



RESEARCH ARTICLE

REVISED **Comparing QuantiFERON-TB Gold Plus with QuantiFERON-TB Gold in-tube for diagnosis of latent tuberculosis infection among highly TB exposed gold miners in South Africa [version 3; peer review: 2 approved, 1 approved with reservations]**

Previously titled: 'Comparing the performance of QuantiFERON-TB Gold Plus with QuantiFERON-TB Gold in-tube among highly TB exposed gold miners in South Africa'

Thobani NtshiqID¹, Violet ChihotaID^{1,2}, Raoul MansukhaniID³, Lindiwe Nhlangulela¹, Kavindhra Velen¹, Salome Charalambous^{1,2}, Pholo Maenetje¹, Thomas R. Hawn⁴, Robert Wallis¹, Alison D. GrantID^{2,3,5}, Katherine Fielding^{2,3}, Gavin Churchyard^{1,2}

¹Implementation Research Division, The Aurum Institute, Johannesburg, Gauteng, 2193, South Africa

²School of Public Health, University of the Witwatersrand, Johannesburg, Gauteng, 2193, South Africa

³Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom, WC1E 7HT, UK

⁴Department of Medicine, University of Washington, Seattle, Seattle, New York, 98195, USA

⁵Africa Health Research Institute, Laboratory Medicine & Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, KwaZulu-Natal, 4041, South Africa

V3 **First published:** 11 Mar 2021, **5:66**
<https://doi.org/10.12688/gatesopenres.13191.1>
Second version: 08 Apr 2022, **5:66**
<https://doi.org/10.12688/gatesopenres.13191.2>
Latest published: 18 Aug 2022, **5:66**
<https://doi.org/10.12688/gatesopenres.13191.3>

Abstract

Background: QuantiFERON-TB-Gold-in-tube (QFT-GIT) is an interferon-gamma release assay (IGRA) used to diagnose latent tuberculosis infection. Limited data exists on performance of QuantiFERON-TB Gold-Plus (QFT-Plus), a next generation of IGRA that includes an additional antigen tube 2 (TB2) while excluding TB7.7 from antigen tube 1 (TB1), to measure TB specific CD4+ and CD8+ T lymphocytes responses. We compared agreement between QFT-Plus and QFT-GIT among highly TB exposed goldminers in South Africa.

Methods: We enrolled HIV-negative goldminers in South Africa, aged ≥33 years with no prior history of TB disease or evidence of silicosis. Blood samples were collected for QFT-GIT and QFT-Plus. QFT-GIT was considered positive if TB1 tested positive; while QFT-Plus was positive

Open Peer Review

Approval Status ✓ ? ✓

	1	2	3
version 3			
(revision)	✓	?	✓
18 Aug 2022	view	view	view
	↑		
version 2			
(revision)	?		
08 Apr 2022	view		
	↑		
version 1			
11 Mar 2021	?		
	view		

if both or either TB1 or TB2 tested positive, as per manufacturer's recommendations. We compared the agreement between QFT-Plus and QFT-GIT using Cohen's Kappa. To assess the specific contribution of CD8+ T-cells, we used TB2 – TB1 differential values as an indirect estimate. A cut-off value was set at 0.6. Logistic regression was used to identify factors associated with having TB2-TB1>0.6 difference on QFT-Plus.

Results: Of 349 enrolled participants, 304 had QFT-Plus and QFT-GIT results: 205 (68%) were positive on both assays; 83 (27%) were negative on both assays while 16 (5%) had discordant results. Overall, there was 94.7% (288/304) agreement between QFT-Plus and QFT-GIT (Kappa = 0.87). 214 had positive QFT-Plus result, of whom 202 [94.4%, median interquartile range (IQR): 3.06 (1.31, 7.00)] were positive on TB1 and 205 [95.8%, median (IQR): 3.25 (1.53, 8.02)] were positive on TB2. A TB2-TB1>0.6 difference was observed in 16.4% (35/214), with some evidence of a difference by BMI; 14.9% (7/47), 9.8% (9/92) and 25.3% (19/75) for BMI of 18.5-24.9, 18.5-25 and >30 kg/m², respectively (P=0.03).

Conclusion: In a population of HIV-negative goldminers, QFT-Plus showed high agreement with QFT-GIT, suggesting similar performance.

Keywords

Latent Tuberculosis Infection, QFT-GIT, QFT-Plus, TST, Performance, Goldmines, South Africa

1. **Mark Hatherill** , University of Cape Town, Cape Town, South Africa

2. **Lucia Barcellini**, University of Milan, Milan, Italy

3. **Sonali Sarkar**, JIPMER, Puducherry, India

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Thobani Ntshiqqa (thobanintshiqqa@yahoo.com)

Author roles: **Ntshiqqa T:** Conceptualization, Formal Analysis, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Chihota V:** Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Mansukhani R:** Data Curation, Formal Analysis, Software, Writing – Original Draft Preparation, Writing – Review & Editing; **Nhlangulela L:** Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Velen K:** Conceptualization, Formal Analysis, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Charalambous S:** Conceptualization, Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Maenetje P:** Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Hawn TR:** Formal Analysis, Funding Acquisition, Investigation, Methodology, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Wallis R:** Conceptualization, Funding Acquisition, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Grant AD:** Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Fielding K:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Software, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Churchyard G:** Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This study was funded by the Bill & Melinda Gates Foundation [OPP1116635].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Ntshiqqa T *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Ntshiqqa T, Chihota V, Mansukhani R *et al.* **Comparing QuantiFERON-TB Gold Plus with QuantiFERON-TB Gold in-tube for diagnosis of latent tuberculosis infection among highly TB exposed gold miners in South Africa [version 3; peer review: 2 approved, 1 approved with reservations]** Gates Open Research 2022, 5:66 <https://doi.org/10.12688/gatesopenres.13191.3>

First published: 11 Mar 2021, 5:66 <https://doi.org/10.12688/gatesopenres.13191.1>

REVISED Amendments from Version 2

The difference between the current and previously published versions is the removal of the word “performance” in the study title. The study title now reads as follows: Comparing QuantiFERON-TB Gold Plus with QuantiFERON-TB Gold in-tube for diagnosis of latent tuberculosis infection among highly TB exposed gold miners in South Africa. The word performance was also removed from the introduction, objectives, and methods sections both in the abstract and main manuscript to reflect that agreement between QFT-Plus and QFT-GIT was measured rather than performance.

Any further responses from the reviewers can be found at the end of the article

Introduction

Latent tuberculosis infection (LTBI) is the seedbed from which tuberculosis (TB) cases arise. LTBI is defined as an asymptomatic state characterized with a persistent immune response to stimulation by *Mycobacterium tuberculosis* (Mtb) antigens with no evidence of active TB¹⁻³. LTBI is typically characterized by a positive tuberculin skin test (TST) *in vivo*, involving intradermal injection of purified protein derivative from Mtb strain and/or a positive interferon-gamma release assay (IGRA)^{4,5}. Better tests are needed to identify persons at increased risk of developing TB disease.

IGRAs measure released interferon-gamma from cluster of differentiation (CD) T-lymphocytes specific to Mtb complex antigens but not produced by *Mycobacterium bovis* BCG vaccine strains⁶. QuantiFERON-TB Gold In-Tube assay (QFT-GIT) is designed to elicit interferon-gamma response from CD4+ helper T lymphocytes in a single TB antigen tube containing long peptides from ESAT-6, CPF-10 and TB7.7 antigens (Qiagen, Germantown, MD)⁵⁻⁷. QuantiFERON-TB Gold Plus assay (QFT-Plus) is a next generation IGRA that contains peptides from only the ESAT-6 and CFP-10 antigens comprising a TB1 tube, identical to the QFT-GIT, with the exception of TB7.7, and stimulates CD4+ T cells, and an additional antigen tube, TB2, which has a cocktail of both long and short ESAT-6 and CFP-10 peptides to elicit interferon-gamma release from both CD4+ helper T lymphocytes and CD8+ cytotoxic T lymphocytes⁵⁻⁸.

CD8+ cytotoxic T lymphocytes stimulating peptide was included in the QFT-Plus assay to improve on the sensitivity of QFT-GIT. Currently, limited data exist comparing the performance of these assays in high TB burdened settings. We compared the agreement between QFT-Plus and QFT-GIT among highly Mtb exposed goldminers in South Africa.

Methods**Study setting and population**

The study was conducted in the South African goldmines at the Occupational Health Centre (OHC), in Orkney, North West Province among goldminers, attending for their annual medical examination between July 2015 and December 2016.

Study design and procedures – parent study

In a cohort study, herein described as the parent study, we enrolled miners to identify those who were uninfected with Mtb despite being highly exposed to Mtb, to compare epidemiological factors between Mtb uninfected and infected miners and to collect specimens from Mtb uninfected and infected miners to determine gene expression and immunological profiles associated with being Mtb uninfected in future analysis.

Goldminers, attending OHC for their annual medical examination were pre-screened for the study to identify those aged 33–60 years who had worked in the mining industry for at least 15 years. Following informed consent, a full screen was conducted. Miners were included if they did not have symptoms suggestive of TB, no prior or current history of treatment for active TB disease, no history of or not currently taking isoniazid preventive therapy, no silicosis, had body mass index (BMI) >18.5, no serious medical conditions, HIV negative and no current treatment for cancer, no treatment with steroid tablets, inhalers or injections.

Blood samples were collected intravenously by trained professional phlebotomy nurses amongst those who met the inclusion criteria for QFT (QFT-Plus and QFT-GIT; Qiagen, Hilden Germany), peripheral blood mononuclear cell (PBMC), and transcriptomic (PAXgene) testing. Under the first version of the protocol (enrolments from 10 July 2015 to 29 October 2015), participants gave blood samples for QFT at enrolment.

A sputum sample was also collected for mycobacterial culture testing (BACTEC MGIT 960 system, BD Diagnostic Systems, Sparks, MD, USA) to exclude subclinical TB. A questionnaire was administered in a private room by a trained research assistant to collect demographic characteristics and information on factors associated with being TB uninfected. Blood samples collected were tested at the Aurum Clinical Research laboratory for LTBI using QFT-GIT and QFT-Plus. A 6 ml of whole blood sample was collected intravenously from each participant into a single lithium heparin tube. Samples were then transported to Aurum Clinical Research laboratory where they were aliquoted into 1 ml tubes: three tubes for QFT-GIT test kit (QFT-GIT nil, QFT-GIT TB, QFT-GIT mitogen with catalogue numbers 0594-0201 and 0594-0501) and four for QFT-Plus (QFT-Plus nil, QFT-Plus TB1, QFT-Plus TB2, QFT-Plus mitogen with catalogue number 622120). Tubes were then placed in a pre-heated 37°C portable incubator for 16–24 hours, within eight hours of collection. Subsequently, samples were centrifuged to separate plasma for same day testing. Alternatively, samples were stored at -80°C for up to 48–72 hours prior testing. Plasma was tested by interferon-gamma enzyme-linked immunosorbent assay (ELISA), performed using Biotek microplate reader model EL x 800 using Gen 5 software. Biotek microplate washer model EL x 508 and ThermoStar shaker were also used.

Study design – sub-study

In this sub-study, using cross-sectional data from the parent study, we compared the agreement between QFT-Plus and QFT-GIT using QFT measurements which were all done at baseline.

Study definitions

QFT-GIT was considered positive if TB1 tested positive; while QFT-Plus was positive if both TB1 and TB2 tested positive or if either TB1 or TB2 tested positive, as per manufacturer's recommendations. To assess the specific contribution of CD8+ T-cells, we used TB2–TB1 differential values as an indirect estimate. A cut-off value was set at 0.6 in order to reduce the bias of the intrinsic variability of the test⁹.

Statistical methods

All eligible participants from the parent study were included in the analysis. Binary outcomes (positive/negative) from QFT-Plus and QFT-GIT were compared using the percentage agreement and Kappa statistic. TB1 and TB2 responses for those QFT-Plus positive were summarized by QFT-GIT status using median and interquartile range (IQR) and percentage positive (measurement minus nil response > 0.35). Logistic regression was used to identify factors associated with having TB2–TB1 > 0.6. Results were summarized using odds ratios (OR) with their corresponding 95% confidence intervals (CI) and p-values. Due to a small number of outcomes a multivariable analysis was not conducted. Data were analyzed using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP).

Ethical statement

The study received ethical clearance from the University of Witwatersrand Human Research Ethics Committee (WHREC Ref: 150217), London School of Hygiene & Tropical Medicine, UK (LSHTM Ethics Ref: 9279), University of Washington, USA (IRB number 33335) and North West Health Research and Ethics Committee (DOH-27-0515-4991). We sought informed consent from all study participants using written informed consent and information sheets available in the most commonly used local languages. Participants who were unable to read or write were asked to make a mark or thumbprint in the presence of a witness. This study was conducted according to Good Clinical Practice guidelines, in accordance with the requirements of the funders and respective ethics committees.

Results

Process flow

We approached 25,627 miners, 17,030 (66.5%) agreed to be pre-screened, of whom 3,534 (20.8%) satisfied the pre-screen criteria and were eligible for full screening, following informed consent (Figure 1). Overall, 2,980 (84.3%) were offered consent, of whom 1,749 (58.7%) consented and 1,231 (41.3%) declined to take part in the study; 554 (15.7%) were not offered consent as they were lost in the OHC queue.

Following the full screen, 349/1,749 (20.0%) met the inclusion criteria and were enrolled into the study. Of the 349, 304 had a baseline QFT-Plus and QFT-GIT.

Demographic characteristics

Of the 349 participants enrolled into the study, the median age was 48 years (IQR 45, 53 years), median years in the workforce was 24 (IQR 18, 28 years) and 98.6% (344) were male

(Table 1). Overall, 92.0% (321) were of Black/African ethnicity, 72.5% (253) had a BCG scar present, 66.8% (233) were born in South Africa and a minority lived in a mine hostel (28.7%; 100). Subsample of the 349 participants who had a baseline QFT-Plus and QFT-GIT result (n=304) had similar demographic characteristics to the overall sample¹⁰.

Comparison of QuantiFERON-TB Gold-Plus vs. Gold in-tube

Of the 304 who had QFT-Plus and QFT-GIT results, 214 (70.4%) had a positive QFT-Plus result, 205 (67.4%) were positive on both assays; 83 (27.3%) were negative on both assays, while 16 (5.3%) had discordant results (seven QFT-Plus negative/QFT-GIT positive; nine QFT-Plus positive/QFT-GIT negative) (Table 2). Overall, the agreement between QFT-Plus and QFT-GIT was 94.7% (288/304) and Kappa was 0.87.

Assessing the specific contribution of CD8+ T-cells

Of the 214 who had positive QFT-Plus results, 202 (94.4%) were positive on TB1, while 205 (95.8%) were positive on TB2 (Table 2). The median (IQR) values for TB1 and TB2 among those who were QFT-Plus positive were 3.06 (1.31, 7.00) and 3.25 (1.53, 8.02), respectively. The median difference between the TB1 and TB2 was 0.00 (-0.17, 0.39). A >0.6 difference between TB2 and TB1 was observed in 16.4% (35/214) of those who were positive on QFT-Plus (Table 2). Only BMI status was found to be associated with TB2–TB1 > 0.6; unadjusted OR 1.94 (95% CI: 0.74–5.05) for BMI > 30 kg/m² versus BMI between 18.5–24.9 (Table 3).

Discussion

In this study, conducted in South African goldmines, the overall agreement between QFT-Plus and QFT-GIT was high at 94.7%, suggesting that QFT-GIT may have similar performance to QFT-Plus; consistent with previous evaluation studies conducted in low TB incidence settings which showed similar diagnostic performance and high overall agreement between QFT-Plus and its predecessor^{5,8,11–15}. The high concordance (or low discordance) in our study may be because our study was in HIV negative adults among whom QFT-GIT would be expected to have relatively high sensitivity. A greater difference between the two tests might be expected in populations where QFT-GIT typically has poor sensitivity e.g. children and people with advanced HIV disease^{11,16–18}. Overall, there were 16 discordant pairs (5.3%); seven were QFT-Plus negative/QFT-GIT positive and nine were QFT-Plus positive/QFT-GIT negative. It is interesting that only 56.3% were QFT-Plus positive/QFT-GIT negative and not higher. The discordancy rate found in our study was consistent with findings from Theel *et al.* and Moon *et al.* studies, which showed discordancy rates of 3.1% and 4.4%, respectively^{5,12}. However, of the five discordant pairs in the Theel *et al.* study, 60.0% (3/5) were QFT-Plus negative/QFT-GIT positive and 40.0% (2/5) were QFT-Plus positive/QFT-GIT negative; while in the Moon *et al.* study, 25.6% (11/43) were QFT-Plus negative/QFT-GIT positive and 74.4% (32/43) were QFT-Plus positive/QFT-GIT negative in the 43 discordant pairs. This is thought to be due to several factors, broadly classified as preanalytical, analytical, postanalytical, manufacturing,

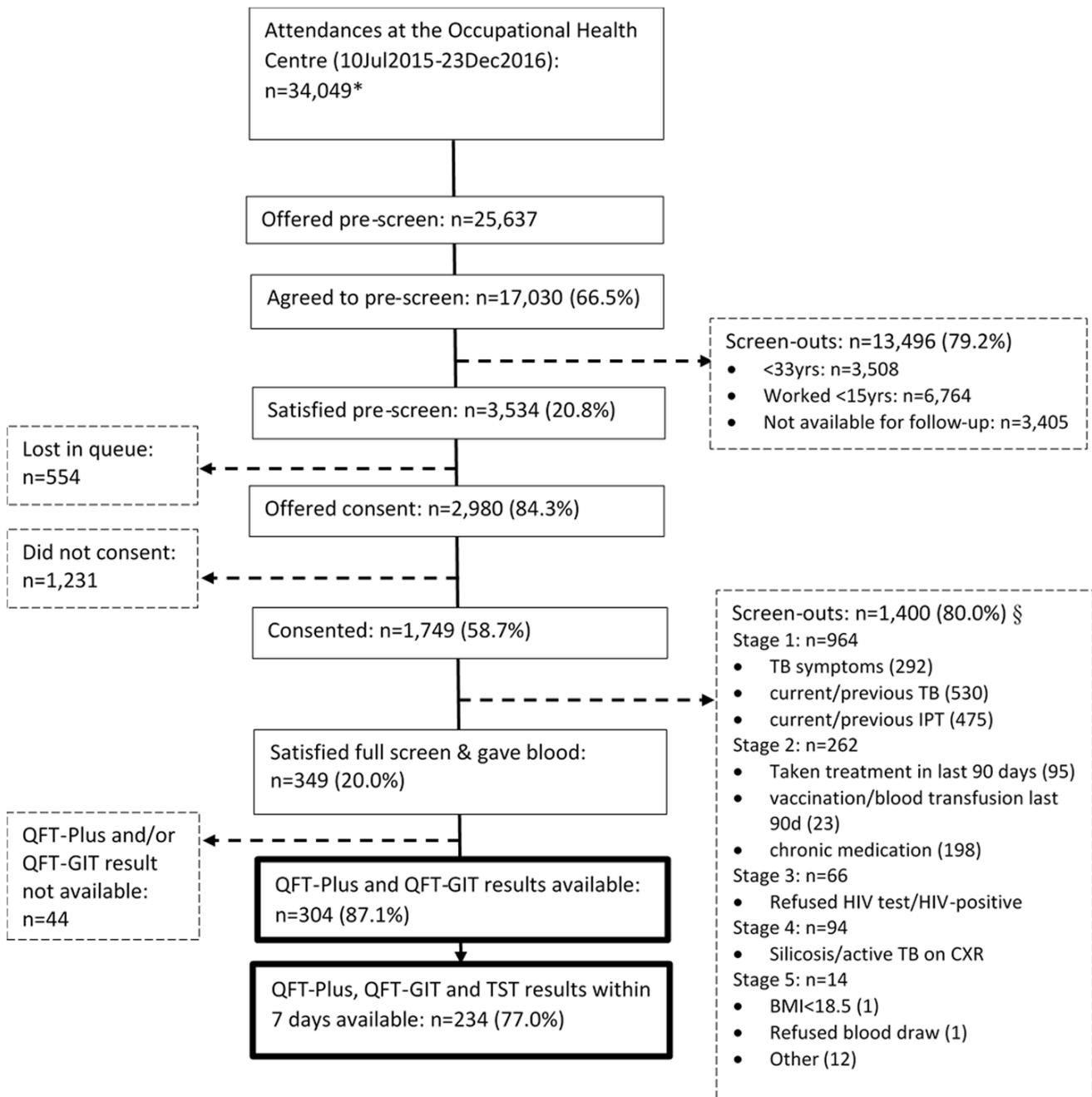


Figure 1. Participant flow chart at enrolment. *Data from the daily attendance register. Includes repeat attendances during this time period. §Screen out questions were asked in stages; if screened out at a stage no screening was conducted for subsequent stages. Within a stage more than one screen-out reason may apply. Yrs, years; IPT, isoniazid preventive therapy; BMI, body mass index; QFT-Plus, QuantiFERON-TB Gold-Plus; QFT-GIT, QuantiFERON-TB Gold In-Tube assay.

immunological, and interferon-gamma levels bordering on the binary 0.35 IU/ml cutoff for assay positivity^{9,19-21}.

We observed a TB2–TB1 difference >0.6 among 35 (16.4%) individuals who had QFT-Plus positive results, associated with obesity. Barcellini *et al.* had also observed a similar

TB2–TB1 difference >0.6 in a small proportion of TB contacts who had a positive QFT-Plus results 18 (15.1%); suggesting presence of Mtb-specific CD8+ T lymphocytes, which may be indicative of a higher antigenic burden^{14,22-27}. The presence of Mtb-specific CD8+ T lymphocytes in latently infected miners may therefore be predictive of Mtb active replication

Table 1. Summary of demographic characteristics at enrolment.

Variable		Participants Enrolled	Participants enrolled with a QFT-Plus and QFT-GIT result
	N	349	304
Age, years	Median (IQR)	48 (45-52)	48 (44-52)
Sex	Male	344 (98.6%)	300 (98.7%)
BCG scar	No	84 (24.1%)	74 (24.3%)
	Yes	253 (72.5%)	221 (72.7%)
	Indeterminate	12 (3.4%)	9 (3.0%)
Country of birth	South Africa	233 (66.8%)	204 (67.1%)
	Lesotho	59 (16.9%)	50 (16.4%)
	Mozambique	37 (10.6%)	32 (10.5%)
	Other	20 (5.7%)	18 (5.9%)
Ethnicity	Black/African	321 (92.0%)	279 (91.8%)
Hostel	No	170 (48.7%)	155 (51.0%)
	Hostel	100 (28.7%)	83 (27.3%)
	Other mine house	79 (22.6%)	66 (21.7%)
Years worked underground	Median (IQR)	24 (18-28)	24 (17.5-28)
Sleeping arrangement	Alone	51 (14.6%)	46 (15.1%)
	1 person	206 (59.0%)	182 (59.9%)
	> 1 person	92 (26.4%)	76 (25.0%)
Occupation	Unskilled	271 (77.7%)	236 (77.6%)

IQR, interquartile range; QFT-Plus, QuantiFERON-TB Gold-Plus; QFT-GIT, QuantiFERON-TB Gold.

Table 2. Comparison of QuantiFERON-TB Gold-Plus vs. QuantiFERON-TB Gold in-tube.

QFT-GIT	N	QFT-Plus		QFT-Plus interferon-gamma concentration, among those positive on QFT-Plus					
		Positive	Negative	Positive result*: TB1 (%)	Positive result*: TB2 (%)	TB1-nil Median (IQR)	TB2-nil Median (IQR)	TB2 - TB1 median difference (IQR)	TB2 - TB1 >0.6
Positive	212	205	7	198/205 ^a (96.6%)	200/205 ^b (97.6%)	3.06 (1.31, 7)	3.25 (1.53, 8.02)	0.00 (-0.16, 0.39)	34/205 (16.6%)
Negative	92	9	83	4/9 ^c (44.4%)	5/9 ^d (55.6%)	0.35 (0.18, 0.53)	0.37 (0.28, 0.45)	0.02 (-0.23, 0.30)	1/9 (11.1%)
Total	304 [^]	214	90	202/214 (94.4%)	205/214 (95.8%)	2.89 (1.18, 6.97)	2.95 (1.17, 7.79)	0.00 (-0.17, 0.39)	35/214 (16.4%)

[^] n=304 who have QFT-Plus and QFT-GIT. Percentage agreement 94.7% (95%CI: 91.6– 97.0%); Kappa 0.87.

^a Of the 198, n=5 are positive on TB1 alone; ^b of the 200, n=7 are positive on TB2 alone; ^c of the four, all are positive on TB1 alone; ^d of the five, all are positive on TB2 alone.

^e Of the 154, n=3 are positive on TB1 alone; ^f of the 157, n=6 are positive on TB2 alone; ^g of the three, all are positive on TB1 alone; ^h of the three, all are positive on TB2 alone.

* Defined as TB1-nil>0.35 or TB2-nil>0.35.

QFT-Plus, QuantiFERON-TB Gold-Plus; QFT-GIT, QuantiFERON-TB Gold in-tube; IQR interquartile range; CI confidence interval.

Table 3. Risk factors for having TB2-TB1>0.6 on QFT-plus.

Variable	N	TB2-TB1 >0.6 n (%)	Univariable analysis		
			Crude OR	95% CI	p-value*
Age group, years					0.85
<45	48	7 (14.6)	1		
45–49	71	13 (18.3)	1.31	0.48 - 3.58	
≥50	95	15 (15.8)	1.10	0.42 - 2.91	
Gender					0.66
Male	210	34 (16.2)	1		
Female	4	1 (25.0)	1.73	0.17 - 17.09	
Ethnicity					0.20
Black/African	209	33 (15.8)	1		
Other	5	2 (40.0)	3.56	0.57 - 22.11	
Marital status					0.14
Married	193	29 (15.0)	1		
Other	21	6 (28.6)	2.26	0.81 - 6.31	
Country of birth					0.85
South Africa	141	25 (17.7)	1		
Lesotho	39	5 (12.8)	0.68	0.24 - 1.92	
Mozambique	23	3 (13.0)	0.70	0.19 - 2.52	
Other	11	2 (18.2)	1.03	0.21 - 5.07	
Occupational level					0.17
Unskilled	177	26 (14.7)	1		
Skilled	37	9 (24.3)	1.87	0.79-4.41	
Years worked underground					0.99
<20	61	10 (16.4)	1		
20-29	112	18 (16.1)	0.98	0.42 - 2.27	
≥30	41	7 (17.1)	1.05	0.36 - 3.03	
Type of mine housing					0.31
Not staying in mine house	102	17 (16.7)	1		
Hostel	68	8 (11.8)	0.67	0.27 - 1.64	
Other mine housing	44	10 (22.7)	1.47	0.61 - 3.53	
Sleeping arrangement					0.30
Alone	38	5 (13.2)	1		
1 person	129	25 (19.4)	1.59	0.56 - 4.48	
>1 person	47	5 (10.6)	0.79	0.21 - 2.94	

Variable	N	TB2-TB1 >0.6 n (%)	Univariable analysis		
			Crude OR	95% CI	p-value*
BCG Scar					0.39
Yes/indeterminate [‡]	159	28 (17.6)	1		
No	55	7 (12.7)	0.68	0.28-1.66	
BMI, kg/m²					0.03
18.5-24.9	47	7 (14.9)	1		
25-29.9	92	9 (9.8)	0.62	0.22 - 1.78	
≥30	75	19 (25.3)	1.94	0.74 - 5.05	

* P-value from the likelihood ratio test; ‡, n=4 indeterminate.

QFT-Plus, QuantiFERON-TB Gold-Plus; OR, odds ratio; CI confidence interval.

and may be indicative of higher likelihood of disease progression²³. In Barcellini *et al.* study, sleeping in the same room and the European origin were significantly associated with TB2–TB1 difference >0.6 (27). In this study, only obesity was associated with TB2–TB1 difference >0.6, suggesting a greater TB2 response among obese participants. However, multivariable analysis was not conducted, due to the small number of outcomes. A much bigger sample size may be needed to investigate relative prognostic value of the TB1 and TB2 antigen tubes and further explore the association between TB2–TB1 difference >0.6 and BMI.

Study limitations

This sub-analysis was based on a cross-sectional sample and did not include follow-up to confirm the LTBI status due to low number of individuals available to provide blood sample to conduct Mtb infection testing. The sample size of 304 was relatively small to make strong inferences. In addition, the study was conducted among HIV-negative goldminers who were most likely to have experienced prolonged and high exposure to Mtb. However, results may be generalizable as the performance of a diagnostic test is independent of setting and prevalence of infection. The lack of a gold-standard test for Mtb infection means that where results were discordant, we cannot know which (if either) was correct.

Conclusion

Among HIV-negative goldminers in South Africa, QFT-Plus showed high agreement with QFT-GIT, suggesting similar performance. For most discordant results, interferon-gamma concentrations bordered on the binary cut-off for assay positivity.

Data availability

LSHTM Data Compass: Data set for the comparison of the performance of QuantiFERON-TB Gold Plus with QuantiFERON-T

Gold in-tube among highly TB exposed gold miners in South Africa. <https://doi.org/10.17037/DATA.00001891>¹⁰.

This project contains the following underlying data:

- Hetu-dataset.txt
- Hetu_data_codebook.html
- Hetu_data_userguide.html

Due to ethical concerns, dataset access is restricted to ensure privacy and confidentiality of participant data. However, raw data is available upon request under a custom data sharing agreement and will require authorization from Principal Investigators (Professor Violet Chihota: VChihota@auruminstitute.org and Professor Katherine Fielding: Katherine.Fielding@lshtm.ac.uk). Once access is granted, the files will be made available on LSHTM Data Compass.

The data codebook and user guide are available under the terms of the [Creative Commons Attribution 3.0 International license](https://creativecommons.org/licenses/by/3.0/) (CC-BY 3.0).

Acknowledgements

The authors are thankful to the North West Department of Health and gold mining companies for the gracious endorsement for undertaking of this study. We also thank all the participants who participated in the HETU and the following lab and field researchers: Ithabeleng Morojele, Keolebogile Ntshamane, Tebogo Rampai, Ndumiso Sithole, Zamakhabako Mhlanga, Ntombomzi Motsoeneng, James Seseng, Samuel Tlhabakwane, Abel Qas, Kabelo Leshoro, Samantha Naicker, Martha Albani, Ken Clarke, Palesa Mosweu, Lethogonolo Seabela, Nondumiso Langa.

References

1. **Latent tuberculosis infection: updated and consolidated guidelines for programmatic management.** Geneva: World Health Organization; Licence: CC BY-NC-SA 3.0 IGO. 2018; [cited 2019 Mar 6].
[Reference Source](#)
2. Riley RL, Mills CC, Nyka W, *et al.*: **Aerial Dissemination Of Pulmonary Tuberculosis A Two-Year Study Of Contagion In A Tuberculosis Ward.** *Am J Epidemiol.* 1959; **70**(2): 185–96.
[Publisher Full Text](#)
3. Vynnycky E, Fine PE: **The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection.** *Epidemiol Infect.* 1997; **119**(2): 183–201.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
4. Pai M, Deninger CM, Kik SV, *et al.*: **Gamma Interferon Release Assays for Detection of *Mycobacterium tuberculosis* Infection.** *Clin Microbiol Rev.* 2014; **27**(1): 3–20.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. Theel ES, Hilgart H, Breen-Lyles M, *et al.*: **Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube Interferon Gamma Release Assays in Patients at Risk for Tuberculosis and in Health Care Workers.** *J Clin Microbiol.* 2018; **56**(7): e00614–18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Tsiouris SJ, Coetzee D, Toro PL, *et al.*: **Sensitivity Analysis and Potential Uses of a Novel Gamma Interferon Release Assay for Diagnosis of Tuberculosis.** *J Clin Microbiol.* 2006; **44**(8): 2844–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Andersen P, Munk ME, Pollock JM, *et al.*: **Specific immune-based diagnosis of tuberculosis.** *Lancet.* 2000; **356**(9235): 1099–104.
[PubMed Abstract](#) | [Publisher Full Text](#)
8. Yi L, Sasaki Y, Nagai H, *et al.*: **Evaluation of QuantiFERON-TB Gold Plus for Detection of *Mycobacterium tuberculosis* infection in Japan.** *Sci Rep.* 2016; **6**: 30617.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Metcalfe JZ, Cattamanchi A, McCulloch CE, *et al.*: **Test Variability of the QuantiFERON-TB Gold In-Tube Assay in Clinical Practice.** *Am J Respir Crit Care Med.* 2013; **187**(2): 206–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Fielding K: **Data set for the comparison of the performance of QuantiFERON-TB Gold Plus with QuantiFERON-T Gold in-tube among highly TB exposed gold miners in South Africa.** [Data Collection]. London School of Hygiene & Tropical Medicine, London, United Kingdom. 2020.
<http://www.doi.org/10.17037/DATA.00001891>
11. Telisinghe L, Amofa-Sekyi M, Maluzi K, *et al.*: **The sensitivity of the QuantiFERON®-TB Gold Plus assay in Zambian adults with active tuberculosis.** *Int J Tuberc Lung Dis.* 2017; **21**(6): 690–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. Moon HW, Gaur RL, Tien SSH, *et al.*: **Evaluation of QuantiFERON-TB Gold-Plus in Health Care Workers in a Low-Incidence Setting.** *J Clin Microbiol.* 2017; **55**(6): 1650–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Hoffmann H, Avsar K, Göres R, *et al.*: **Equal sensitivity of the new generation QuantiFERON-TB Gold plus in direct comparison with the previous test version QuantiFERON-TB Gold IT.** *Clin Microbiol Infect.* 2016; **22**(8): 701–3.
[PubMed Abstract](#) | [Publisher Full Text](#)
14. Barcellini L, Borroni E, Brown J, *et al.*: **First evaluation of QuantiFERON-TB Gold Plus performance in contact screening.** *Eur Respir J.* 2016; **48**(5): 1411–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
15. Petruccioli E, Vanini V, Chiacchio T, *et al.*: **Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold In-tube assays in subjects with or without tuberculosis.** *Tuberculosis (Edinb).* 2017; **106**: 38–43.
[PubMed Abstract](#) | [Publisher Full Text](#)
16. Shao L, Zhang W, Zhang S, *et al.*: **Potent immune responses of Ag-specific Vgamma2Vdelta2+ T cells and CD8+ T cells associated with latent stage of *Mycobacterium tuberculosis* coinfection in HIV-1-infected humans.** *AIDS.* 2008; **22**(17): 2241–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Rose MV, Kimaro G, Nissen TN, *et al.*: **QuantiFERON®-TB Gold In-Tube Performance for Diagnosing Active Tuberculosis in Children and Adults in a High Burden Setting.** *PLoS One.* 2012; **7**(7): e37851.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Santin M, Muñoz L, Rigau D: **Interferon-γ Release Assays for the Diagnosis of Tuberculosis and Tuberculosis Infection in HIV-Infected Adults: A Systematic Review and Meta-Analysis.** *PLoS One.* 2012; **7**(3): e32482.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Whitworth WC, Hamilton LR, Goodwin DJ, *et al.*: **Within-Subject Interlaboratory Variability of QuantiFERON-TB Gold In-Tube Tests.** *PLoS One.* 2012; **7**(9): e43790.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Detjen AK, Loebenberg L, Grewal HMS, *et al.*: **Short-Term Reproducibility of a Commercial Interferon Gamma Release Assay.** *Clin Vaccine Immunol.* 2009; **16**(8): 1170–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Banaei N, Gaur RL, Pai M: **Interferon Gamma Release Assays for Latent Tuberculosis: What Are the Sources of Variability?** *J Clin Microbiol.* 2016; **54**(4): 845–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
22. Rozot V, Vigano S, Mazza-Stalder J, *et al.*: ***Mycobacterium tuberculosis*-specific CD8+ T cells are functionally and phenotypically different between latent infection and active disease.** *Eur J Immunol.* 2013; **43**(6): 1568–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Day CL, Abrahams DA, Lerumo L, *et al.*: **Functional capacity of *Mycobacterium tuberculosis*-specific T cell responses in humans is associated with mycobacterial load.** *J Immunol.* 2011; **187**(5): 2222–32.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. Lewinsohn DA, Heinzel AS, Gardner JM, *et al.*: ***Mycobacterium tuberculosis*-specific CD8+ T Cells Preferentially Recognize Heavily Infected Cells.** *Am J Respir Crit Care Med.* 2003; **168**(11): 1346–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
25. Chiacchio T, Petruccioli E, Vanini V, *et al.*: **Polyfunctional T-cells and effector memory phenotype are associated with active TB in HIV-infected patients.** *J Infect.* 2014; **69**(6): 533–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
26. Lancioni C, Nyendak M, Kiguli S, *et al.*: **CD8+ T Cells Provide an Immunologic Signature of Tuberculosis in Young Children.** *Am J Respir Crit Care Med.* 2012; **185**(2): 206–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Barcellini L, Borroni E, Brown J, *et al.*: **First independent evaluation of QuantiFERON-TB Plus performance.** *Eur Respir J.* 2016; **47**(5): 1587–90.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:   

Version 3

Reviewer Report 07 August 2023

<https://doi.org/10.21956/gatesopenres.15091.r34041>

© 2023 Sarkar S. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Sonali Sarkar

JIPMER, Puducherry, India

The authors have compared the agreement between QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube Interferon Gamma Release Assays in South African gold miners with the premise that the results might vary from the multiple studies done in low burden settings.

The assumption that the agreement between the two tests can vary is a valid given the knowledge that the spectrum of the condition under study has some influence on sensitivity and specificity of the tests performed. Having said that, it is also noted that the artifactual variability that is introduced by excluding participants in whom the TB infection is difficult to diagnose, such as those with body mass index (BMI) <18.5, having serious medical conditions, HIV positive and current treatment for cancer, treatment with steroid tablets, inhalers or injections, overestimates the accuracy of the tests.(1) There are multiple studies reporting the agreement between the two tests in lower incidence setting. Results from repetition of a similar study in a high-burden setting was expected and does not contribute to the clinical decision of using the QFT-Plus in similar or other settings. The inputs required for justification for the use of QFT-Plus would come from directional hypothesis with minimal acceptable criteria set in calculation of sample size (2). An attempt at sample size calculation should have been done to justify the validity of the results.

Minor comments regarding the study are as follows.

1. The flowchart of the number of participants does not match with the numbers stated in the text.
2. Typographical error in line 3 of the results section under the demographic characteristics, work-face to be corrected as work-force.

References

1. Leeflang MM, Rutjes AW, Reitsma JB, Hooft L, Bossuyt PM. Variation of a test's sensitivity and specificity with disease prevalence. CMAJ. 2013 Aug 6;185(11):E537-44. doi: 10.1503/cmaj.121286. Epub 2013 Jun 24. PMID: 23798453; PMCID: PMC3735771.

2. Korevaar, D.A., Gopalakrishna, G., Cohen, J.F. *et al.* Targeted test evaluation: a framework for designing diagnostic accuracy studies with clear study hypotheses. *Diagn Progn Res* **3**, 22 (2019). <https://doi.org/10.1186/s41512-019-0069-2>

References

1. Leeflang MM, Rutjes AW, Reitsma JB, Hooft L, et al.: Variation of a test's sensitivity and specificity with disease prevalence. *CMAJ*. 2013; **185** (11): E537-44 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Korevaar DA, Gopalakrishna G, Cohen JF, Bossuyt PM: Targeted test evaluation: a framework for designing diagnostic accuracy studies with clear study hypotheses. *Diagn Progn Res*. 2019; **3**: 22 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology of Tuberculosis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 02 August 2023

<https://doi.org/10.21956/gatesopenres.15091.r34036>

© 2023 Barcellini L. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Lucia Barcellini

University of Milan, Milan, Italy

The study compared the performance of QuantiFERON-TB Gold-Plus (QFT-Plus) with the standard QFT-GIT assay in diagnosing latent tuberculosis infection in goldminers. QFT-Plus showed high agreement (94.7%) with QFT-GIT, suggesting similar performance.

The microbiological procedure used in the study is reliable, and the inclusion of subclinical TB testing with a culture is a strong aspect.

However, the inclusion criteria have led to a significant reduction in the population included, and they are not entirely clear.

While some criteria are clear, such as excluding previous TB patients or those currently on prophylaxis, the rationale behind others is less transparent.

If the objective was to assess the agreement between the two tests, having a more diverse sample, including different age groups, individuals with low BMI, and those with comorbidities, could actually be advantageous.

Please, clarify the specific rationale behind the chosen criteria and /or discuss them as a limitation.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology: diagnostic test for TB

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 25 August 2022

<https://doi.org/10.21956/gatesopenres.15091.r32392>

© 2022 Hatherill M. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Mark Hatherill 

South African Tuberculosis Vaccine Initiative (SATVI), University of Cape Town, Cape Town, South Africa

This revision is satisfactory, I have no further comments.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 06 June 2022

<https://doi.org/10.21956/gatesopenres.14885.r31933>

© 2022 Hatherill M. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Mark Hatherill

South African Tuberculosis Vaccine Initiative (SATVI), University of Cape Town, Cape Town, South Africa

The authors have revised statements that “*QFT-Plus showed similar performance to QFT-GIT*” to state that QFT-Plus showed high agreement with QFT-GIT, suggesting similar performance, since performance of these tests to differentiate true Mtb infection was not measured.

However, in version 2 the abstract, introduction and methods still state incorrectly that the study compared performance of QFT-Plus with QFT-GIT. This needs to be corrected to reflect that agreement was tested, not performance.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: tuberculosis vaccinology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 04 June 2021

<https://doi.org/10.21956/gatesopenres.14396.r30700>

© 2021 Hatherill M. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Mark Hatherill

South African Tuberculosis Vaccine Initiative (SATVI), University of Cape Town, Cape Town, South Africa

Ntshiqqa *et al.* report a comparison of QuantiFERON-TB Gold Plus with QuantiFERON-TB Gold in-tube among highly TB exposed gold miners, showing that agreement was high (94%).

Major comments:

- The data analysed are from a subset of a larger parent study and the 304 participants available for direct comparison form only 1.8% of the 17,030 who consented to screening; and the study participants included in the analysis are all very highly exposed to Mtb over a long period. i.e. It is not clear if the study population is representative of other settings, or to what extent the results are generalisable.

Minor comments:

- Abstract: Typo 33 years of age?
- Study limitations: The statement that “it is unlikely that the LTBI status would change over a relatively short period of follow-up” does not justify the lack of confirmatory testing.
- Discussion: No TST information is included in the title, abstract or discussion, but is included in the methods and main results. Suggest TST information is either included in the title and abstract, and concordance/discordance with the IGRA tests is interpreted and discussed further, or TST is removed entirely from this manuscript.
- The observation that TB2-TB1 was associated with BMI deserves further discussion.
- Conclusion (abstract and main text): Here and elsewhere - suggest statements that “QFT-Plus showed similar performance to QFT-GIT” should be revised to state that QFT-Plus showed high agreement with QFT-GIT, or qualified as per the Discussion “suggesting similar performance” since performance of these tests to differentiate true Mtb infection was not measured.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: tuberculosis vaccinology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
