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Performance of the QuantiFERON®-TB Gold In-Tube assay in tuberculin skin test converters: a prospective cohort study

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SUMMARY

OBJECTIVE: To investigate diagnostic agreement of the QuantiFERON®-TB Gold In-Tube (QFT-GIT) test in adult tuberculin skin test (TST) converters in a high tuberculosis (TB) burden setting.

SETTING AND DESIGN: We performed a case-cohort study from 2014 to 2016 in Uganda among residents who were not infected with *Mycobacterium tuberculosis*. Participants were followed up for 1 year, when they were retested to determine TST conversion. All TST converters and a random sample of participants from baseline were offered QFT-GIT testing.

RESULTS: Of 368 enrolled participants, 61 (17%) converted their TST by 1 year. Among 61 converters, 42 were tested using QFT-GIT, 64% of whom were QFT-GIT-positive. Of 307 participants with a persistent negative TST, 48 were tested using QFT-GIT, 83% of whom were QFT-negative. Overall concordance of TST and QFT-GIT was moderate ($\kappa = 0.48$, 95%CI 0.30–0.66). Converters with a conversion of ⩾15 mm had a higher proportion of concordant QFT-GIT results (79%) than converters with increments of 10–14.9 mm (52%).

CONCLUSION: Concordance between TST and QFT-GIT was moderate among TST converters in this urban African population. These findings call for improved tests that more accurately measure conversion to tuberculous infection.

KEY WORDS: *Mycobacterium tuberculosis*; interferon-gamma release assays; tuberculous infection; transmission

IN AFRICA, up to 50% of tuberculosis (TB) cases remain undetected,[1] which can lead to substantial TB transmission. The magnitude of this transmission is best measured through incident cohorts, as these designs quantify the rate of new infections as opposed to new cases. The incidence of infection is difficult to quantify, however, due to limitations in current diagnostic tests for tuberculous infection.

There is currently no gold standard test for the diagnosis of tuberculous infection.[2] The tuberculin skin test (TST) is widely used, but false-positive results may occur in some populations due to previous sensitization to environmental mycobacteria or bacille Calmette-Guérin (BCG) vaccination.[3–5] Furthermore, false-negative tests may occur due to malnutrition, coinfection with other micro-organisms or through errors in testing technique.[6,7] The interferon-gamma release assay (IGRA) is another type of test used to diagnose tuberculous infection. IGRAs use antigens from the region of difference 1 of the mycobacterial genome, which are absent in BCG[8] and many pathogenic environmental mycobacteria.[9] However, inconsistency of IGRA results after repeat testing is common, and may be caused by a wide array of factors.[4]

While the operating characteristics of IGRAs have been compared against the TST in numerous cross-sectional and prevalence studies,[10–13] their performance has not been compared fully in incidence cohorts among TST converters, especially in adults from low- and middle-income countries. In only one study from South Africa, a commercial IGRA—the QuantiFERON®-TB Gold In-Tube test (QFT-GIT; Qiagen, Hilden, Germany)—was evaluated in adolescents who experienced TST conversion, and the concordance between the two tests was excellent.[14]

To assess whether this high level of agreement was present among adults, we compared the performance and concordance of QFT-GIT and TST among converters identified in a prospective cohort study of Ugandan adults.

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STUDY POPULATION AND METHODS

We performed a case-cohort study within a cohort of adult African individuals with no evidence of latent tuberculous infection (LTBI). Between June 2014 and August 2015, a cohort of 422 individuals was recruited from among the residents of the Lubaga Division of Kampala, Uganda. The inclusion criteria were TST induration $\leqslant 5$ mm at baseline, age 18–65 years, and no signs or symptoms of active TB. A standard clinical evaluation was performed at baseline to assess the risk of acquiring new tuberculous infection. Participants were evaluated at quarterly intervals to assess risk of disease. At 1 year (median 374 days, interquartile range [IQR] 367–386), participants were retested using the TST to evaluate for new infection, as defined by TST conversion. Controls were randomly selected from the parent cohort at baseline. All TST converters and randomly selected controls were offered QFT-GIT testing as a way to validate conversion. Blood samples (3 ml) were collected for QFT-GIT before placement of the TST to preclude potential QFT-GIT boosting using TST.[15]

Demographic, social, and clinical characteristics were obtained through interviews performed by trained field workers using standardized questionnaires. The TST was performed by placing five tuberculin units of purified protein derivative on the left forearm of the participants using the Mantoux method.[16] Two field workers independently measured the diameter of the induration within 48–72 h using the 'ball-point' technique and digital calipers to reduce digit preference bias. Samples for QFT-GIT testing were collected in 1 ml tubes provided with the kit, and were transported to and received by the laboratory at room temperature within approximately 2 h of blood collection. The tubes were incubated at 37°C for 16–24 h when the plasma was separated and stored at –80° until an enzyme-linked immunosorbent assay was performed.

Values for optical density were used to calculate interferon-gamma (IFN-γ) results in international units per milliliter (IU/ml) using QFT-GIT Analysis v2.17. Human immunodeficiency virus (HIV) testing was performed in accordance with the Ugandan Ministry of Health national testing algorithms. All participants were administered Determine™ HIV-1/2 (Abbott, Waltham, MA, USA) and HIV 1/2 STAT-PAK™ (Medford, NY, USA); Uni-Gold™ HIV (Trinity Biotech, Bray, Ireland) testing was performed in case of discrepant results for the initial tests.

TST conversion was defined as having a second TST reading of $\geqslant 10$ mm with an increase in reaction size of $\geqslant 10$ mm from the first to the second reading.[17] If the conditions mentioned above were not met, subjects were classified as persistent TST-negative. QFT-GIT results were defined as positive if the difference in IFN-γ values between the TB antigen and nil values was $\geqslant 0.35$ IU/ml and $\geqslant 25\%$ of the nil value.[18] QFT-GIT results were defined as negative if the difference in IFN-γ values between the TB antigen and nil values was <0.35 IU/ml or if the same difference is >0.35 IU/ml and $<25\%$ of the nil value. A test result was considered to be indeterminate if the difference in the mitogen and nil values was <0.50 IU/ml, or whenever the nil value was >8.0 IU/ml. For sensitivity analyses, we used two additional definitions of QFT-GIT positivity: TB antigen minus nil IFN-γ values of >0.20 IU/ml and >0.70 IU/ml.[19,20]

Analytic approach

Baseline characteristics of the study population and the subset of subjects with available QFT-GIT results were summarized with proportions and measures of central tendency. Cumulative incidence of TST conversion at 1 year was estimated as a proportion with 95% confidence intervals (CIs). Concordance between TST and QFT-GIT results was assessed using Cohen's κ, and κ values were interpreted using previously defined cut-off values.[21] We estimated the κ statistic in groups stratified by age, sex, baseline HIV status and income. We further compared the proportion of TST converters with a concordant QFT-GIT result among those with conversion reactions of 10–14.9 mm and those with reactions of $\geqslant 15$ mm.

The median IFN-γ value was estimated for each conversion group. Any IFN-γ value with a numeric value below zero was transformed to zero. IQRs were calculated to demonstrate distributions within each conversion subgroup and for the following groups: QFT-GIT–/TST–, QFT-GIT–/TST+, QFT-GIT+/TST–, and QFT-GIT+/TST+. In the sensitivity analysis, we estimated TST conversion and the concordance of TST and QFT-GIT results using alternative definitions of QFT-GIT positivity (see Appendix).*

All analyses were carried out using SAS v9.4 (Statistical Analysis System, Cary, NC, USA) and R v3.3.1 (R Foundation for Statistical Computing, Vienna, Austria, 2016).

Ethical considerations

Written informed consent was provided by all participants before study inclusion. Institutional review board clearance was obtained from the Ethics Committee at Makerere University School of Public Health, Kampala, Uganda, and the University of Georgia, Athens, GA, USA. TST converters were referred for TB evaluation by study medical personnel. If TB was suspected, the participant was referred to the National Tuberculosis Control Program for

* The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2018/00000022/00000009/art0007

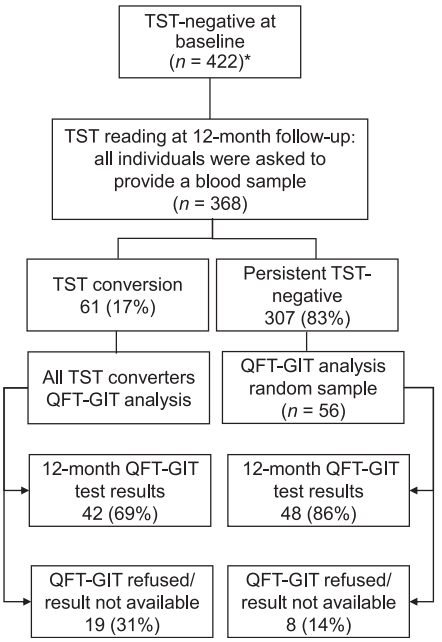

Figure 1 Flow diagram of study selection and TST and QFT-GIT test results. A total of 368 participants with no tuberculosis disease and a baseline TST result of ⩽5 mm were enrolled and had a second TST after 12 months of follow-up. All individuals were asked to provide a blood sample. QFT-GIT was administered in a subsample of these participants ($n = 90$).* Excluded: 25 refused second test, 19 withdrew before 12 months and 10 could not be traced. QFT-GIT = QuantiFERON®-TB Gold In-Tube; TST = tuberculin skin test.

treatment; otherwise they were offered isoniazid treatment by the study personnel.

RESULTS

The cohort comprised 368 participants who had TST data at baseline and follow-up (Figure 1). The median age of the participants was 24 years (IQR 21–30); 54% were male and 21 (6%) were HIV-positive. At the 12-month follow-up visit, no participants were diagnosed with TB disease. In all, 61 of 368 participants converted their TST by 1 year, leading to a cumulative annual incidence of tuberculous infection of 17% (95%CI 13–20). Among TST converters, the median skin test induration change was 13.6 mm (IQR 12.1–16.1). There were 307 participants with persistent negative TST at 12 months, 269 of whom showed no change from the baseline TST, 6 had an induration size smaller than the first one, and 32 had a median increment of 5.1 mm (IQR 3.0–7.1), not meeting the criteria for conversion.

Of the 61 TST converters, 42 (69%) had a corresponding QFT-GIT; of the 305 persistent TST-negative participants, 56 were randomly sampled and 48 (86%) were tested using QFT-GIT. The baseline characteristics in this subset of 90 participants with QFT-GIT results were similar to the overall cohort, except that the proportion of males was slightly higher (60%) (Table 1).

Of the 42 converters tested with QFT-GIT, 27 (64%) tested positive. Of the 48 participants with persistent negative TST, 40 (83%) also had a negative QFT-GIT. Test results were discordant in 15 (36%) of the TST converters and in 8 (17%) of those with persistent TST-negative results. The overall concordance between the TST and QFT-GIT was moderate ($\kappa = 0.48$; Table 2). The agreement between the tests was moderate when stratifying by age and HIV serostatus, but there were important differences in stratum-specific κ values when stratifying by sex and income (Table 2). The tests appeared to have good agreement in women and among individuals in the lower income bracket.

There was greater agreement between TST and QFT-GIT results among TST converters with an increment of ⩾15 mm than among converters with an induration size of 10–14.9 mm. Of the 19 converters with an increment of ⩾15 mm, 15 (79%, 95%CI 60–98) had a positive QFT-GIT (Figure 2 and Appendix Figure A.1). In contrast, among the 23 converters with increments between 10 and 14.9 mm, only 12 (52%, 95%CI 31–73) had a positive QFT-GIT. The median IFN-γ value was greatest in the QFT-GIT+/TST+ strata (median 3.79, IQR 0.76–8.66; Appendix Figure A.2), followed by individuals with a QFT-GIT+/TST– (median 1.11, IQR 0.73–6.32). The median IFN-γ results for the QFT-GIT–/TST+ and QFT-GIT–/TST– were respectively 0.01 (IQR 0.00–0.03) and 0.01 (IQR 0.00–0.06).

DISCUSSION

In this prospective cohort study of adults performed in a community with a high TB burden, we used QFT-GIT to confirm TST conversion, and found only moderate diagnostic agreement ($\kappa = 0.48$). We did, however, observe greater concordance among individuals with follow-up TST reactions of ⩾15 mm.

The main source of discrepancy between tests occurred among converters. Overall agreement was only 64%, with 36% of TST converters testing negative on the QFT-GIT. It is possible to explain negative QFT-GIT results if some of the TST conversion events represent false-positive conversions. Boosting of the TST as a result of repeated testing has been found to be a potential cause of false-positive TST conversion, especially in BCG-vaccinated individuals, such as our study subjects.[22] We assessed this possibility, however, in our cohort and found that boosting occurred in only 2% of participants.[23] Another potential cause of false-positive TST results is the presence of non-tuberculous mycobacteria (NTM); however, a study conducted in Ugandan children and adolescents showed

Table 1 Baseline characteristics of the total study population and in a subset of subjects with an available QFT-GIT assay result at 12-month follow-up

Category	Participants *n* (%)	Participants with QFT-GIT assay *n* (%)
n	368	90
Male sex	200 (54)	54 (60)
Age, years		
18–24	185 (50)	40 (44)
>24	183 (50)	50 (56)
Median [IQR]	24 [21–30]	26 [21–32]
Marital status		
Single/never married	169 (46)	42 (47)
Married in monogamous union	143 (39)	30 (33)
Married in a polygamous union	21 (6)	5 (6)
Separated/divorced	28 (8)	9 (10)
Widowed	7 (2)	4 (4)
Literacy		
Ability to read	354 (96)	87 (97)
Ability to write	349 (95)	85 (94)
Monthly income, USh*		
<199 999	213 (58)	54 (60)
⩾200 000	155 (42)	36 (40)
Baseline HIV status		
Negative	341 (93)	81 (90)
Positive	21 (6)	9 (10)
Refused	6 (2)	0
BCG-vaccinated†		
Yes (verbal/confirmed)	214 (91)	83 (93)
No	7 (3)	2 (2)
Unknown	14 (6)	4 (4)

* USh199 999 = US$55.3.
† TB vaccination data available for 235/368 individuals.
QFT-GIT = QuantiFERON®-TB Gold In-Tube; IQR = interquartile range; USh = Ugandan shilling; HIV = human immunodeficiency virus; BCG = bacille Calmette-Guérin; TB = tuberculosis.

that NTM represented <5% of mycobacterial infection in this setting.[24]

It is also possible that the QFT-GIT tests represent false-negative results. In serial testing, it has been shown that IGRA results may be unstable,[4] so TST converters who tested negative with QFT-GIT may test positive at another time. A longitudinal study conducted in South Africa found that annual risk of QFT-GIT reversion occurred in 5% of the study populations that had previously converted.[14] More rigorous algorithms have recently been proposed to optimize QFT-GIT testing and interpretation when measuring conversion, in which a stringent QFT-GIT converter will be an individual who converts from <0.2 IU/ml at baseline to >0.70 IU/ml after 1 year.[19,25] In our study, alternative cut-offs of >0.2 IU/ml and >0.70 IU/ml for QFT-GIT at follow-up did not result in improved concordance with TST conversion. Importantly, we did not have a baseline QFT-GIT result to assess conversion using QFT-GIT.

Our findings differ from those reported in a cohort study by Andrews et al. conducted in South Africa evaluating QFT-GIT and TST conversion in which the concordance was excellent ($\kappa = 0.74$).[14] There are three main differences between the studies that may affect the findings. First, Andrews et al. followed adolescents, whereas we excluded individuals aged <18 years. It is likely that the history of exposure to *Mycobacterium tuberculosis* is greater among the older cohort, given the age-assorted, social mixing in the African setting.[26] Second, as Andrews et al. used 5 mm as the cut-off for TST conversion, it is likely that they diagnosed a greater number of TST conversions. Third, we only measured QFT-GIT at one time point, i.e., at the end of 1 year of observation. In short, although the two studies present distinct findings, they do not contradict each other, but instead complement one another.

When we examined the relationship between the size of the TST reaction and the absolute value of the QFT-GIT result, we observed that the size of the TST conversion was associated with the size of the QFT-GIT values. These findings are consistent with those reported in cross-sectional studies in which TST induration was a predictor for a positive QFT-GIT result.[27] These observations are interesting and may suggest that the blood level of IFN-γ provides information about the nature of LTBI and risk for active disease. Various investigators have reported a correlation between the absolute level of IFN-γ and the risk for developing disease,[28–30] above and beyond the dichotomous definition.[31,32]

Our analysis raises questions about the value of using QFT-GIT to confirm TST conversion in high-endemic settings. Our findings suggest that QFT-GIT was most sensitive in individuals with the largest

Table 2 Concordance of QFT-GIT test results* at 12-month follow-up by participant's TST conversion† status: all individuals and selected subgroups

QFT-GIT result	TST converter ($n = 42$)	Persistent TST-negative ($n = 48$)	Total ($n = 90$)	κ (95%CI)
Total				
Positive	27	8	35	0.48 (0.30 to 0.66)
Negative	15	40	55	
Age group, years				
<25				
Positive	14	2	16	0.55 (0.31 to 0.80)
Negative	7	17	24	
⩾25				
Positive	13	6	19	0.42 (0.16 to 0.67)
Negative	8	23	31	
Sex				
Male				
Positive	12	5	17	0.34 (0.10 to 0.59)
Negative	12	25	37	
Female				
Positive	15	3	18	0.67 (0.42 to 0.91)
Negative	3	15	18	
Baseline HIV				
HIV-positive				
Positive	2	2	4	0.53 (0.02 to 1.00)
Negative	0	5	5	
HIV-negative				
Positive	25	6	31	0.48 (0.29 to 0.67)
Negative	15	35	50	
Monthly income, USh				
<199 999				
Positive	21	4	25	0.63 (0.42 to 0.84)
Negative	6	23	29	
⩾200 000				
Positive	6	4	10	0.22 (–0.09 to 0.53)
Negative	9	17	26	

* QFT-GIT tests were administered only at the 12-month follow-up visit, and results are stratified here by TST conversion status. QFT-GIT results were defined as positive if the difference in interferon-γ values between the TB antigen and nil values was ⩾0.35 IU/ml and ⩾25% of the nil value.
† Defined as having a second TST reading at the 12-month follow-up visit of ⩾10 mm, with an increase in reaction of ⩾10 mm from first to second reading.
QFT-GIT = QuantiFERON®-TB Gold In-Tube; TST = tuberculin skin test; CI = confidence interval; HIV = human immunodeficiency virus; USh = Ugandan shilling; IU = international unit.

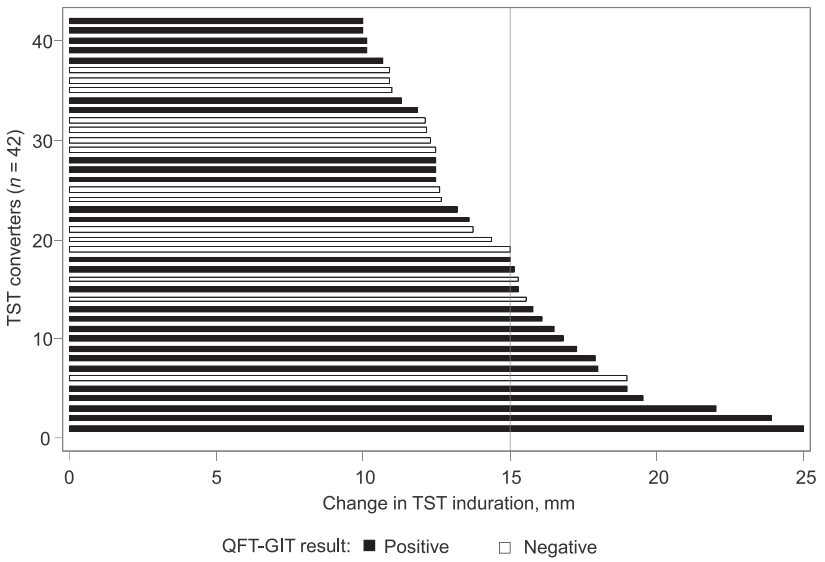

Figure 2 Change in TST induration in 42 TST converters. Each line represents a participant with TST conversion after the 12-month follow-up visit and the change in induration size (in mm) from baseline to the 12-month follow-up reading. Individuals with a positive QFT-GIT test result are indicated in black. Individuals with a negative QFT-GIT result are indicated in white. The vertical line represents the cut-off (15 mm) in change in induration size after 12 months. QFT-GIT = QuantiFERON®-TB Gold In-Tube; TST = tuberculin skin test.

conversions (i.e., >15 mm), or those with greater likelihood of new infection. In individuals with moderate conversion reactions (i.e., 10–14.9 mm), QFT-GIT was less sensitive than TST in delineating new infection. At this time, we agree with the recent statement by the World Health Organization (WHO) that 'IGRA should not replace TST in low-income and other middle-income countries'.[33] The use of the C-Tb skin test (Statens Serum Institut, Copenhagen, Denmark) as a measure of LTBI may mitigate concerns raised for both TST and QFT-GIT, but requires further validation in TB-endemic settings.[34]

There were several limitations to our analyses. The most important one was that QFT-GIT results were not available for participants from baseline. As we did not collect QFT-GIT at both baseline and follow-up, we could not assess the concordance between TST conversion and QFT-GIT conversion. Moreover, sampling bias may have been present, as approximately one in three participants declined QFT-GIT or no QFT-GIT result was available. This bias would be differential only if the unobserved QFT-GIT result was associated with withdrawal from the study; from our analysis, this seems unlikely, so any bias would be non-differential. A survival bias may have been present in our prospective analysis if patients with progressive primary disease died without our knowledge. Finally, invalid results from QFT-GIT are possible as QFT-GIT is known to have several sources of pre-analytical and analytical variability.[4,35] We implemented standard operating procedures for QFT-GIT collection and analysis; however, random variations are likely to persist.[35]

In summary, in TST converters, a large change in induration size was associated with higher concordance with QFT-GIT results. A QFT-GIT test may not be useful in confirming TST conversion in highly endemic settings, such as Uganda. The moderate agreement of QFT-GIT among TST converters calls for improved tests that more accurately measure conversion to tuberculous infection.

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Conflicts of interest: none declared.

References

1 World Health Organization. Global tuberculosis report, 2015. WHO/HTM/TB/2015.22. Geneva, Switzerland: WHO, 2015.
2 Lalvani A. Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest 2007; 131: 1898–1906.
3 Lange C, Mori T. Advances in the diagnosis of tuberculosis. Respirology 2010; 15: 220–240.
4 Pai M, Denkinger C M, Kik S V, et al. Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. Clin Microbiol Rev 2014; 27: 3–20.
5 Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis 2006; 10: 1192–1204.
6 Munoz L, Stagg H R, Abubakar I. Diagnosis and management of latent tuberculosis infection. Cold Spring Harb Perspect Med 2015; 5: a017830.
7 Lardizabal A A, Reichman L B. Diagnosis of latent tuberculosis infection. Microbiol Spectr 2017; 5(1).
8 Behr M, Wilson M, Gill W, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science 1999; 284: 1520–1523.
9 Andersen P, Munk M, Pollock J, Doherty T. Specific immune-based diagnosis of tuberculosis. Lancet 2000; 356: 1099–1104.
10 Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med 2007; 146: 340–354.
11 Legesse M, Ameni G, Mamo G, Medhin G, Bjune G, Abebe F. Community-based cross-sectional survey of latent tuberculosis infection in Afar pastoralists, Ethiopia, using QuantiFERON-TB Gold In-Tube and tuberculin skin test. BMC Infect Dis 2011; 11: 89.
12 Weinfurter P, Blumberg H M, Goldbaum G, et al. Predictors of discordant tuberculin skin test and QuantiFERON®-TB Gold In-Tube results in various high-risk groups. Int J Tuberc Lung Dis 2011; 15: 1056–1061.
13 Ghassemieh B J, Attia E F, Koelle D M, Mancuso J D, Narita M, Horne D J. Latent tuberculosis infection test agreement in the National Health and Nutrition Examination Survey. Am J Respir Crit Care Med 2016; 194: 493–500.
14 Andrews J R, Hatherill M, Mahomed H, et al. The dynamics of QuantiFERON-TB gold in-tube conversion and reversion in a cohort of South African adolescents. Am J Respir Crit Care Med 2015; 191: 584–591.
15 van Zyl-Smit R N, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. PLOS ONE 2009; 4: e8517.
16 Rieder H L, Chadha V K, Nagelkerke N J, Van Leth F, vanderWerf M J. Guidelines for conducting tuberculin skin test surveys in high-prevalence countries. Int J Tuberc Lung Dis 2011; 15 (Suppl 1): S1–S25.
17 Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. Am J Respir Crit Care Med 2000; 161: S221–S247.
18 Qiagen. QuantiFERON®-TB Gold (QFT®) ELISA Package Insert 03/2016. Hilden, Germany: Qiagen, 2016.
19 Nemes E, Rozot V, Geldenhuys H, et al. Optimization and interpretation of serial QuantiFERON testing to measure acquisition of *Mycobacterium tuberculosis* infection. Am J Respir Crit Care Med 2017; 196: 638–648.
20 Jonsson J, Westman A, Bruchfeld J, Sturegard E, Gaines H, Schon T. A borderline range for QuantiFERON Gold In-Tube results. PLOS ONE 2017; 12: e0187313.
21 Viera A J, Garrett J M. Understanding interobserver agreement: the kappa statistic. Fam Med 2005; 37: 360–363.
22 Menzies D. Interpretation of repeated tuberculin tests: boosting, conversion, and reversion. Am J Respir Crit Care Med 1999; 159: 15–21.

23 Sekandi J N, Zalwango S, Nkwata A K, et al. Low prevalence of tuberculin skin test boosting among community residents in Uganda. Am J Trop Med Hyg 2018; 98: 379–381.
24 Asiimwe B B, Bagyenzi G B, Ssengooba W, et al. Species and genotypic diversity of non-tuberculous mycobacteria isolated from children investigated for pulmonary tuberculosis in rural Uganda. BMC Infect Dis 2013; 13: 88.
25 Banaei N, Pai M. Detecting new *Mycobacterium tuberculosis* infection. Time for a more nuanced interpretation of QuantiFERON conversions. Am J Respir Crit Care Med 2017; 196: 546–547.
26 Dodd P J, Looker C, Plumb I D, et al. Age- and sex-specific social contact patterns and incidence of *Mycobacterium tuberculosis* infection. Am J Epidemiol 2016; 183: 156–166.
27 Mahan C S, Johnson D F, Curley C, van der Kuyp F. Concordance of a positive tuberculin skin test and an interferon gamma release assay in bacille Calmette-Guerin vaccinated persons. Int J Tuberc Lung Dis 2011; 15: 174–178, i.
28 Andrews J R, Nemes E, Tameris M, et al. Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study. Lancet Respir Med 2017; 5: 282–290.
29 Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Nienhaus A. Negative and positive predictive value of a whole-blood interferon-gamma release assay for developing active tuberculosis: an update. Am J Respir Crit Care Med 2011; 183: 88–95.
30 Altet N, Dominguez J, Souza-Galvao M L, et al. Predicting the development of tuberculosis with the tuberculin skin test and QuantiFERON testing. Ann Am Thorac Soc 2015; 12: 680–688.
31 Villar-Hernández R, Latorre I, Altet N, Domínguez J. Could IFN-γ levels in serial QuantiFERON predict tuberculosis development in young children? J Lab Precis Med 2017; 2(11).
32 Rangaka M X, Wilkinson K A, Glynn J R, et al. Predictive value of interferon-gamma release assays for incident active tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12: 45–55.
33 World Health Organization. Guidelines on the management of latent tuberculosis infection. Geneva, Switzerland: WHO, 2015.
34 Ruhwald M, Aggerbeck H, Gallardo R V, et al. Safety and efficacy of the C-Tb skin test to diagnose *Mycobacterium tuberculosis* infection, compared with an interferon-gamma release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial. Lancet Respir Med 2017; 5: 259–268.
35 Banaei N, Gaur R L, Pai M. Interferon-gamma release assays for latent tuberculosis: what are the sources of variability? J Clin Microbiol 2016; 54: 845–850.

APPENDIX

CONCORDANCE ANALYSIS USING ALTERNATIVE QFT-GIT POSITIVITY DEFINITIONS: SUPPLEMENTARY ANALYSIS

QFT-GIT cut-off >0.7 IU/ml

The change in cut-off resulted in a moderate concordance ($\kappa = 0.50$, 95% confidence interval [CI] 0.32–0.68), similar to the one obtained using the standard definitions (Table A.1).

QFT-GIT cut-off >0.2 IU/ml

The change in cut-off resulted in a weak concordance ($\kappa = 0.39$, 95%CI 0.20–0.58), lower than the one obtained using the standard definition (Table A.2).

Table A.1 Concordance of QFT-GIT test results* at the 12-month follow-up visit by TST conversion† status: all individuals and selected subgroups‡

Variable	QFT-GIT result	TST converter	Persistent TST-negative	Total	κ (95%CI)
Overall	Positive	26	6	32	0.50 (0.32 to 0.68)
	Negative	16	42	58	
Age group, years					
<25	Positive	13	2	15	0.51 (0.25 to 0.76)
	Negative	8	17	25	
⩾25	Positive	13	4	17	0.49 (0.25 to 0.74)
	Negative	8	25	33	
Sex					
Male	Positive	11	4	15	0.34 (0.10 to 0.58)
	Negative	13	26	39	
Female	Positive	15	2	17	0.72 (0.50 to 0.95)
	Negative	3	16	19	
Baseline HIV					
HIV-positive	Positive	2	2	4	0.53 (0.02 to 1.00)
	Negative	0	5	5	
HIV-negative	Positive	24	4	28	0.50 (0.32 to 0.68)
	Negative	16	37	53	
Monthly income, USh					
<199 999	Positive	20	3	23	0.63 (0.42 to 0.83)
	Negative	7	24	31	
⩾200 000	Positive	6	3	9	0.27 (–0.03 to 0.58)
	Negative	9	18	27	

* QFT-GIT results were defined as positive if the difference in interferon-γ values between the TB antigen and nil value was >0.70 IU/ml.
† Defined as having a second skin test reading at the 12-month follow-up visit of ⩾10 mm, with an increase in reaction of ⩾10 mm from the first to the second reading.
‡ Analysis using a more conservative definition of QFT-GIT positivity (>0.7 IU/ml).
QFT-GIT = QuantiFERON®-TB Gold In-Tube; TST = tuberculin skin test; CI = confidence interval; HIV = human immunodeficiency virus; USh = Ugandan shilling; IU = international unit.

Table A.2 Concordance of QFT-GIT test results* at the 12-month follow-up visit by TST conversion† status: all individuals and selected subgroups‡

Variable	QFT-GIT result	TST converter	Persistent TST-negative	Total	κ (95%CI)
Total	Positive	27	12	39	0.39 (0.20 to 0.58)
	Negative	15	36	51	
Age group, years					
<25	Positive	14	2	16	0.55 (0.30 to 0.80)
	Negative	7	17	24	
⩾25	Positive	13	10	17	0.27 (0.00 to 0.54)
	Negative	8	19	27	
Sex					
Male	Positive	12	9	21	0.20 (–0.06 to 0.46)
	Negative	12	21	33	
Female	Positive	15	3	18	0.67 (0.42 to 0.91)
	Negative	3	15	18	
Baseline HIV					
HIV-positive	Positive	2	3	5	0.37 (–0.08 to 0.82)
	Negative	0	4	4	
HIV-negative	Positive	25	9	34	0.41 (0.21 to 0.60)
	Negative	15	32	47	
Monthly income, USh					
<199 999	Positive	21	5	26	0.59 (0.38 to 0.81)
	Negative	6	22	28	
⩾200 000	Positive	6	7	13	0.07 (–0.26 to 0.39)
	Negative	9	14	23	

* Defined as positive if the difference in interferon-γ values between the TB antigen and nil value was >0.20 IU/ml.
† TST converter was defined as having a second skin test reading at the 12-month follow-up visit of ⩾10 mm, with an increase in induration size of ⩾10 mm from the first to the second reading.
‡ Analysis using alternative definition of QFT-GIT positivity (>0.2 IU/ml).
QFT-GIT = QuantiFERON®-TB Gold In-Tube; TST = tuberculin skin test; CI = confidence interval; HIV = human immunodeficiency virus; USh = Ugandan shilling; IU = international unit.

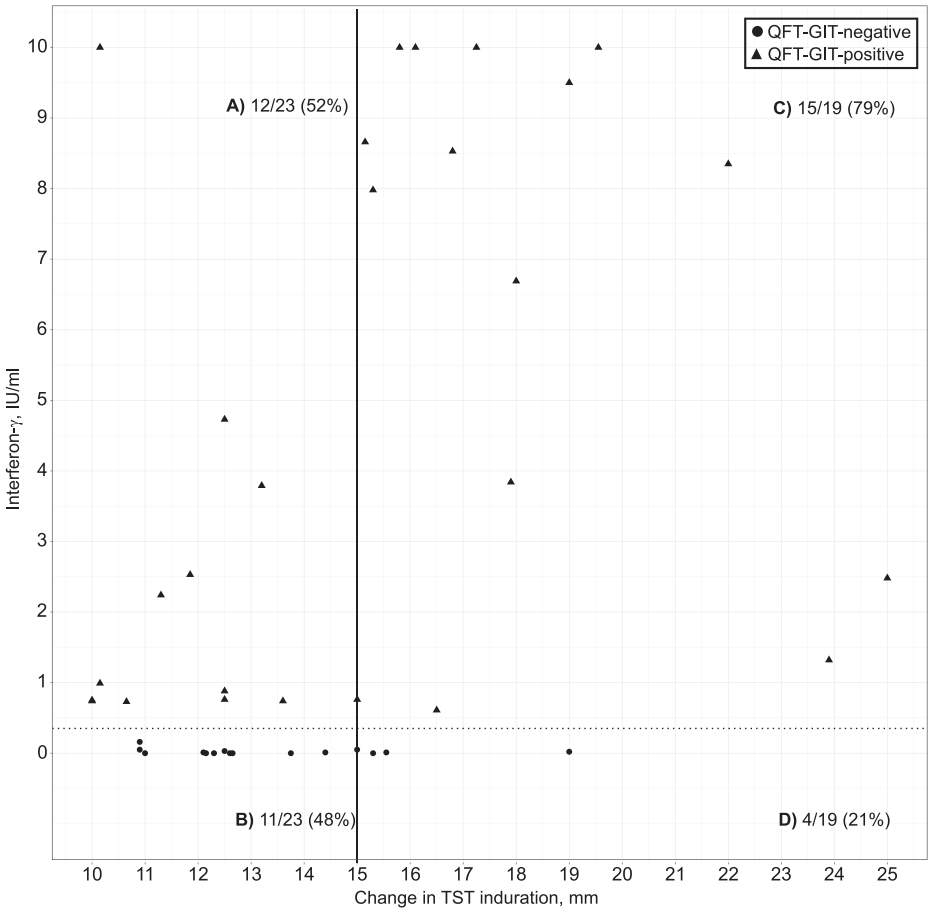

Figure A.1 Scatter plot of change in TST induration size and QFT-GIT test results at the 12-month follow-up visit among 42 TST converters. We assessed the relationship between the increment in TST among converters and the result of QFT-GIT by plotting a graph. A QFT-GIT-positive test was defined as a TB antigen minus nil interferon-γ value that was ⩾0.35 IU/ml. This cut-off is represented here by the horizontal black dashed line. The vertical line represents the cut-off point at 15 mm in change of induration size after 12 months. Triangles indicate TST converters with a positive QFT-GIT result; dots represent TST converters with a negative QFT-GIT result at the 12-month follow-up visit. The proportions shown represent: **A)** positive and **B)** negative QFT-GIT results among TST converters with a change of TST induration of 10–14.99 mm ($n = 23$); **C)** positive and **D)** negative QFT-GIT results among TST converters with a change in TST induration size of ⩾15 mm ($n = 19$). QFT-GIT = QuantiFERON®-TB Gold In-Tube; TST = tuberculin skin test; TB = tuberculosis; IU = international unit.

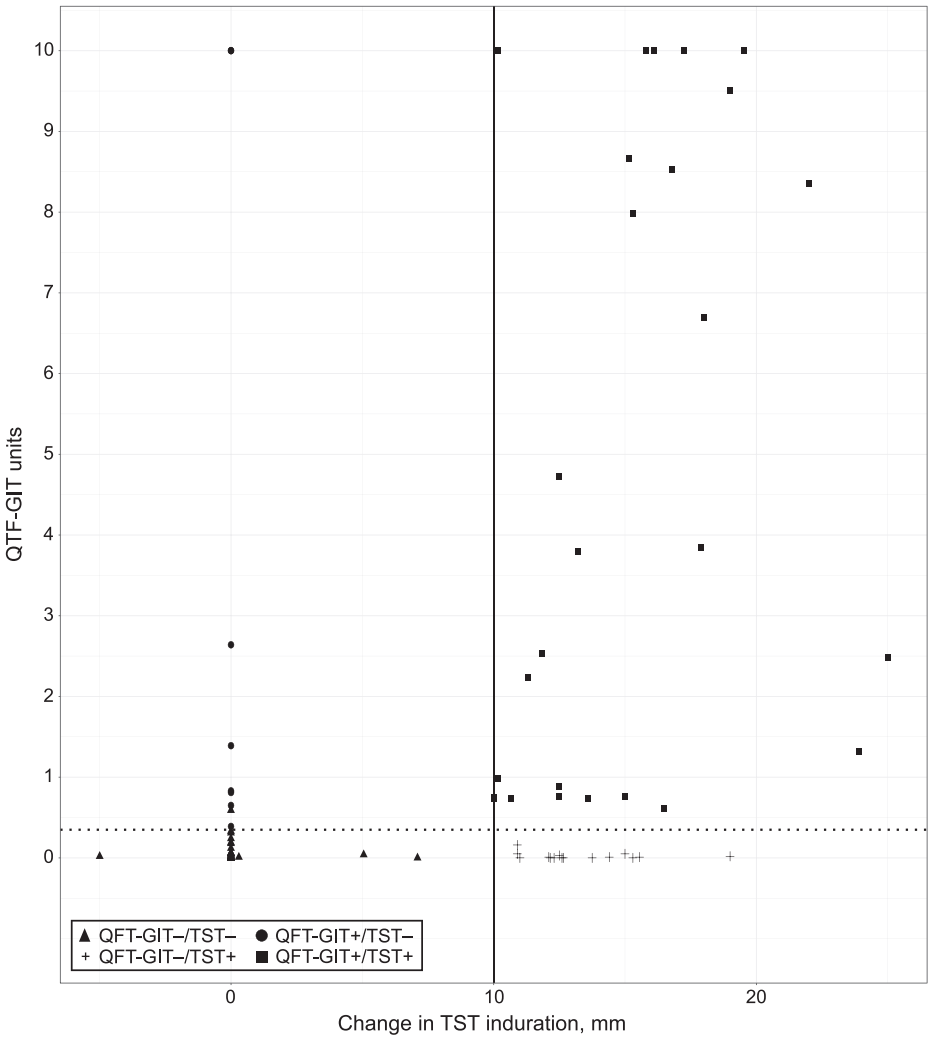

Figure A.2 Scatter plot of change in induration size (in mm) and IFN-γ values for QFT-GIT results among TST converters and persistent negatives ($n = 90$). A QFT-GIT-positive test was defined as a TB antigen minus nil IFN-γ value that was ⩾0.35 IU/ml. This cut-off is represented here by the horizontal black dashed line. The solid vertical line separates persistent TST-negatives from TST converters (second TST reading of ⩾10 mm, with an increase in reaction of ⩾10 mm). Black triangles indicate concordant negative QFT-GIT/TST ($n = 40$). Black crosses indicate discordant QFT-GIT–/TST+ ($n = 15$). Black circles indicate discordant QFT-GIT+/TST– ($n = 8$) and black squares represent concordant positive QFT-GIT/TST ($n = 27$). QFT-GIT = QuantiFERON®-TB Gold In-Tube; – = negative; TST = tuberculin skin test; + = positive; TB = tuberculosis; IU = international unit; IFN = interferon.

RÉSUMÉ

OBJECTIF : Etudier l'accord diagnostique du QuantiFERON®-TB Gold In-Tube (QFT-GIT) chez des adultes qui ont eu une conversion du test cutané à la tuberculine (TST) dans un contexte lourdement frappé par la tuberculose.

CONTEXTE ET SCHÉMA : Nous avons réalisé une étude cas-cohorte de 2014 à 2016 en Ouganda parmi des résidents qui n'ont pas été infectés par *Mycobacterium tuberculosis*. Les participants ont été suivis pendant 1 an puis ont été retestés pour identifier la conversion du TST. Tous les participants qui ont eu une conversion du TST et un échantillon aléatoire de participants de départ ont été invités à faire un test QFT-GIT.

RÉSULTATS : Sur 368 participants enrôlés, 61 (17%) ont eu une conversion du TST en 1 an. Parmi ces 61 participants, 42 ont eu un test QFT-GIT, dont 64% ont été QFT-GIT positifs. Sur 307 participants dont le TST est resté négatif, 48 ont été testés par QFT-GIT, dont 83% ont été QFT-GIT négatifs. La concordance d'ensemble du TST et de QFT-GIT a été modérée ($\kappa =$ 0,48, IC95% 0,30–0,66). Les patients qui ont eu une conversion du TST avec une réaction de $\geqslant$15 mm ont eu une proportion plus élevée de résultats concordants avec QFT-GIT (79%) que ceux dont la réaction allait de 10 à 14,9 mm (52%).

CONCLUSION : La concordance entre TST et QFT-GIT parmi les participants qui avaient eu une conversion du TST a été modérée parmi ces participants dans cette population urbaine d'Afrique. Ces résultats sont en faveur de tests plus performants qui mesurent de manière plus précise la survenue de l'infection tuberculeuse.

RESUMEN

OBJETIVO: Investigar la concordancia diagnóstica de la prueba QuantiFERON®-TB Gold en Tubo (QFT-GIT) en los adultos que han convertido la prueba cutánea de la tuberculina (TST) en un entorno con alta carga de morbilidad por tuberculosis.

MARCO DE REFERENCIA Y MÉTODO: Se llevó a cabo un estudio de casos en una cohorte del 2014 al 2016 en Uganda, en los residentes que no presentaban infección por *Mycobacterium tuberculosis*. Al final del seguimiento de 1 año se repitió la TST con el fin de detectar las conversiones. Se ofreció la prueba QFT-GIT a todos los residentes que habían convertido la reacción TST y a una muestra aleatoria de los participantes iniciales.

RESULTADOS: De los 368 participantes en el estudio, 61 habían convertido su reacción TST 1 año después (17%). De estos 61 participants, se practicó la prueba QFT-GIT en 42 y de ellos el 64% obtuvo un resultado positivo. De los 307 participantes en quienes la reacción TST permaneció negativa, se practicó la prueba QFT-GIT en 48, de los cuales el 83% obtuvo un resultado negativo. La concordancia global de la TST y la QFT-GIT fue moderada ($\kappa = 0{,}48$; IC95% 0,30–0,66). Quienes convirtieron la TST con una reacción de $\geqslant$15 mm exhibieron una proporción más alta resultados concordantes de la QFT-GIT (79%) que quienes presentaron una conversión con una reacción TST de 10 mm a 14,9 mm (52%).

CONCLUSIÓN: La concordancia entre la TST y la prueba QFT-GIT fue moderada en las personas de esta población urbana de África que habían convertido la TST. Estos resultados demuestran la necesidad de contar con mejores pruebas que midan con mayor exactitud la conversión hacia la infección tuberculosa.