigen and nil values were higher than those of QFT-GIT ($P<0.001$), but no significant difference was observed for TB antigen minus nil values. Second, TB antigen minus nil, TB antigen, and nil values were statistically higher in TBF than in TBF tubes/QFT ELISA tests ($P<0.001$), and similarly, total TB antigen and nil values were higher in QFT tubes/TBF ELISA than in QFT-GIT tests ($P<0.001$). In other words, IFN-γ values measured

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using the ELISA kit of TBF were higher than those measured with the ELISA of QFT-GIT when the same tubes were used. Third, total TB antigen minus nil, TB antigen, and nil values were higher in QFT-GIT than in TBF tubes/QFT ELISA tests. This implies that the ELISA of QFT-GIT generates higher IFN-γ values in combination with the QFT-GIT tubes than with the TBF tubes. On the other hand, there were no differences in values between TBF and QFT tubes/TBF ELISA tests, except for the nil values of negative subgroups; therefore, the ELISA kit of TBF was not affected by the tubes used in the incubation stage.

Bland and Altman plots among the two IGRAs and the cross-manner tests between two IGRAs for the TB antigen minus nil antigen values are illustrated in Fig. 2B to 2F. In all the plots except for those between the TBF tube/QFT ELISA and the QFT tube/TBF ELISA, more than 95.0% of difference values were within 2SD; the percentages were 96.5% (410/425) for those between the TBF and the TBF tube/QFT ELISA, 96.0% (408/425) for those between the TBF and the QFT tube/TBF ELISA, 96.0% (408/425) for those between the QFT and the TBF tube/QFT ELISA, 96.0% (408/425) for those between the QFT and the QFT tube/TBF ELISA, and 94.1% (400/425) for those between the TBF tube/QFT ELISA and the QFT tube/TBF ELISA.

Discussion

In this study, we revealed that the performance of TBF, a newly developed IGRA, was comparable to that of the QFT-GIT, the most widely used assay in the same technical category, in the diagnosis of LTBI in HCWs. TBF showed a comparable positive rate of LTBI detection to QFT-GIT, and the κ value between the two IGRAs represented a “strong” degree of correlation. Recently, several ELISA-based IGRAs, using three tubes, were developed and released in the Korean market and accordingly, this provided a wider range of choice to clinical laboratories.

HCWs with LTBI are a potential risk group for transmission of *M. tuberculosis* to other HCWs and patients. Thus, LTBI screening and treatment for HCWs is an important component of TB infection control in hospitals (14-16). The Republic of Korea, where this study was performed, has an intermediate *M. tuberculosis* burden, with an annual notification rate of new TB cases of 60.4 per 100,000 population in 2016 (2, 5). The Korea Centers for Disease Control and Prevention (KCDC) recommends that HCWs working in departments with increased risk of TB infection receive annual screening for LTBI (5). In these circumstances, the diagnostic performance of IGRAs detecting LTBI is fundamental for TB control.

Although the quantitative IFN-γ values from both IGRAs evaluated in this study are well correlated and the qualitative performance of the TBF was comparable to the QFT-GIT, discordant results were observed. In our quantitative comparison, we found that TB antigen and nil tube values were higher in TBF than in QFT-GIT test, and these differences appear to originate from both the tube (incubation) and ELISA stages. By comparing these assays in a cross-manner, we revealed that the ELISA kits of TBF provided higher values in IFN-γ

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265 measurements than the ELISA of QFT-GIT. The incubation time and temperature during the
266 ELISA stage were differ between two IGRAs, and this difference might contribute to the
267 higher measurements observed in the ELISA of TBF.

268 TBF used recombinant whole protein of TB specific antigens, but antigens of QFT-GIT were
269 peptide forms. Whole recombinant proteins were degraded into diverse small peptides,
270 multiple epitopes can be presented onto cell surface. The multiple epitopes could stimulate T
271 cells more effectively and might consequently lead to better sensitivity or higher IFN-γ values.
272 In addition, some *HLA* genotype had low affinity in binding with both ESAT-6 and CFP10
273 epitopes (17, 18), hence multiple epitopes might give advantages to the performance of IGRA
274 test. However, in spite of those differences, the better performance or higher IFN-γ values of
275 TBF tubes comparing to the QFT-GIT tubes were not found in the present study. Rather,
276 QFT-GIT tubes appeared to generate higher IFN-γ values, as using TBF tubes with the QFT-
277 GIT ELISA resulted in lower values. Although there were differences in TB antigen and nil
278 values between the tested two IGRAs, those difference were removed when the TB antigen
279 minus nil value calculation was performed, resulting in an acceptable concordance rate
280 between the IGRAs.

281 Of the 425 HCW samples, 20 showed discordant results between the two IGRAs. The six
282 TBF(+)/QFT-GIT(-) samples may be a result of the higher IFN-γ values generated by the
283 TBF ELISA. We speculate this because the majority of these samples were negative in TBF
284 tubes/QFT ELISA and QFT tubes/TBF ELISA cross-manner tests (five and four out of six,
285 respectively). The tendency towards higher IFN-γ values in the ELISA of TBF is also
286 illustrated by the finding that two QFT-GIT-negative samples were positive with QFT
287 tubes/TBF ELISA. Thirteen samples which showed TBF(-)/QFT-GIT(+) result may have

arose due to a tube problem rather than an ELISA problem, wherein IFN-γ generation was low in TBF tubes and clearly high in QFT-GIT tubes. This finding implies that there would be a small group of individuals who are not detected by TBF. However, as we did not record or collect the subject characteristics extensively, we were not able to assess the possible attributing factors to these discordant results. Regarding the gray zone (0.2–0.7 IU/mL, TB Ag-Nil), three samples showed discordant TB Ag-Nil values of IGRAs outside of the gray zone: values lower than 0.2 and higher than 0.7 for each IGRA, and all of these samples showed TBF(-)/QFT-GIT(+). Four samples showed discordant results, but both values from the two IGRAs were within the gray zone, and the remaining 13 samples showed values within the gray zone from only one of the IGRAs.

There is no "gold standard" for IGRAs; it is therefore impossible to declare which IGRA was more "accurate". Although our study did not assess the repeatability or variability of the two IGRAs, Metcalfe et al. reported that QFT-GIT had considerable variability in qualitative results, from ±0.24 IU/mL for the samples with responses in the borderline range of 0.25 to 0.8 IU/mL, to ±0.6 IU/mL for all samples (9, 19, 20). The difference of laboratory or research setting and thus it was difficult to apply as it was to our study. Indeed, applying these findings to our study could potentially allow reclassification of most of the discordant samples (19/20), with the exception of one sample (TB antigen minus nil values of 1.0 IU/mL in QFT-GIT and 0.09 IU/mL in TBF). The high variability of IGRA results arises from the fact that IGRAs have various potential sources of variability (9, 20-22). For example, pre-analytical sources of variability include the time of blood collection, inadequate disinfection of the skin, the order of tube filling, the volume of blood drawn, vigorous shaking, delay in

sample incubation, and transport of blood specimen at lower ambient temperature. Analytical sources of variability, or those in the ELISA stage include matrix effect, imprecision of pipetting, errors in centrifugation, problems during washing steps, imprecision in final signal measurement, and whether automated or manual methods are used. Furthermore, post-analytical errors can occur during manual data entry. As might be expected, manufacturing defects, such as faulty antigen tubes, can also occur, and this problem has been documented in QFT-GIT (23, 24). Additional research focusing on these possible sources of error and repeatability in the TBF assay is therefore warranted because these questions have not been addressed in our study.

Recently, QuantiFERON-TB Gold Plus (QFT-Plus, QIAGEN), the next generation of QFT-GIT assay was released, which is a four-tube-based IGRA (25-27). This assay contains peptides from only the ESAT-6 and CFP-10 antigens, with two TB antigen tubes (TB1 and TB2). Additionally, each tube is designed to elicit IFN-γ release from CD4+ and CD8+ T cells for accurate LTBI detection in individuals with low CD4+ T cell counts (25-28). However, in the screening programs in HCWs or military service personnel, most subjects have normal immune status and thus several reports that evaluated QFT-Plus performance in HCWs reported that it showed a high correlation with, and results comparable to those of QFT-GIT (26, 29). In addition, the QFT-Plus is more expensive and require more resource for their analysis, therefore three-tube IGRAs remain a cost-effective way for HCW screening. Also, it is predicted that QFT-GIT reagents will be discontinued, thus another three-tube IGRA, such as TBF, offers an alternative choice based on its comparable assay performance with respect to that of QFT-GIT.

This study had several limitations. First, we did not assess reproducibility, as described above.

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Second, we did not evaluate disease status affecting immune status, clinical characteristics, including smoking status, drugs taken, other than TB medication, or past medical histories of the study subjects. These factors may contribute to indeterminate or inaccurate IGRA results. However, we assumed that few HCWs in our study were impacted by these conditions or factors, because among the 425 subjects, only one subject resulted in indeterminate results. The third limitation concerns our focus on HCWs rather than immunocompromised patients, who are a high-risk group of TB infection or LTBI. Further TBF evaluation should be performed in this patient population. Finally, the exact TB exposure history was not collected individually for each subject, and because the subjects worked in different wards or departments, the degree of TB exposure could differ.

In conclusion, TBF showed comparable and acceptable clinical performance for detecting LTBI when compared to QFT-GIT, and thus TBF represents a useful, alternative ELISA-based IGRA, especially for large-scale LTBI screening in HCWs. Both tubes and ELISA of the IGRAs appeared to affect the assay performance, and although TBF showed higher TB antigen and nil values than QFT-GIT, the differences were eliminated by calculating TB antigen minus nil values. As some discordant results between the IGRAs were also present, caution would be needed while interpreting data, especially for the results near the cut-off value.

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Figure legends

Fig 1. Scatter plot for the TB antigen minus nil antigen values from the samples which showed discordant results between the STANDARD E TB-Feron ELISA (TBF) and the QuantiFERON-TB Gold In-Tube (QFT-GIT). Among 20 discordant results, 13 samples (65.0%) were negative with TBF and positive with QFT-GIT and 6 samples (30.0%) showed positive TBF with negative QFT-GIT results. There was one sample which showed indeterminate result with TBF and negative with QFT-GIT (This data was not plotted). The dashed line designates the cut-off value for the determination of positive results from IGRAs (0.35 IU/mL).

Fig 2. Bland and Altman plots for the TB antigen minus nil antigen values (IU/mL) among the STANDARD E TB-Feron ELISA (TBF), the QuantiFERON-TB Gold In-Tube (QFT-GIT), and the two cross-manner tests (TBF tubes/QFT ELISA and QFT tubes/TBF ELISA). The dashed line and the dotted line designate the mean value and two standard deviation of difference between the differences of the results from two assays. The samples with discordant results between the TBF and the QFT-GIT were plotted using crosses (×), and the others were plotted using filled circles (●).

Fig 3. The distribution of interferon-γ (TB antigen minus nil values) of the samples measured in the STANDARD E TB-Feron ELISA (TBF), the QuantiFERON-TB Gold In-Tube (QFT-GIT) and the two cross-manner tests (TBF tubes/QFT ELISA and QFT tubes/TBF ELISA). The dashed line designates the cut-off value for the determination of positive results

from IGRAs (0.35 IU/mL). (A) The samples which showed positivity in TBF but negativity in QFT-GIT (N=6). (B). The samples which had negative results in TBF but positive results in QFT-GIT (N=13).

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Table 1. Qualitative comparisons between the STANDARD E TB-Feron ELISA and the QuantiFERON-TB Gold In-Tube in a total of 425 health care workers.

		TBF				PPA	NPA	Kappa
		Positive	Negative	Indeterminate	Total			
QFT-GIT	Positive	42 (9.9%)	13 (3.1%)	0 (0.0%)	55 (12.9%)	81.6%	97.4%	0.78* (0.69-0.87)†
	Negative	6 (1.4%)	363 (85.4%)	1 (0.2%)	370 (87.1%)			
	Indeterminate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
	Total	48 (11.3%)	376 (88.5%)	1 (0.2%)	425 (100.0%)			

Abbreviations: TBF, STANDARD E TB-Feron ELISA; PPA, positive percentage agreement; NPA, negative percentage agreement; QFT-GIT, QuantiFERON-TB Gold In-Tube.

* $P<0.001$

† 95% confidence interval

477 Table 2. Qualitative result comparisons for STANDARD E TB-Feron ELISA (TBF) and QuantiFERON-TB Gold In-Tube (QFT-GIT) to the
478 results from TBF tubes/QFT-GIT ELISA and QFT-GIT tubes/TBF ELISA.

		TBF tubes / QFT ELISA					QFT tubes / TBF ELISA				
		Positive	Negative	Indeterminate	Total	Kappa	Positive	Negative	Indeterminate	Total	Kappa
TBF	Positive	34 (8.0%)	14 (3.3%)	0 (0.0%)	48 (11.3%)	0.75* (0.65-0.86)†	43 (10.1%)	5 (1.2)	0 (0.0%)	48 (11.3%)	0.72* (0.67-0.77)†
	Negative	4 (0.9%)	372 (87.5%)	0 (0.0%)	376 (88.5%)		20 (4.7)	354 (83.3%)	2 (0.5%)	376 (88.5%)	
	Indeterminate	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)		0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)	
	Total	38 (8.9%)	387 (91.1%)	0 (0.0%)	425 (100.0%)		63 (14.8%)	360 (84.7%)	2 (0.5%)	425 (100.0%)	
QFT-GIT	Positive	36 (86.8%)	19 (4.5%)	0 (0.0%)	55 (12.9%)	0.75* (0.64-0.85)†	54 (12.7%)	1 (0.2%)	0 (0.0%)	55 (12.9%)	0.88* (0.82-0.95)†
	Negative	2 (0.5%)	368 (86.9%)	0 (0.0%)	370 (87.1%)		9 (2.1%)	359 (84.5%)	2 (0.5%)	370 (87.1%)	
	Indeterminate	0	0	0	0		0	0	0	0	

		(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
		38	387	0	425	63	360	2	425
	Total	(8.9%)	(91.1%)	(0.0%)	(100.0%)	(14.8%)	(84.7%)	(0.5%)	(100.0%)

Abbreviations: TBF, STANDARD E TB-Feron ELISA; QFT-GIT, QuantiFERON-TB Gold In-Tube; QFT ELISA, QuantiFERON®-TB Gold In-Tube ELISA.

* $P<0.001$

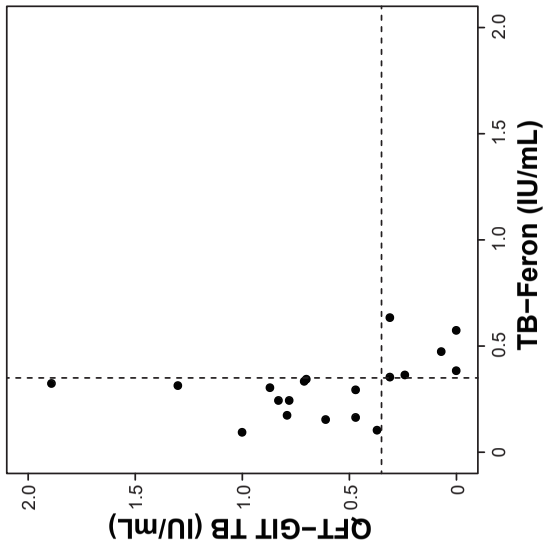
† 95% confidence interval

484 Table 3. Median interferon-γ values measured from TB antigen tube minus nil tube, TB antigen tube, and nil tube of the STANDARD E TB-
485 Feron ELISA (TBF) and the QuantiFERON-TB Gold In-Tube (QFT-GIT) to the results from TBF tubes/QFT-GIT ELISA and QFT-GIT
486 tubes/TBF ELISA.

Tubes		TBF	QFT-GIT	TBF tubes /QFT ELISA	QFT tubes /TBF ELISA	*P* value	Kruskal-Wallis grouping†
TB antigen minus	Total	0.00 [0.00-0.08]	0.01 [0.00-0.09]	0.00 [0.00-0.03]	0.01 [0.00-0.09]	<0.001	a,a,b,a
Nil	Positive	1.02 [0.42-2.24]	1.30 [0.71-4.53]	1.06 [0.56-4.36]	1.20 [0.59-3.31]	0.089	-
	Negative	0.00 [0.00-0.04]	0.00 [0.00-0.04]	0.00 [0.00-0.02]	0.00 [0.00-0.04]	<0.001	a,a,b,a
TB Ag	Total	0.23 [0.17-0.35]	0.13 [0.07-0.31]	0.07 [0.05-0.14]	0.22 [0.16-0.39]	<0.001	a,b,c,a
	Positive	1.28 [0.70-2.57]	1.52 [0.86-6.16]	1.15 [0.61-4.45]	1.53 [0.86-2.92]	0.298	-
	Negative	0.22 [0.17-0.29]	0.11 [0.07-0.19]	0.07 [0.04-0.11]	0.20 [0.16-0.30]	<0.001	a,b,c,a
Nil	Total	0.21 [0.17-0.29]	0.09 [0.06-0.14]	0.06 [0.04-0.10]	0.19 [0.15-0.27]	<0.001	a,b,c,a
	Positive	0.25 [0.18-0.35]	0.12 [0.06-0.24]	0.07 [0.04-0.13]	0.21 [0.18-0.40]	<0.001	a,b,c,a
	Negative	0.21 [0.17-0.27]	0.09 [0.06-0.15]	0.06 [0.04-0.10]	0.19 [0.15-0.26]	<0.001	a,b,c,d

487 Abbreviations: TBF, STANDARD E TB-Feron ELISA; QFT-GIT, QuantiFERON-TB Gold In-Tube; QFT ELISA, QuantiFERON-TB Gold In-Tube
488 ELISA.

489 [†] The same letters indicate non-significant differences between groups based on the Mann-Whitney U or Scheffe's multiple comparison tests.



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