Fourth generation QuantiFERON-TB Gold-Plus: What is the evidence?

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Abstract

QuantiFERON-TB Gold Plus (QFT-Plus) is the latest generation of interferon-gamma release assays (IGRAs) to receive approval from the US FDA, replacing its predecessor QuantiFERON-TB Gold In-Tube (QFT-GIT). The novelty of QFT-Plus is that it elicits a response from CD8 T-cells in addition to CD4 T-cells, thus collecting a broader response from T-cell subsets compared with QFT-GIT. It was developed with the aim to improve detection of *M. tuberculosis* infection (LTBI), especially among recently exposed, immunocompromised hosts and young children. In this mini review, we summarize the performance of QFT-Plus compared with QFT-GIT among active TB patients (a surrogate for LTBI), high-risk populations, and low-risk individuals based on recent publications. Studies comparing QFT-Plus to QFT-GIT currently do not support superior performance of QFT-Plus in individuals with active TB and LTBI. The difference in sensitivity between QFT-Plus and QFT-GIT in active TB patients was not significant in nearly all studies and ranged from -4.0 to 2.0%. Among high-risk groups, the agreement between QFT-Plus and QFT-GIT was 89.9 to 96.0% (kappa 0.80 to 0.91). The specificity in the low-risk population was slightly lower in QFT-Plus than QFT-GIT with a difference ranging from -7.4 to 0%. Further studies are needed to accurately evaluate the sensitivity of QFT-Plus in immunocompromised hosts and children. In addition, further evidence is required to validate a modified interpretation of QFT-Plus for the identification of false-positive results in low-risk healthcare workers.

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Introduction

Up to one quarter of the global population is estimated to be infected with *Mycobacterium tuberculosis* (Mtb) (1), 5-10% of whom will progress to active tuberculosis (TB) during their lifetime (https://www.who.int/publications-detail/who-consolidated-guidelines-on-tuberculosis-module-1-prevention-tuberculosis-preventive-treatment). To achieve the End TB Strategy target of a 90% reduction in TB incidence rate by 2035, the World Health Organization (WHO) recommends the testing and preventive treatment of latent TB infection (LTBI) in high-risk groups (https://www.who.int/publications-detail/who-consolidated-guidelines-on-tuberculosis-module-1-prevention-tuberculosis-preventive-treatment). These groups include people living with HIV, household contacts of people with active TB and patients initiating immunotherapy, receiving dialysis or preparing for transplant (https://www.who.int/publications-detail/who-consolidated-guidelines-on-tuberculosis-module-1-prevention-tuberculosis-preventive-treatment). Widespread LTBI testing is required to achieve this target goal.

Current testing options for LTBI include the conventional tuberculin skin test (TST) and more recently introduced interferon-gamma (IFN-γ) release assays (IGRAs). IGRAs are in vitro blood tests which measure IFN-γ release by antigen-specific T-cells in response to stimulation by Mtb antigens. Advantages and limitations of IGRAs have been covered in prior reviews (2, 3). Unlike the TST, IGRAs do not cross react with Bacille Calmette–Guérin (BCG) vaccine and nontuberculous mycobacteria with the exception of *M. kansasii*, *M. szulgai* and *M. marinum* (https://www.quantiferon.com/us/wp-

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content/uploads/sites/13/2020/01/L1095849-R06-QFT-Plus-ELISA-IFU.pdf). However, IGRAs share some of the limitations of the TST. Neither can reliably distinguish LTBI from active TB, both have reduced sensitivity in immunocompromised patients, and neither has an adequate positive predictive value for progression to active TB (2). In addition, IGRAs have shown lower specificity and more variability than TST in low-risk subjects especially low-risk North American healthcare workers (2).

The most widely used IGRAs are the QuantiFERON (Qiagen, Venlo, Netherlands) and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom). The latest IGRA to receive FDA approval is the fourth generation QuantiFERON-TB Gold Plus (QFT-Plus) assay, a replacement for the QuantiFERON-TB Gold In-Tube (QFT-GIT). This review will focus solely on QFT-Plus.

QFT-Plus is an enzyme-linked immunosorbent assay (ELISA)-based whole blood test which measures the IFN-γ response of T-cells to the ESAT-6 and CFP-10 peptide antigens. The measured response is in international units (IU) per milliliter (mL). Unlike QFT-GIT, it does not contain TB7.7 antigen and the formulation of antigen varies between QFT-Plus and QFT-GIT such that antigen is sprayed in QFT-Plus vs. resin coated in QFT-GIT (https://www.quantiferon.com/us/wp-content/uploads/sites/13/2020/01/L1095849-R06-QFT-Plus-ELISA-IFU.pdf). The QFT-Plus assay consists of four tubes, rather than the three tubes of QFT-GIT: a negative control (nil) tube which measures background IFN-γ response, a positive control (mitogen) tube, which measures antigen-independent T-cell response, the TB1 antigen

tube, which contains ESAT-6 and CFP-10 peptide antigens to primarily detect the CD4 T-cell response, and the TB2 antigen tube, which contains additional shorter peptides from ESAT-6 and CFP-10 to detect both CD4 and CD8 T-cell responses. The TB1 antigen tube is essentially the same as the QFT-GIT TB antigen tube with the exception of TB7.7 antigen missing from the former. As shown in Table 1, results of the QFT-Plus assay, like QFT-GIT, are reported qualitatively as positive, negative or indeterminate.

The modification of QFT-GIT to additionally detect a CD8 T-cell response was included to broaden the immune response to Mtb antigen in hope of improving assay sensitivity for detection of Mtb infection, especially among recent contacts, immunocompromised hosts and young children. (https://www.quantiferon.com/us/wp-content/uploads/sites/13/2020/01/L1095849-R06-QFT-Plus-ELISA-IFU.pdf). Prior studies have shown greater frequency of antigen-specific CD8 T-cells, producing IFN-γ and other cytokines, in active TB compared to LTBI (4-6) and among recent contacts of TB patients compared to TB patients and healthy controls (7). Increased mycobacterial bacillary load has also been reported to produce a greater CD8 T-cell response (6). There is evidence that active TB in children can be distinguished from TB exposure by the magnitude of the CD8 T-cell response, especially in those under 5 years of age (8). In HIV-infected individuals, monofunctional CD8 T-cell responses to Mtb antigens were observed, even with low CD4 cell count (9-11). However, alongside these potential benefits, the QFT-Plus assay requires an additional blood collection tube and extra ELISA well so its adoption over QFT-GIT decreases testing throughput and increases

the per-test cost in most settings. Thus, it is crucial that modifications made to QFT-Plus improve its clinical performance and justify the added costs of labor and reagents.

In this mini review, we summarize the emerging literature on performance of QFT-Plus compared with QFT-GIT among patients with active TB (a surrogate for LTBI), high-risk patients, and low-risk individuals. PubMed electronic database was searched until December 2019. We focused on cross-sectional studies with head-to-head comparisons to obtain an accurate assessment of QFT-Plus compared with QFT-GIT since performance characteristics of QFT-GIT are well-studied and summarized in several meta-analyses.

Sensitivity in active TB patients

Several investigators have conducted head-to-head studies comparing the sensitivity of QFT-Plus to QFT-GIT in patients with active TB (Table 2). Both microbiological and clinical reference standards were used. Except for one pediatric study discussed below, all other studies were conducted in adult patients and had very low representation of HIV coinfection and immunocompromising conditions.

Collectively, these studies show nearly identical sensitivities between QFT-Plus (range, 85% to 100%) and QFT-GIT (range, 85% to 100%). As shown in Table 2, the difference in sensitivity ranged from -4.0 to 2.0%.

Quantitatively, IFN-γ response in QFT-GIT (TB Ag-Nil) was shown to be significantly higher than QFT-Plus for either TB1 or TB2 antigen tube minus Nil (Table 2). This finding is most likely due to reformulation of the antigens in QFT-Plus (sprayed in QFT-Plus vs. resin coated in QFT-GIT). However, removal of TB 7.7 antigen from QFT-Plus could also account for a lower response in TB1 and TB2 compared with QFT-GIT. Furthermore, in several studies, higher positivity rate and higher median IFN-γ level was reported with TB2 tube compared with TB1 (Table 2) (12-14), which is likely due to stimulation of both CD8 and CD4 T-cells in TB2. Response to TB2 antigen alone in absence of TB1 response has also been reported (15-17).

Sensitivity of QFT-Plus compared to QFT-GIT in patients coinfected with HIV and TB remains poorly characterized. A study conducted in Zambia showed 85% sensitivity with QFT-Plus among culture-positive, active TB patients who were HIV positive (n=68) (17). While the study did not include a head-to-head comparison with QFT-GIT, the authors argued that QFT-Plus has higher sensitivity than QFT-GIT in HIV coinfected patients given that 63% sensitivity with QFT-GIT was observed in an earlier study in the same setting (18). Similar to QFT-GIT, this study also showed that the positivity rate decreases in HIV infected patients with decreasing CD4 T-cell count (17). Thus, although QFT-Plus may appear to have enhanced sensitivity compared with QFT-GIT in HIV-positive TB patients, head-to-head comparison of QFT-Plus to QFT-GIT with adjustment for CD4 T-cell count is needed to accurately demonstrate higher sensitivity of QFT-Plus in this population.

A single study compared the sensitivity of QFT-Plus to QFT-GIT in children with TB. This small study conducted in Eswatini showed identical sensitivity between QFT-Plus and QFT-GIT among children with active TB based on microbiological and clinical reference standards (Table 2) (19).

Detection of latent infection

Performance of QFT-Plus compared to QFT-GIT for diagnosis of LTBI has been assessed in high-risk populations including close contacts of active TB cases, immigrants from high risk countries, immunocompromised individuals such as HIV infected, individuals having received a solid organ or hematopoietic stem cell transplant, patients on immunotherapy, children <5 years, and institutionalized individuals (https://www.cdc.gov/tb/topic/basics/risk.htm). As shown in Table 3, except for one study, all other studies have demonstrated significant agreement between the two tests (≥93.7%). Kappa values overall ranged from 0.80 to 0.91. Lack of discordance between QFT-Plus and QFT-GIT indicates that QFT-Plus has a comparable sensitivity to QFT-GIT for detection of LTBI (Table 3). Most discordant results were due to TB response close to assay cut-off in the range of 0.2-0.7 IU/ml (20-23). In the only pediatric study among 46 children with household Mtb exposure, agreement between the QFT-Plus and QFT-GIT was 96% and the positivity rate was identical (19). One study reported ≥10% higher positivity rate with QFT-Plus compared with QFT-GIT, however, the positivity rate with TB1 and TB2 were identical (25). Given that TB1 contains the same antigens as those in QFT-GIT except for the exclusion of TB7.7, this suggests that higher positivity observed with QFT-Plus over QFT-GIT may have been due to antigen

formulation (spraying in QFT-Plus vs resin coating in QFT-GIT) rather than higher sensitivity of QFT-Plus due to assay design (https://www.quantiferon.com/us/wp-content/uploads/sites/13/2020/01/L1095849-R06-QFT-Plus-ELISA-IFU.pdf).

The difference in IFN-γ response between TB2 and TB1 in QFT-Plus has been used by some investigators as a surrogate for CD8 T-cell response (20, 21, 25). A difference (TB2-TB1) >0.6 IU/ml was considered as the threshold for CD8 T-cell response. Using this approach, some studies have shown an association between CD8 T-cell response and exposure intensity, proximity to index case and proximity to time of infection (21, 25). However, these findings have not been reproducible in other studies (20). This may be in part explained by the fact that TB1 antigens also elicit a CD8 T-cell response through class 1 MHC antigen presentation (13). Further studies are needed to show whether TB2-TB1 difference can be used as an accurate measure of CD8 T-cell response.

Specificity in low-risk populations

Several studies have compared the specificity of QFT-Plus to QFT-GIT in low-risk populations. This group includes healthy adults with no or low risk factors for TB exposure and healthcare workers in low TB incidence settings. The risk was assessed by TB questionnaires obtained before study enrollment. Specificity was estimated by measuring the percent negativity for QFT-Plus and QFT-GIT. Overall, these studies show comparable specificity between QFT-Plus and QFT-GIT (Table 4). The specificity of QFT-Plus is slightly lower than QFT-GIT in some studies, but the difference is not

statistically significant, and no clear pattern has emerged in these studies. One study showed that the specificity of QFT-Plus is not affected by infection by *M. avium* complex and *M. abscessus* group, the two most common NTM (26).

QFT-Plus and QFT-GIT were qualitatively and quantitatively highly concordant in low-risk HCWs (Table 4). Positivity rate in 626 HCWs with no risk factors for LTBI was 3.0% for QFT-Plus using the manufacturer's interpretation compared with 2.1% for QFT-GIT. CDC recently withdrew the recommendation for serial TB screening with IGRA in low-risk HCWs due to high conversion and reversion rates, and higher false-positive rates compared with TST (27, 28). Moon and colleagues have proposed a conservative interpretation of QFT-Plus, based on positivity of both TB1 and TB2 vs. manufacturer's interpretation where either tube can be positive, to increase assay specificity in low-risk HCWs (29). Application of this approach led to a reduction in the positivity rate in no-risk HCWs from 3.0% to 1.0%. Follow-up testing of eleven HCWs with discordant results between TB1 and TB2 in QFT-Plus showed reversion to negative results in ten cases with no progression to active TB in any of the participants. If confirmed in other studies, the conservative interpretation of QFT-Plus in low-risk populations may represent a viable approach to identifying false-positive results in low-risk individuals without the need for repeat testing.

Sources of variability

Sources of variability impacting IGRA are classified into pre-analytical, analytical, postanalytical, manufacturing and immunological (3). Although sources of variability

were largely investigated and described for the QFT-GIT, these might apply to QFT-Plus as well. Further research and modeling are needed to investigate and quantify the variability introduced from known sources due to addition of the second antigen tube in QFT-Plus. Agarwal and colleagues have recently identified a previously unrecognized source of variability for QFT-Plus due to the method of blood collection (30). Blood was collected directly in QFT-Plus tubes (plus-direct) and also in a separate blood collection tube from where blood was transferred to the QFT-Plus tubes (plus-transfer). Positive rate for plus-direct was 12% compared with 17% for plus-transfer method. Agreement between plus-direct and plus-transfer was 85% (kappa 0.37, $p<0.001$). This finding supports variability in QFT-Plus and highlights the need for consistent blood collection methods in individuals undergoing serial testing.

Predictive value of QFT-Plus

No study has yet been published on the predictive value of positive QFT-Plus on progression from latent infection to active TB. Two studies evaluating the prognostic performance of QFT-Plus- The Correlate of Risk Targeted Intervention Study in HIV uninfected (CORTIS-01) (https://clinicaltrials.gov/ct2/show/NCT02735590) and HIV infected (CORTIS-HR) (https://zivahub.uct.ac.za/articles/CORTIS-HR_Statistical_Analysis_Plan/11792079) have recently been completed in South Africa. Findings from these trials are currently being analyzed and should be published soon.

Conclusion

Although QFT-Plus was launched with the promise of improved performance over QFT-GIT through the addition of CD8 T-cell response, studies directly comparing QFT-Plus with QFT-GIT in TB patients, high-risk groups, and low-risk population have not revealed any significant improvement in its performance. Further research in immunocompromised individuals and children is needed to determine the performance of QFT-Plus in these groups.

Unanswered questions

Although studies described in this mini review have advanced our understanding on the performance of QFT-Plus, there are a number of questions that remain unanswered. The following represent areas in need of further research to complete our understanding of QFT-Plus.

1. **Sensitivity in HIV coinfected individuals.** Head-to-head comparison of QFT-Plus to QFT-GIT with adjustment for CD4 count is needed in patients with active TB and HIV to assess whether QFT-Plus has a higher sensitivity in the HIV-coinfected individuals.

2. **Sensitivity in children.** Head-to-head comparison of QFT-Plus to QFT-GIT with sufficient number of children with TB disease is needed to accurately assess QFT-Plus sensitivity in this group.

3. **Predictive value.** Head-to-head comparison of QFT-Plus to QFT-GIT is needed to determine the predictive value of QFT-Plus for progression to active TB. Studies are

also needed to determine whether the CD8 T-cell response derived from QFT-Plus can accurately identify patients with recent and high intensity exposure who are at a greater risk of progressing to active TB.

4. **Conservative interpretation.** Further studies are needed to validate the conservative interpretation of QFT-Plus in low-risk populations and to define quantitative cut-offs that enhance its accuracy.

5. **Reproducibility.** Further research is needed to assess reproducibility of QFT-Plus and investigate sources of variabilities introduced with the addition of second tube and reformulation of peptide antigens.

References

1. Houben RM, Dodd PJ. 2016. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. PLoS Med 13:e1002152.

2. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, Metcalfe JZ, Cattamanchi A, Dowdy DW, Dheda K, Banaei N. 2014. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev 27:3-20.

3. Banaei N, Gaur RL, Pai M. 2016. Interferon Gamma Release Assays for Latent Tuberculosis: What Are the Sources of Variability? J Clin Microbiol 54:845-850.

297 4. Rozot V, Vigano S, Mazza-Stalder J, Idrizi E, Day CL, Perreau M, Lazor-Blanchet C,
298 Petruccioli E, Hanekom W, Goletti D, Bart PA, Nicod L, Pantaleo G, Harari A. 2013.
299 Mycobacterium tuberculosis-specific CD8+ T cells are functionally and phenotypically
300 different between latent infection and active disease. Eur J Immunol 43:1568-1577.
301 5. Day CL, Moshi ND, Abrahams DA, van Rooyen M, O'rie T, de Kock M, Hanekom WA.
302 2014. Patients with tuberculosis disease have Mycobacterium tuberculosis-specific CD8
303 T cells with a pro-apoptotic phenotype and impaired proliferative capacity, which is not
304 restored following treatment. PLoS One 9:e94949.
305 6. Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'rie T, Pienaar
306 B, de Kock M, Kaplan G, Mahomed H, Dheda K, Hanekom WA. 2011. Functional
307 capacity of Mycobacterium tuberculosis-specific T cell responses in humans is
308 associated with mycobacterial load. J Immunol 187:2222-2232.
309 7. Nikolova M, Markova R, Drenska R, Muhtarova M, Todorova Y, Dimitrov V, Taskov
310 H, Saltini C, Amicosante M. 2013. Antigen-specific CD4- and CD8-positive signatures in
311 different phases of Mycobacterium tuberculosis infection. Diagn Microbiol Infect Dis
312 75:277-281.
313 8. Lancioni C, Nyendak M, Kiguli S, Zalwango S, Mori T, Mayanja-Kizza H, Balyejusa S,
314 Null M, Baseke J, Mulindwa D, Byrd L, Swarbrick G, Scott C, Johnson DF, Malone L,
315 Mudido-Musoke P, Boom WH, Lewinsohn DM, Lewinsohn DA, Tuberculosis Research
316 Unit. 2012. CD8+ T cells provide an immunologic signature of tuberculosis in young
317 children. Am J Respir Crit Care Med 185:206-212.
318 9. Sutherland JS, Young JM, Peterson KL, Sanneh B, Whittle HC, Rowland-Jones SL,
319 Adegbola RA, Jaye A, Ota MO. 2010. Polyfunctional CD4(+) and CD8(+) T cell

responses to tuberculosis antigens in HIV-1-infected patients before and after anti-retroviral treatment. J Immunol 184:6537-6544.

10. Chiacchio T, Petruccioli E, Vanini V, Cuzzi G, Pinnetti C, Sampaolesi A, Antinori A, Girardi E, Goletti D. 2014. Polyfunctional T-cells and effector memory phenotype are associated with active TB in HIV-infected patients. J Infect 69:533-545.

11. Ongaya A, Huante MB, Mwangi P, Keiser PH, Amukoye E, Endsley JJ. 2013. Mycobacterium tuberculosis-specific CD8+ T cell recall in convalescing TB subjects with HIV co-infection. Tuberculosis 93 Suppl:S60-5.

12. Horne DJ, Jones BE, Kamada A, Fukushima K, Winthrop KL, Siegel SAR, Kovacs A, Anthony P, Meekin KA, Bhat S, Kerndt P, Chang A, Koelle DM, Narita M. 2018. Multicenter study of QuantiFERON((R))-TB Gold Plus in patients with active tuberculosis. Int J Tuberc Lung Dis 22:617-621.

13. Petruccioli E, Chiacchio T, Pepponi I, Vanini V, Urso R, Cuzzi G, Barcellini L, Cirillo DM, Palmieri F, Ippolito G, Goletti D. 2016. First characterization of the CD4 and CD8 T-cell responses to QuantiFERON-TB Plus. J Infect 73:588-597.

14. Barcellini L, Borroni E, Brown J, Brunetti E, Codecasa L, Cugnata F, Dal Monte P, Di Serio C, Goletti D, Lombardi G, Lipman M, Rancoita PM, Tadolini M, Cirillo DM. 2016. First independent evaluation of QuantiFERON-TB Plus performance. Eur Respir J 47:1587-1590.

15. Petruccioli E, Vanini V, Chiacchio T, Cuzzi G, Cirillo DM, Palmieri F, Ippolito G, Goletti D. 2017. Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold In-tube assays in subjects with or without tuberculosis. Tuberculosis 106:38-43.

342 16. Hong JY, Park SY, Kim A, Cho SN, Hur YG. 2019. Comparison of QFT-Plus and
343 QFT-GIT tests for diagnosis of M. tuberculosis infection in immunocompetent Korean
344 subjects. J Thorac Dis 11:5210-5217.
345 17. Telisinghe L, Amofa-Sekyi M, Maluzi K, Kaluba-Milimo D, Cheeba-Lengwe M,
346 Chiwele K, Kosloff B, Floyd S, Bailey SL, Ayles H. 2017. The sensitivity of the
347 QuantiFERON((R))-TB Gold Plus assay in Zambian adults with active tuberculosis. Int J
348 Tuberc Lung Dis 21:690-696.
349 18. Raby E, Moyo M, Devendra A, Banda J, De Haas P, Ayles H, Godfrey-Faussett P.
350 2008. The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay
351 in Zambian adults with active tuberculosis. PLoS One 3:e2489.
352 19. Kay AW, DiNardo AR, Dlamini Q, Kahari J, Mndzebele T, Mtetwa G, Ustero P,
353 Maphalala G, Mandalakas AM. 2019. Evaluation of the QuantiFERON-Tuberculosis
354 Gold Plus Assay in Children with Tuberculosis Disease or Following Household
355 Exposure to Tuberculosis. Am J Trop Med Hyg 100:540-543.
356 20. Venkatappa TK, Punnoose R, Katz DJ, Higgins MP, Banaei N, Graviss EA, Belknap
357 RW, Ho CS. 2019. Comparing QuantiFERON-TB Gold Plus with Other Tests To
358 Diagnose Mycobacterium tuberculosis Infection. J Clin Microbiol 57:10.
359 21. Pieterman ED, Liqui Lung FG, Verbon A, Bax HI, Ang CW, Berkhout J, Blaauw G,
360 Brandenburg A, van Burgel ND, Claessen A, van Dijk K, Heron M, Hooghiemstra M,
361 Leussenkamp-Hummelink R, van Lochem E, van Loo IHM, Mulder B, Ott A, Pontesilli
362 O, Reuwer A, Rombouts P, Saegeman V, Scholing M, Vainio S, de Steenwinkel JEM.
363 2018. A multicentre verification study of the QuantiFERON((R))-TB Gold Plus assay.
364 Tuberculosis 108:136-142.

365 22. Zhang H, Xin H, Wang D, Pan S, Liu Z, Cao X, Wang J, Li X, Feng B, Li M, Yang Q,
366 Zhang M, Jin Q, Gao L. 2019. Serial testing of Mycobacterium tuberculosis infection in
367 Chinese village doctors by QuantiFERON-TB Gold Plus, QuantiFERON-TB Gold in-
368 Tube and T-SPOT.TB. J Infect 78:305-310.
369 23. Chien JY, Chiang HT, Lu MC, Ko WC, Yu CJ, Chen YH, Hsueh PR. 2018.
370 QuantiFERON-TB Gold Plus Is a More Sensitive Screening Tool than QuantiFERON-
371 TB Gold In-Tube for Latent Tuberculosis Infection among Older Adults in Long-Term
372 Care Facilities. J Clin Microbiol 56:10.1128/JCM.00427-18.
373 24. Tsuyuzaki M, Igari H, Okada N, Suzuki K. 2019. Variation in interferon-gamma
374 production between QFT-Plus and QFT-GIT assays in TB contact investigation. Respir
375 Investig 57:561-565.
376 25. Barcellini L, Borroni E, Brown J, Brunetti E, Campisi D, Castellotti PF, Codecasa LR,
377 Cugnata F, Di Serio C, Ferrarese M, Goletti D, Lipman M, Rancoita PM, Russo G,
378 Tadolini M, Vanino E, Cirillo DM. 2016. First evaluation of QuantiFERON-TB Gold Plus
379 performance in contact screening. Eur Respir J 48:1411-1419.
380 26. Siegel SAR, Cavanaugh M, Ku JH, Kawamura LM, Winthrop KL. 2018. Specificity of
381 QuantiFERON-TB Plus, a New-Generation Interferon Gamma Release Assay. J Clin
382 Microbiol 56:10.
383 27. Sosa LE, Njie GJ, Lobato MN, Bamrah Morris S, Buchta W, Casey ML, Goswami
384 ND, Gruden M, Hurst BJ, Khan AR, Kuhar DT, Lewinsohn DM, Mathew TA, Mazurek
385 GH, Reves R, Paulos L, Thanassi W, Will L, Belknap R. 2019. Tuberculosis Screening,
386 Testing, and Treatment of U.S. Health Care Personnel: Recommendations from the

National Tuberculosis Controllers Association and CDC, 2019. MMWR Morb Mortal Wkly Rep 68:439-443.

28. Knierer J, Gallegos Morales EN, Schablon A, Nienhaus A, Kersten JF. 2017. QFT-Plus: a plus in variability? - Evaluation of new generation IGRA in serial testing of students with a migration background in Germany. J Occup Med Toxicol 12:1-016-0148-z. eCollection 2017.

29. Moon HW, Gaur RL, Tien SS, Spangler M, Pai M, Banaei N. 2017. Evaluation of QuantiFERON-TB Gold-Plus in Health Care Workers in a Low-Incidence Setting. J Clin Microbiol 55:1650-1657.

30. Agarwal S, Nguyen DT, Lew JD, Graviss EA. 2018. Performance and variability of QuantiFERON Gold Plus assay associated with phlebotomy type. PLoS One 13:e0207892.

31. Hoffmann H, Avsar K, Gores R, Mavi SC, Hofmann-Thiel S. 2016. 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 22:701-703.

32. Yi L, Sasaki Y, Nagai H, Ishikawa S, Takamori M, Sakashita K, Saito T, Fukushima K, Igarashi Y, Aono A, Chikamatsu K, Yamada H, Takaki A, Mori T, Mitarai S. 2016. Evaluation of QuantiFERON-TB Gold Plus for Detection of Mycobacterium tuberculosis infection in Japan. Sci Rep 6:30617.

33. Gallegos Morales EN, Knierer J, Schablon A, Nienhaus A, Kersten JF. 2017. Prevalence of latent tuberculosis infection among foreign students in Lubeck, Germany tested with QuantiFERON-TB Gold In-Tube and QuantiFERON-TB Gold Plus. J Occup Med Toxicol 12:12-017-0159-4.

410 34. Ryu MR, Park MS, Cho EH, Jung CW, Kim K, Kim SJ, Oh HY, Huh W, Jang HR,
411 Koh WJ, Park HY, Kim YH, Sinn DH, Choi JO, Joh JW, Kim JM, Kim SJ, Park JB, Kang
412 ES. 2018. Comparative Evaluation of QuantiFERON-TB Gold In-Tube and
413 QuantiFERON-TB Gold Plus in Diagnosis of Latent Tuberculosis Infection in
414 Immunocompromised Patients. J Clin Microbiol 56:10.
415 35. Moon HW, Gaur RL, Tien SS, Spangler M, Pai M, Banaei N. 2017. Evaluation of
416 QuantiFERON-TB Gold-Plus in Health Care Workers in a Low-Incidence Setting. J Clin
417 Microbiol 55:1650-1657.

418 **Table 1.** Result interpretation of QFT-Plus and QFT-GIT
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Result	QFT-Plus	QFT-GIT	Interpretation
Positive	Nil ≤8.0; and TB1 and/or TB2 minus Nil ≥0.35 and ≥25% of Nil	Nil ≤8.0; and TB Antigen minus Nil ≥0.35 and ≥25% of Nil	*M. tuberculosis* infection likely
Negative	Nil ≤8.0, Mitogen minus Nil ≥0.5; and TB1 and TB2 minus Nil <0.35 or ≥0.35 and <25% of Nil	Nil ≤8.0, Mitogen minus Nil ≥0.5; and TB Antigen minus Nil <0.35 or ≥0.35 and <25% of Nil	*M. tuberculosis* infection is not likely
Indeterminate	Nil >8.0; or Nil ≤8.0 and TB1 and TB2 minus Nil <0.35 or ≥0.35 and <25% of Nil and Mitogen minus Nil <0.5	Nil >8.0; or Nil ≤8.0 and TB Antigen minus Nil <0.35 or ≥0.35 and <25% of Nil and Mitogen minus Nil <0.5	Likelihood of *M. tuberculosis* cannot be determined

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424 **Table 2.** Head-to-head comparison of sensitivity between QFT-Plus and QFT-GIT in

425 patients with active TB disease

Study reference	Country	Sample size	No. (%) of IC hosts	Adult/ Pediatric (Median age)	Sensitivity			Median or Mean IFN-γ (IU/mL)		
					QFT-Plus	QFT-GIT	Difference (95% CI)	TB 1 in QFT-Plus	TB 2 in QFT-Plus	TB Ag in QFT-GIT
(31)	Germany	24 (MRS)	4 (7.0)	Adult (NA)	95.8%	95.8%	0.0% (-11.3 to 11.3)	3.10	3.70	4.67
		33 (CRS)			84.8%	84.8%	0.0% (-17.3 to 17.3)			
(12)	USA and Japan	164 (MRS)	4 (2.4)	Adult (71)	93.0%	94.3%	-1.3% (-6.6 to 4.0)	3.07	3.56	4.45
(13)	Italy	27 (23 MRS, 4 CRS)	0 (0.0)	Adult (38)	85.0%	89.0%	-4.0% (-21.9 to 13.9)	NA	NA	NA
(15)	Italy	69 (49 MRS, 20 CRS)	0 (0.0)	Adult (35)	90.0%	88.0%	2.0% (-8.4 to 12.4)	1.90	2.50	2.60
(32)	Japan	162 (MRS)	9 (5.5)	Adult (59)	91.1%	90.7%	0.4% (-5.9 to 6.7)	2.36	2.85	4.24
(16)	South Korea	33 (16 MRS, 17 CRS)	0 (0.0)	Both (17)	93.9%	93.9%	0.0% (-11.5 to 11.5)	10.00	10.00	NA
(19)	Eswatini	5 MRS	5 (41.7)	Pediatric (NA)	80.0%	80.0%	0.0% (-49.6 to 49.6)	NA	NA	NA
		7 CRS			14.0%	14.0%	0.0% (-36.4 to 36.4)			

MRS, microbiological reference standard which includes patients with positive culture, nucleic acid test or histopathological findings consistent with active TB, both pulmonary or extrapulmonary; CRS, clinical reference standard which includes patients with clinical and radiological symptoms and signs consistent with active TB in the absence of bacteriological confirmation by culture, nucleic acid test or histopathology after excluding other diseases;

IC, immunocompromised; CI, confidence interval; IFN-γ, interferon-gamma; IU, international units; mL, milliliter; No., number; NA, not available

436 **Table 3.** Agreement between QFT-Plus and QFT-GIT among high-risk groups
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Study reference	Country	Sample size (% IC)	Adult/Pediatric (Median age)	Test indications	Test positivity proportion			Agreement (Kappa)
					QFT-Plus	QFT-GIT	Difference (95% CI)	
(25)	Italy	119 (9.2)	Adult (38)	TB contacts with TST conversion	57.1%*	47.1%	10.0% (-2.6 to 22.6)	89.9% (0.80)
(20)	USA	508 (4.0)	Both (32)	TB contacts; immigrants from high burden countries; HIV+	23.0%	20.0%	3.0% (-2.0 to 8.0)	94.0% (0.81)
(21)	Netherlands and Belgium	1031 (17.0)	Adult (44**)	Pre-immunotherapy; TB contacts; TB exclusion; routine screening	14.5%	14.8%	-0.3% (-3.3 to 2.7)	95.0% (0.83)
(24)	Japan	412 (NA)	Adult (44)	TB contacts	7.5%	5.8%	1.7% (-1.7 to 5.1)	NA (0.82)
(33)	Germany	134 (NA)	Adult (25**)	Immigrants from high-risk countries	8.2%	8.2%	0.0% (-6.6 to 6.6)	NA (0.85)
(22)	China	616 (NA)	Adult (47)	At-risk health care workers	31.2%	27.9%	3.3% (-1.8 to 8.4)	94.8% (0.87)
(23)	Taiwan	229 (NA)	Adult (80)	Individuals in long term care facility	32.3%	28.8%	3.5% (-4.9 to 11.9)	93.9% (0.86)
(34)***	South Korea	169 (100.0)	Adult (54)	Pre-organ transplant	37.9%	37.3%	0.6% (-9.7 to 10.9)	93.7% (0.86)

		105 (100.0)	Adult (53)	Pre-stem cell transplant	17.1%	15.2%	1.9% (-8.1 to 11.9)	
		43 (100.0)	Both (45)	Pre-immunotherapy	23.3%	20.9%	2.4% (-15.1 to 19.9)	
(19)	Eswatini	46 (2.0)	Children <15 years (NA)	TB contacts	32.6%	32.6%	0.0% (-19.2 to 19.2)	96.0% (0.91)

IC, immunocompromised; CI, confidence interval; Kappa, kappa coefficient;

NA, not available

*No difference in positivity rate was observed between TB1 and TB2

**Mean age was provided

***Overall positivity rate for QFT-Plus 27.8% and QFT-GIT 29.0%

445 **Table 4.** Comparison of specificity between QFT-Plus and QFT-GIT among low-risk

446 populations

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Study reference	Country	Sample size	Specificity		
			QFT-Plus	QFT-GIT	Difference (95% CI)
(26)	USA	262 non-HCW including 51 NTM patients	98.1%	98.9%	-0.8% (-2.8 to 1.3)
(31)	Germany	77 low-risk HCW	87.0%	89.6%	-2.6% (-12.7 to 7.5)
(15)	Italy	19 non-HCW	100%	100%	0.0% (0.0 to 0.0)
(16)	South Korea	27 non-HCW	92.6%	100%	-7.4% (-17.3 to 2.5)
(32)	Japan	212 non-HCW	97.0%	98.6%	-1.6% (-4.4 to 1.2)
(35)	USA	626 no-risk HCW	97.0%	97.9%	-0.9% (-2.6 to 0.8)

448 HCW, healthcare worker; NTM, nontuberculous mycobacteria; CI, confidence interval

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