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MYCOBACTERIOLOGY AND AEROBIC ACTINOMYCETES

Comparing QuantiFERON-TB Gold Plus with Other Tests To Diagnose *Mycobacterium tuberculosis* Infection

Thara K. Venkatappa,[a] Rose Punnoose,[b] Dolly J. Katz,[a] Michael P. Higgins,[c] Niaz Banaei,[d] Edward A. Graviss,[e] Robert W. Belknap,[c] Christine S. Ho,[a] for the Tuberculosis Epidemiologic Studies Consortium

[a]Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
[b]Northrop Grumman, Atlanta, Georgia, USA
[c]Denver Health and Hospital Authority, Denver, Colorado, USA
[d]Department of Pathology, Stanford University School of Medicine, Stanford, California, USA
[e]Department of Pathology and Genomic Medicine, Houston Methodist Research Institute, Houston, Texas, USA

ABSTRACT The fourth-generation QuantiFERON test for tuberculosis infection, QuantiFERON-TB Gold Plus (QFT-Plus) has replaced the earlier version, QuantiFERON-TB Gold In-Tube (QFT-GIT). A clinical need exists for information about agreement between QFT-Plus and other tests. We conducted this study to assess agreement of test results for QFT-Plus with those of QuantiFERON-TB Gold In-Tube (QFT-GIT), T-SPOT.TB (T-SPOT), and the tuberculin skin test (TST). Persons at high risk of latent tuberculosis infection (LTBI) and/or progression to tuberculosis (TB) disease were enrolled at the 10 sites of the Tuberculosis Epidemiologic Studies Consortium from October 2016 through May 2017; each participant received all four tests. Cohen's kappa (κ) and Wilcoxon signed-rank test compared qualitative and quantitative results of QFT-Plus with the other tests. Test results for 506 participants showed 94% agreement between QFT-Plus and QFT-GIT, with 19% positive and 75% negative results. When the tests disagreed, it was most often in the direction of QFT-GIT negative/QFT-Plus positive. QFT-Plus had similar concordance as QFT-GIT with TST (77% and 77%, respectively) and T-SPOT (92% and 91%, respectively). The study showed high agreement between QFT-GIT and QFT-Plus in a direct comparison. Both tests had similar agreement with TST and T-SPOT.

KEYWORDS IGRA, QFT-Plus, latent TB infection, TBESC, TST

Molecular epidemiologic data show that >80% of tuberculosis (TB) cases in the United States are due to reactivation of latent tuberculosis infection (LTBI) rather than to recent transmission. A strong focus on diagnosis and treatment of LTBI is therefore a key step toward TB elimination in the United States, defined as <1 case annually per million population (1). Accurate diagnosis of LTBI is difficult due to the lack of a gold standard test. The tuberculin skin test (TST) is subject to false-positive results, because many of the proteins in tuberculin are also in the bacillus Calmette-Guérin (BCG) TB vaccine and in nontuberculous mycobacteria (2, 3). Blood assays called interferon gamma release assays (IGRAs) contain unique *Mycobacterium tuberculosis* antigens that enhance specificity (4). Two types of IGRAs are available in the United States: QuantiFERON (Qiagen, Germantown, MD, USA) and T-SPOT.TB (T-SPOT; Oxford Immunotec, Inc., Marlborough, MA, USA).

On 8 June 2017, the Food and Drug Administration (FDA) approved a fourth-generation QFT test, QuantiFERON-TB Gold Plus (QFT-Plus), to replace QuantiFERON-TB Gold In-Tube (QFT-GIT). QFT-GIT has a single TB antigen tube with a mixture of synthetic peptides of three *M. tuberculosis* antigens: early secreted antigenic target-6 (ESAT-6), culture filtrate protein-10 (CFP-10), and TB 7.7. QFT-Plus has two TB antigen

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Address correspondence to Thara K. Venkatappa, kyx2@cdc.gov.

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tubes, TB1 and TB2. Both tubes contain long ESAT-6 and CFP-10 peptides that stimulate $CD4^+$ helper T cells. TB2 also has short ESAT-6 and CFP-10 peptides that stimulate $CD8^+$ cytotoxic T cells. The presence of ESAT-6- and CFP-10-stimulated $CD8^+$ lymphocytes has reportedly been associated with recent *M. tuberculosis* infection (5, 6).

The purpose of this study was to assess agreement of QFT-Plus with QFT-GIT, TST, and T-SPOT in a population at high risk for LTBI and/or progression to TB disease.

MATERIALS AND METHODS

Study participants. Participants were part of a CDC-funded study of the ability of three tests for TB infection to predict progression to TB disease (https://www.cdc.gov/tb/topic/research/tbesc/default.htm, ClinicalTrials.gov: NCT01622140). From July 2012 through May 2017, health department and university clinics affiliated with the Tuberculosis Epidemiologic Studies Consortium (TBESC) enrolled approximately 22,000 persons at high risk for LTBI and/or progression to TB disease in 11 states. Inclusion criteria were close contacts (defined as persons identified during an ongoing contact investigation who spent ≥8 h within 1 week with a person with infectious TB disease), non-U.S.-born persons from countries whose populations in the United States have high TB rates (≥100/100,000 population) (see Table S1 in the supplemental material), recent arrivals (within 5 years) from countries whose U.S. populations have medium TB rates (10/100,000 to 99/100,000), persons who spent at least 30 days in a high-risk country within 5 years of enrollment, persons belonging to a local population with a documented ≥25% LTBI prevalence (e.g., homeless persons), and HIV-positive persons.

Exclusion criteria were individuals with known current active TB disease, persons with previous anaphylactic reaction to tuberculin, persons currently under treatment for LTBI, individuals planning to permanently leave the U.S. within 2 years of enrollment (e.g., tourists and visiting scholars), and foster children. Participants received all three FDA-approved tests (TST, T-SPOT, and QFT-GIT), and individuals with any positive result were actively followed for 24 months for progression to TB disease.

Participants were enrolled in this substudy from October 2016 through May 2017 and received QFT-Plus in addition to the 3 FDA-approved tests. Results of QFT-Plus were not used for diagnosis, because the study was conducted before FDA approval. The study was approved by the CDC and local institutional review boards (IRBs). All participants provided written informed consent, assent, and/or parental permission. Phlebotomy was followed by TST placement with Aplisol (JHP Pharmaceuticals, LLC, Rochester, MI, USA) or Tubersol (Sanofi Pasteur Limited, Toronto, ON, Canada) (5 TU per 0.1 ml) based on clinic practice. For study purposes, TSTs read 44 to 76 h after placement were accepted. TST results were interpreted as positive using CDC guidelines: ≥5 mm for close contacts and persons with HIV infection or other immunosuppressive conditions and ≥10 mm for all others. (https://www.cdc.gov/tb/publications/factsheets/testing/skintestresults.htm).

Blood collection and laboratory procedures. Blood for the QFT was drawn at 13 locations and processed at ten laboratories. Procedures varied across sites but were consistent with manufacturers' guidelines. Blood was drawn directly into QFT tubes or into lithium heparin tubes and then transferred to QFT tubes in the laboratory. Some sites used the nil and mitogen tubes from either the GIT or Plus kit for both tests (5-tube format); others used separate nil and mitogen tubes (7-tube format). Whole blood samples were incubated (16 to 24 h) at 37°C within 16 h of collection and then processed immediately or stored at 4°C until the enzyme-linked immunosorbent assay (ELISA) was performed. Gamma interferon (IFN-γ) levels (international units per milliliter [IU/ml]) were quantified for QFT-GIT samples with 4-point or 8-point standard curves and for QFT-Plus samples with a 4-point standard curve. IFN-γ values of >10 IU/ml were treated as 10 IU/ml, since the ELISA cannot accurately measure values >10 IU/ml (7). Results were reported as TB antigen minus nil values. According to manufacturer's guidelines, a result was positive if TB antigen minus nil was ≥0.35 IU/ml and ≥25% of the nil (QFT-GIT, http://www.quantiferon.com/wp-content/uploads/2017/04/English_QFT_ELISA_R04_082016.pdf) and TB1 and/or TB2 minus nil was ≥0.35 IU/ml and ≥25% of the nil (QFT-Plus, https://www.quantiferon.com/us/wp-content/uploads/sites/13/2018/09/QFT-Plus-ELISA-IFU-L1095849-R04.pdf).

For the T-SPOT, blood was drawn into lithium heparin tubes and shipped to Oxford Immunotec's central processing laboratory in Memphis, TN, for processing within 32 h of phlebotomy. Based on FDA-approved U.S. cutoffs, a positive result was ≥8 spots, 5 to 7 spots was borderline, and ≤4 spots was negative (http://www.tspot.com/wp-content/uploads/2012/01/PI-TB-US-v4.pdf).

Statistical analyses. Analyses were conducted overall and for U.S. and non-U.S.-born persons, recent immigrants and refugees with abnormal overseas chest radiographs but negative overseas sputum smears and cultures (class B1 immigrants, https://www.cdc.gov/immigrantrefugeehealth/exams/ti/panel/tuberculosis-panel-technical-instructions.html) (8), and close contacts to TB cases. Borderline and invalid T-SPOT results and indeterminate QFT results were excluded from test agreement analyses. Test agreement for qualitative results was evaluated by proportion of concordant results and Cohen's kappa (κ) coefficient. Quantitative IFN-γ levels for QuantiFERON were reported with median and interquartile range (IQR) values; differences between paired samples were assessed with the Wilcoxon signed-rank test. For QFT-Plus, the $CD8^+$ T-cell response was calculated by subtracting the TB1 from the TB2 IFN-γ levels (9). All analyses were conducted with SAS (version 9.4, Cary, NC, USA).

RESULTS

Of the 520 participants enrolled, data for 11 were excluded from analysis because they did not complete enrollment and for one who had TB disease at enrollment.

TABLE 1 Demographics of study participants

Characteristic[a] (*N* = 508)	*n*	%
Male	250	49
Non-U.S. born	436	86
Class B1 immigrant	49	10
Close contact	48	9
Age[b] (yrs)		
≤5	19	4
6–14	58	11
15–24	107	21
25–44	208	41
45–64	99	19
≥65	17	3
Medical risk factors		
Diabetes	33	7
HIV/AIDS	8	2
Steroid use	8	2

[a]Class B1 immigrant, immigrant with abnormal chest radiograph but negative sputum smears and cultures in overseas examinations; close contact, ≥8 h/week exposure to TB case and includes U.S. and non-U.S.-born persons; HIV, human immunodeficiency virus.
[b]Median age, 32 years (range, 1 to 86).

Among the 508 participants included in the analysis, the median age was 32 years (interquartile range [IQR], 19 to 44.5 years); 49% were male and 86% were non-U.S. born, including 31 (65%) of the 48 close contacts (Table 1). The most common countries of birth were the United States (14%), Philippines (14%), Myanmar (8%), Cambodia (6%), Honduras, (6%), Mexico (6%), and Somalia (5%). The remaining 41% of enrollees were from 50 other countries; the reported birth country for 1 participant was missing.

Among all participants, 35% were positive by TST, 23% by QFT-Plus, 20% by QFT-GIT, and 15% by T-SPOT (Table 2).

Agreement of QuantiFERON-GIT and QuantiFERON-Plus. Two QFT-Plus indeterminate results were excluded from the analysis of agreement, one due to a high nil (10 IU/ml) and the other due to a low mitogen response (0.06 IU/ml).

The remaining 506 results showed 94% agreement between QFT-GIT and QFT-Plus ($\kappa = 0.81$), including 19% that were positive for both tests and 75% that were negative for both tests. Results were similar for non-U.S.-born and U.S.-born participants, with a higher proportion negative by both tests compared to that for positive (Table 3). QFT results for the 77 children <15 years old were 100% concordant; only one child tested positive by both QFTs (see Table S2 in the supplemental material).

Of the 32 (6%) discordant results among all participants, 25 were QFT-GIT negative/QFT-Plus positive. The remaining 7 were QFT-GIT positive/QFT-Plus negative (Table 3). Twenty seven (84%) of the 32 discordant results were in the range of 0.2 to 0.7 IU/ml.

TABLE 2 Percent positive by each test

		No. (%) positive[b]			
Category[a]	Total no.	TST	QFT-Plus	QFT-GIT	T-SPOT
All	508	180 (35)	119 (23)	103 (20)	75 (15)
Non-U.S. born[c]	436	177 (41)	111 (25)	97 (22)	74 (17)
Class B1 immigrant	49	30 (61)	24 (49)	19 (39)	19 (39)
U.S. born[d]	71	3 (4)	8 (11)	6 (8)	1 (1)
Close contact[e]	48	12 (25)	4 (8)	3 (6)	3 (6)

[a]Class B1 immigrant, immigrant with abnormal chest radiograph but negative sputum smears and cultures in overseas examinations; close contact, ≥8 h/week exposure to TB case.
[b]QFT-GIT, QuantiFERON-TB Gold In-Tube; QFT-Plus, QuantiFERON-TB Gold Plus; TST, tuberculin skin test; T-SPOT, T-SPOT.TB.
[c]Includes 49 class B1 immigrants and 31 close contacts.
[d]Includes 17 close contacts.
[e]Includes 31 non-U.S.-born persons.

TABLE 3 Test agreement between QFT-GIT and QFT-Plus results

Category[a]	Total no.	No. (%) with QFT-GIT/QFT-Plus[b] results of:			
		+/+	−/−	+/−	−/+
All[c]	506	94 (19)	380 (75)	7 (1)	25 (5)
Non-U.S. born[d]	434	88 (20)	316 (73)	7 (2)	23 (5)
Class B1 immigrant	49	19 (39)	25 (51)	0 (0)	5 (10)
U.S. born[e]	71	6 (8)	63 (89)	0 (0)	2 (3)
Close contact	48	3 (6)	44 (92)	0 (0)	1 (2)

[a]Class B1 immigrant, immigrant with abnormal chest radiograph but negative sputum smears and cultures in overseas examinations; close contact, ≥8 h/week exposure to TB case.
[b]QFT-GIT, QuantiFERON-TB Gold In-Tube; QFT-Plus, QuantiFERON-TB Gold Plus.
[c]Two indeterminate QFT plus results excluded.
[d]Includes 49 class B1 immigrants and 31 close contacts.
[e]Includes close contacts.

Of the 25 results that were QFT-GIT negative/QFT-Plus positive, 11 were positive by both TB1 and TB2 tubes, five only by TB1, and 9 only by TB2. QFT-GIT showed almost identical concordance with each QFT-Plus antigen tube (see Table S3a).

Agreement between TB1 and TB2 among all 506 participants was 97%, with 1% of the discordance TB1 positive/TB2 negative, and 2% TB1 negative/TB2 positive. The 48 close contacts were 100% concordant for TB1 and TB2 tubes (Table S3b).

Agreement of QuantiFERONs with TST and T-SPOT. QFT-GIT and QFT-Plus showed similar agreement with TST overall and among U.S.-born and non-U.S.-born persons (Table 4). For all participants, QFT-GIT and QFT-Plus had similar agreement with TST, 77% ($\kappa = 0.45$ and $\kappa = 0.46$, respectively). Overall, 36% of participants were tested with Aplisol and 62% with Tubersol; 2% were missing the information. We found similar proportions of Aplisol and Tubersol use in concordant and discordant samples for both the QFT-Plus and QFT-GIT comparisons. For TST results concordant with QFT-Plus, 37% used Aplisol and 61% used Tubersol. For discordant results, 32% used Aplisol and 64% used Tubersol. For TST results concordant with QFT-GIT, 38% used Aplisol and 61% used Tubersol. For discordant results, 35% used Aplisol and 65% used Tubersol.

Twenty-one T-SPOT results were borderline and five were invalid; these were excluded from measurements of agreement. A comparison between QFT-GIT and QFT-Plus showed higher agreement between the two QFTs and T-SPOT than between the QFTs and TST (Table 4). Among all participants, QFT-GIT and QFT-Plus had similar agreement with T-SPOT, 92% ($\kappa = 0.73$) and 91% ($\kappa = 0.71$), respectively. Distributions of QFT-GIT, T-SPOT, and TST results among those positive or negative for QFT-Plus are shown in Tables S4a and S4b.

Ten participants were positive only by QFT-Plus, of whom eight were non-U.S. born. Two were positive by both TB1 and TB2, two were positive only by TB1, and 6 were positive only by TB2. Two other participants were negative by QFT-Plus and positive by the 3 other tests (Table S5).

TABLE 4 Comparing both QFT-GIT and QFT-Plus with TST and T-SPOT

Category	QFT test[a]	Test agreement (*n*/total [%])[b]	
		TST	T-SPOT
All	QFT-GIT	393/508 (77)	445/482[c] (92)
	QFT-Plus	391/506 (77)	437/480[c,d] (91)
Non-U.S. born	QFT-GIT	328/436 (75)	378/410[c] (92)
	QFT-Plus	328/434 (76)	372/408[c,d] (91)
U.S. born	QFT-GIT	64/71 (90)	66/71 (93)
	QFT-Plus	62/71 (87)	64/71 (90)

[a]QFT-GIT, QuantiFERON-TB Gold In-Tube; QFT-Plus, QuantiFERON-TB Gold Plus.
[b]TST, tuberculin skin test; T-SPOT, T-SPOT.TB.
[c]Borderline and invalid results were excluded.
[d]Two indeterminate QFT-Plus results excluded.

TABLE 5 Median gamma interferon levels among those positive by both QFT-GIT and QFT-Plus

QFT-GIT$^+$/QFT-Plus$^+$[a]	Total no.	IFN-γ (median [IQR] IU/ml)		
		QFT-GIT nil	TB1 nil	TB2 nil
All	94	1.83 (0.78, 8.83)	2.55 (0.98, 9.87)	2.90 (1, 9.87)[b,c]
Non-U.S.-born	88	2.05 (0.9, 10)	2.80 (1.10, 9.95)	3.10 (1.05, 9.98)[b]
U.S.-born	6	0.8 (0.38, 1.55)	0.83 (0.42, 1.73)	0.97 (0.53, 1.81)[b]

[a]QFT-GIT, QuantiFERON-TB Gold In-Tube; QFT-Plus, QuantiFERON-TB Gold Plus.
[b]$P < 0.05$ for IFN-γ levels of TB2 compared to QFT-GIT.
[c]$P < 0.05$ for IFN-γ levels of TB1 compared to TB2, the remaining values showed no significant difference (Wilcoxon signed-rank test).

Quantitative results. We assessed variations in IFN-γ levels between QFT-GIT and QFT-Plus and between the TB1 and TB2 tubes in QFT-Plus. Among all participants who had a positive result for both QFT-GIT and QFT-Plus, median IFN-γ concentrations were significantly higher in TB2 tubes than in TB1 and QFT-GIT ($P < 0.05$). Median IFN-γ concentrations were not significantly different in TB1 tubes compared to those in QFT-GIT (Table 5). The median IFN-γ concentrations were 0 IU/ml for all participants who had negative results for both QFT-GIT and QFT-Plus. Among the 102 participants positive by both the TB1 and TB2 tubes, the median CD8$^+$ T-cell response was zero overall, and -0.07 (IQR, -0.17 to 0.27) for 4 close contacts (Table 6).

Two sites used separate nil tubes from QFT-GIT and QFT-Plus kits. For the 102 samples from these two sites, median nil IFN-γ levels were significantly lower in QFT-GIT samples (0.07 IU/ml; IQR, 0.05 to 0.1) than in QFT-Plus samples (0.12 IU/ml; IQR, 0.09 to 0.21, $P < 0.0001$) (see Fig. S1). Despite these differences, the percentage agreement (96%) for QFT-GIT compared to QFT-Plus was almost identical to the percentage agreement (94%) for all participants. Eleven of the 102 samples were positive by both QFT-GIT and QFT-Plus; while these 11 also had significantly different nil values, the reported antigen minus nil values were not significantly different (Table 7).

We assessed the effect of other variations in laboratory practices; none appreciably affected the results among discordant samples (see Table S6).

DISCUSSION

This is the first comparison of QFT-Plus with all other diagnostic tests for *M. tuberculosis* infection: QFT-GIT, TST, and T-SPOT. Overall, QFT-Plus and QFT-GIT showed high agreement; QFT-Plus performed like QFT-GIT compared to TST and T-SPOT.

Despite variations in blood collection and sample processing methods, the 94% agreement between QFT-GIT and QFT-Plus ($\kappa = 0.81$) in this study was comparable to findings in other LTBI studies, which reported agreements ranging from 90% to 95% (9–12). The 6% discordance was primarily in the direction of QFT-GIT negative/QFT-Plus positive; 25 (78%) of the 32 discordant values were in this direction.

TABLE 6 Median CD8$^+$ T-cell response[a]

Category[b]	No. of TB1$^+$ and TB2$^+$	IFN-γ (TB2 − TB1) (median [IQR] IU/ml)	No. of TB1$^-$ and TB2$^-$	IFN-γ (TB2 − TB1) (median [IQR] IU/ml)
All[c]	102	0 (-0.12 to 0.33)	387	0 (-0.01 to 0.02)
Non-U.S. born[d]	96	0 (-0.14 to 0.33)	323	0 (-0.01 to 0.03)
Class B1 immigrant	20	0.01 (-0.05 to 0.25)	25	0.01 (-0.01 to 0.03)
U.S. born[e]	6	0.10 (0.04 to 0.24)	63	0 (-0.01 to 0.02)
Close contact	4	-0.07 (-0.17 to 0.27)	44	0 (-0.01 to 0.02)

[a]CD8$^+$ T-cell response among groups positive (TB1$^+$ or TB2$^+$) or negative (TB1$^-$ or TB2$^-$) for both; 2 antigen tubes of QFT-Plus.
[b]Class B1 immigrant, immigrant with abnormal chest radiograph but negative sputum smears and cultures in overseas examinations; close contact, ≥8 h/week exposure to TB case.
[c]Two indeterminate QFT plus results excluded.
[d]Includes class B1 immigrants and close contacts.
[e]Includes close contacts.

TABLE 7 Comparison of median IFN-γ levels among 11 QFT-GIT positive/QFT-Plus positive that used separate nil tubes

Test[a]	IFN-γ nil (median [IQR] IU/ml)[b]	Antigens tubes	IFN-γ TB antigen-nil (median [IQR] IU/ml)[c]
QFT-GIT	0.06 (0.05, 0.13)	GIT	2.83 (0.86–8.83)
QFT-Plus	0.16 (0.10, 0.40)	Plus TB1	2.98 (0.87–9.45)
		Plus TB2	3.18 (1.02–7.93)

[a]QFT-GIT, QuantiFERON-TB Gold In-Tube; QFT-Plus, QuantiFERON-TB Gold Plus. Separate nil tubes for QFT-GIT and QFT-Plus from respective sets.
[b]$P = 0.001$.
[c]No significant difference between QFT-GIT and QFT-Plus TB antigen minus nil IFN-γ levels (Wilcoxon signed-rank test).

This apparent increase in positive results could be due to a variety of factors. One is the manufacturer guidelines for interpreting a positive QFT-Plus result. Because a positive finding in either tube is considered a positive result, the percent positive will predictably increase (13). By the same measure, a requirement that both tests be positive before concluding that the result is positive would reduce the proportion of positive results. In our study, 14 of 25 (56%) of the QFT-Plus positive/QFT-GIT negative results were due to one positive tube.

The increased positivity could also be due to the changes in the formulation of the QFT-Plus. The new QFT-Plus antigen tubes have a spray-dried antigen coat, while the QFT-GIT antigen tube had a resin coat. The spray drying process was designed to enable better dissolution when mixed with the drawn blood (Masae Kawamura, Qiagen, personal communication). The added $CD8^+$ T-cell-responsive antigens in the TB2 tube may also increase IFN-γ levels, which would increase the number of results considered positive. Among samples positive by both QFT-GIT and QFT-Plus, the median IFN-γ levels were higher in TB1 than in QFT-GIT, but not significantly so (Table 5). Other studies reported significantly higher median QFT-GIT IFN-γ than TB1 and suggested this could be because TB7.7 was excluded from the TB1 tube (12, 14).

Of note, the discordance between the QFT-GIT and the QFT-Plus samples was mainly among results close to the cutoff value for a positive result, as other studies have found (10, 11, 15–17). Results close to the cutoff values are subject to high conversion/reversion rates, which may be attributed to variability in collection and processing of samples (16, 18).

The manufacturer cites a previous independent study to support a suggestion that the $CD8^+$ T-cell response in the TB2 tube can be used as a marker for recent infection (5). Based on that assumption, close contacts would be expected to have higher IFN-γ responses in the TB2 tube after subtraction of the TB1 antigen values. Although other studies have found higher $CD8^+$ T-cell responses (9, 12), we observed a median decrease of 0.07 IU/ml (IQR, −0.17 to 0.27) for TB2 minus TB1 levels among close contacts who were positive by both TB1 and TB2. However, only four of our 48 contacts were positive by QFT-Plus.

Since the $CD8^+$ T-cell response is measured indirectly, caution is required in interpreting the higher IFN-γ values in the TB2 tube. According to the manufacturer, the TB1 tube has antigens that are supposed to elicit a response specifically from $CD4^+$ T cells. Other researchers have used flow cytometry to show that 33% of LTBI patients who were close contacts to infectious TB cases had a $CD8^+$ T-cell response in the TB1 tube (6). The authors suggest that this was probably due to the processing of the TB1 peptides and their subsequent presentation to $CD8^+$ T cells. Another study reported $CD8^+$ T-cell responses to QFT-GIT antigens among individuals with close contact, although these antigens were designed to elicit responses only from $CD4^+$ T cells (5). These findings suggest that the method of subtracting the TB1 IFN-γ levels from the TB2 levels may over- or underestimate the $CD8^+$ T-cell response. A recent study concluded that $CD8^+$ T-cell clones showed specific and targeted responses to the CFP-10 epitopes in the QFT-Plus TB2 tube (19). However,

one of the $CD8^+$ T-cell-responsive CFP-10 epitopes had a significant overlap with the $CD4^+$ T-cell-responsive CFP-10 epitope. Since the QFT-Plus ELISA cannot distinguish the IFN-γ produced by $CD4^+$ and $CD8^+$ T cells when whole blood samples are used, it would be difficult to distinguish if the IFN-γ produced in TB2 was from $CD4^+$ or $CD8^+$ T cells.

In addition, studies investigating responses among close contacts, including the current study, have not rigorously documented whether the contacts were recently infected (i.e., negative at baseline and positive at follow-up testing after exposure to a person with infectious TB). Larger studies on contacts with documented recent infections and a better method to estimate actual $CD4^+$ and $CD8^+$ T-cell responses are needed to validate the $CD8^+$ T-cell response in the TB2 tube.

This study also evaluated agreement between the QFT tests and TST and T-SPOT. Overall and among U.S.-born and non-U.S.-born participants, agreement between QFT-GIT and TST/T-SPOT was almost identical to that between QFT-Plus and TST/T-SPOT. Most studies have shown lower agreement between TST and IGRAs among persons born in countries with high rates of TB. This has been attributed to BCG vaccination, which can produce false-positive TST results (20). Our study also found lower agreement between TST and IGRAs among non-U.S.-born participants. In the comparison of single-test IGRA results, QFT-Plus had the highest proportion of positive results (23%) and T-SPOT the lowest (15%) (Table 2). One possible explanation for the lower proportion of positive TSPOTs is the longer time to processing. The TSPOT manufacturer has developed a reagent (T-Cell Xtend) to preserve the test's accuracy for up to 32 h after collection. A study ($N = 302$) conducted in the United States reported that blood stored for up to 33 h and then processed with Xtend showed similar results compared to samples processed within 0 to 3.5 h of collection without Xtend (21). Further studies are needed to fully assess Xtend's effectiveness.

Ten participants positive by QFT-Plus and negative by QFT-GIT, T-SPOT, and TST are unlikely to have received treatment for LTBI, because the QFT-Plus was performed prior to FDA approval and the result was not used for clinical purposes. Follow-up of these participants through TB registry matches over the next 2 years may provide insight into the sensitivity and specificity of QFT-Plus.

The study's limitations include the small number of close contacts with LTBI; this limited our ability to assess the relevance of $CD8^+$ T-cell-responsive antigens in differentiating recent from remote infection. The strengths of this study were its large size and its ability to compare QFT-Plus with QFT-GIT, T-SPOT, and TST. The study was performed within public health departments serving as TBESC study sites, which makes the results more generalizable to standard public health practice. Overall, our results appeared to be robust to the variability in sample collection and laboratory methods. The findings will reassure clinicians that the new QuantiFERON test, QFT-Plus, is highly concordant with the third generation, QFT-GIT, and similar to QFT-GIT in its concordance with the TST and T-SPOT.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00985-19.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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