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 gold miners in South Africa.

**Methods:** We enrolled HIV-negative gold miners 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 if both or either TB1 or TB2 tested positive, as per manufacturer’s recommendations. We compared the agreement between QFT-Plus and QFT-GIT.
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/m 2, 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

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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 (Mtbb) antigens with no evidence of active TB. LTBI is typically characterized by a positive tuberculin skin test (TST) in vivo, involving intradermal injection of purified protein derivative from Mtbb strain and/or a positive interferon-gamma release assay (IGRA). 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 Mtbb complex antigens but not produced by Mycobacterium bovis BCG vaccine strains. 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, CFP-10 and TB7.7 antigens (Qiagen, Germantown, MD). 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 lymphocytes.

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 Mtbb exposed gold miners 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 gold miners, 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 Mtbb despite being highly exposed to Mtbb, to compare epidemiological factors between Mtbb uninfected and infected miners and to collect specimens from Mtbb uninfected and infected miners to determine gene expression and immunological profiles associated with being Mtbb 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-Plus 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 test6.

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 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 predecessor5,8,11–15. 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 disease11,16–18. 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%, respectively6,12. 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,
immunological, and interferon-gamma levels bordering on the binary 0.35 IU/ml cutoff for assay positivity²⁰–²¹.

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 burden²²–²⁷. The presence of Mtb-specific CD8+ T lymphocytes in latently infected miners may therefore be predictive of Mtb active replication.

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.
Table 1. Summary of demographic characteristics at enrolment.

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

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.

<table>
<thead>
<tr>
<th>QFT-GIT N</th>
<th>QFT-Plus Positive</th>
<th>QFT-Plus Negative</th>
<th>QFT-Plus interferon-gamma concentration, among those positive on QFT-Plus</th>
<th>TB1-nil Median (IQR)</th>
<th>TB2-nil Median (IQR)</th>
<th>TB2 - TB1 median difference (IQR)</th>
<th>TB2 - TB1 &gt;0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive 212</td>
<td>205</td>
<td>7</td>
<td>198/205* (96.6%)</td>
<td>200/205* (97.6%)</td>
<td>3.06 (1.31, 7)</td>
<td>0.00 (-0.16,0.39)</td>
</tr>
<tr>
<td></td>
<td>Negative 92</td>
<td>9</td>
<td>83</td>
<td>4/9* (44.4%)</td>
<td>5/9* (55.6%)</td>
<td>0.35 (0.18, 0.53)</td>
<td>0.02 (-0.23,0.30)</td>
</tr>
<tr>
<td>Total</td>
<td>304*</td>
<td>214</td>
<td>90</td>
<td>202/214 (94.4%)</td>
<td>205/214 (95.8%)</td>
<td>2.89 (1.18, 6.97)</td>
<td>0.00 (-0.17,0.39)</td>
</tr>
</tbody>
</table>

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

* Of the 198, n=5 are positive on TB1 alone; * Of the 200, n=7 are positive on TB2 alone; * of the four, all are positive on TB1 alone; * of the five, all are positive on TB2 alone.

* Of the 154, n=3 are positive on TB1 alone; * of the 157, n=6 are positive on TB2 alone; * of the three, all are positive on TB1 alone; * 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.

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

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 (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, Nдумiso Sithole, Zamakhabako Mhlanga, Ntombomzi Motsoeneng, James Seseng, Samuel Tlhakakwane, Abel Qas, Kabelo Leshoro, Samantha Naicker, Martha Albani, Ken Clarke, Palesa Mosweu, Letlhogonolo Seabela, Nдумiso Langa.

<table>
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<th>Variable</th>
<th>N</th>
<th>TB2-TB1 &gt;0.6 n (%)</th>
<th>Univariable analysis</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>p-value*</th>
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<tbody>
<tr>
<td>BCG Scar</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes/indeterminate</td>
<td>159</td>
<td>28 (17.6)</td>
<td>0.39</td>
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<tr>
<td>No</td>
<td>55</td>
<td>7 (12.7)</td>
<td>0.68</td>
<td>0.28-1.66</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>18.5-24.9</td>
<td>47</td>
<td>7 (14.9)</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-29.9</td>
<td>92</td>
<td>9 (9.8)</td>
<td>0.03</td>
<td>0.22 - 1.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>75</td>
<td>19 (25.3)</td>
<td>0.03</td>
<td>0.74 - 5.05</td>
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<td></td>
</tr>
</tbody>
</table>

* P-value from the likelihood ratio test; ¥, n=4 indeterminate.
QFT-Plus, QuantiFERON-TB Gold-Plus; OR, odds ratio; CI confidence interval.

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Open Peer Review

Current Peer Review Status: ✔

**Version 3**

Reviewer Report 25 August 2022

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

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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.

**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

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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.

**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.

---

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

Ntshiqa *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.