Use of interferon-gamma release assays in a health care worker screening program: Experience from a tertiary care centre in the United States

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BACKGROUND: Interferon-gamma release assays including the QuantiFERON-TB Gold In-Tube test (QFT-GIT [Cellestis Ltd, Australia]) may be used in place of the tuberculin skin test (TST) in surveillance programs for *Mycobacterium tuberculosis* infection control. However, data on performance and practicality of the QFT-GIT in such programs for health care workers (HCWs) are limited.

OBJECTIVES: To assess the performance, practicality and reversion rate of the QFT-GIT among HCWs at a tertiary health care institution in the United States.

METHODS: Retrospective chart review of HCWs at Central Arkansas Veterans Healthcare System (Arkansas, USA) who underwent QFT-GIT testing as a part of their employee screening between November 1, 2008 and October 31, 2009.

RESULTS: QFT-GIT was used to screen 3290 HCWs. The initial QFT-GIT was interpreted as positive for 129 (3.9%) HCWs, negative for 3155 (95.9%) and indeterminate for six (0.2%). Testing with QFT-GIT was repeated in 45 HCWs who had positive results on the initial test. The QFT-GIT reverted to negative in 18 (40.0%) HCWs, all of whom had negative TST status and initial interferon-gamma values of 0.35 IU/mL to 2.0 IU/mL.

CONCLUSIONS: The QFT-GIT test is feasible in large health care setting as an alternative to TST for *M tuberculosis* infection screening in HCWs but is not free from challenges. The major concerns are the high number of positive test results and high reversion rates on repeat testing, illustrating poor short-term reproducibility of positive QFT-GIT test results. These results suggest adopting a borderline zone between interferon-gamma values of 0.35 IU/mL to 2.0 IU/mL, and cautious clinical interpretation of values in this range.

Key Words: Health care workers; Interferon-gamma release assays; Tuberculosis; QuantiFERON Gold In-Tube assay

Health care workers (HCWs) are at increased risk of becoming infected with *Mycobacterium tuberculosis* through occupational exposure. Periodic screening of HCWs for *M tuberculosis* infection (MTBI) is a common practice and an essential component of many hospital infection control programs in the low tuberculosis (TB) prevalence countries including Canada and the United States (1). HCWs are typically screened with a tuberculin skin test (TST) and for symptoms suggestive of TB. Those with symptoms or a positive TST undergo additional testing that typically includes a chest x-ray. Latent *M tuberculosis* infection (LTBI) is diagnosed based on a positive TST result after TB disease has been excluded. The TST has many disadvantages including the assessment of response to a complex mix of antigens included in tuberculin-purified protein derivative that may cross-react following Bacille Calmette-Guérin (BCG) vaccination or exposure to non-TB mycobacteria, technical variations with administration, required return visits for reading and variability in interpretation of test results (2).

L'utilisation des tests de libération d'interféron gamma dans un programme de dépistage chez des travailleurs de la santé : l'expérience d'un centre de soins tertiaires des États-Unis

HISTORIQUE : Les tests de libération d'interféron gamma, y compris le test QuantiFERON-TB Gold In-Tube (QFT-GIT [Cellestis Ltd, Australie]), pourraient peut-être remplacer le test cutané à la tuberculine (TCT) dans les programmes de surveillance pour contrôler l'infection au *Mycobacterium tuberculosis*. Cependant, les données sur le rendement et le caractère pratique du test QFT-GIT dans de tels programmes destinés aux travailleurs de la santé (TdS) sont limités.

OBJECTIFS : Évaluer le rendement, le caractère pratique et le taux de réversion du test de QFT-GIT chez les TdS d'un établissement de soins tertiaires des États-Unis.

MÉTHODOLOGIE : Analyse rétrospective des dossiers des TdS du Central Arkansas Veterans Healthcare System de l'Arkansas, aux États-Unis, qui ont subi le test QFT-GIT dans le cadre du dépistage des employés entre le 1^{er} novembre 2008 et le 31 octobre 2009.

RÉSULTATS : Le test QFT-GIT a servi au dépistage de 3 290 TdS. Le test QFT-GIT initial a été interprété comme positif auprès de 129 TdS (3,9 %), comme négatif chez 3 155 TdS (95,9 %) et comme indéterminé chez six TdS (0,2 %). Le test QFT-GIT a été repris chez 45 TdS qui avaient obtenu des résultats positifs au test initial. Il a donné des résultats négatifs chez 18 TdS (40,0 %), qui avaient tous obtenu un TCT négatif et des valeurs initiales d'interféron gamma de 0,35 UI/mL à 2,0 UI/mL.

CONCLUSIONS : Le test QFT-GIT est faisable dans de grands établissements de santé pour remplacer le TCT dans le dépistage du *M tuberculosis* chez les TdS, mais il n'est pas sans défis. Les principales préoccupations sont le nombre élevé de résultats faux-positifs et le taux élevé de réversion à la reprise du test, qui démontre une mauvaise reproductibilité à court terme des résultats positifs du test QFT-GIT. Ces résultats incitent à préconiser l'adoption d'une zone seuil entre les valeurs d'interféron gamma de 0,35 UI/mL à 2,0 UI/mL et une interprétation clinique prudente des valeurs faisant partie de cette plage.

Interferon-gamma (IFN- γ) release assays (IGRAs) are in vitro tests for MTBI that can be used in place of TST to screen HCWs (3). Two IGRAs are commercially available for the detection of MTBI in the United States including the QuantiFERON-TB Gold In-Tube test (QFT-GIT [Cellestis Ltd, Australia]) and the T-SPOT.TB assay (Oxford Immunotec, United Kingdom) (2,3). QFT-GIT assess immunological responsiveness to specific *M tuberculosis* proteins including early secretory antigenic target-6 (ESAT-6), culture filtrate protein 10 (CFP10) and TB 7.7, which offers several advantages over the TST (2). These antigens do not cross-react with BCG or with most non-TB mycobacteria. The QFT-GIT test requires a single patient visit (2,3).

The Centers for Disease Control and Prevention (CDC, Georgia, USA) published guidelines for using IGRAs and indicated that certain IGRAs, including QFT-GIT may be used in surveillance programs for MTBI including those for HCWs (3). On the contrary, the most

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recent 2010 Canadian guidelines (4) do not recommend the use of IGRAs as a baseline test in serial testing of HCWs. However, data regarding the performance of the QFT-GIT in such screening programs for HCWs in the United States are limited (2,3).

The Central Arkansas Veterans Healthcare System (CAVHS) is a tertiary care academic Veterans Administration hospital affiliated with the University of Arkansas for Medical Sciences (Arkansas, USA) employing more than 3000 individuals. Screening of HCWs for MTBI with the TST, initially at the time of recruitment and annually thereafter, was the standard practice at CAVHS for years following the published guidelines (1); the annual TST conversion rate in the five years before 2008 was less than 0.1%. In November 2008, CAVHS began to use the QFT-GIT test in place of TST for screening both employees and patients for MTBI. After this change, CAVHS were confronted with an unexpectedly high number of positive QFT-GIT test results (>20-fold higher than baseline positive TST results). In the first four weeks, five of 210 HCWs (2.5%) tested positive with the QFT-GIT test. Active TB disease was ruled out by symptom assessment and chest radiographs. This resulted in a clinical decision-making dilemma of whether to offer LTBI treatment because all of the HCWs who tested positive on QFT-GIT testing had a negative TST history. All five of these HCWs underwent repeat testing with QFT-GIT and TST. Not only did all five revert to negative QFT-GIT, but their TST status also remained negative. There was no available literature or data to guide us in this unique situation. Because of positive QFT-GIT results, the infection control team at CAVHS decided to offer repeat QFT-GIT tests to all HCWs whose initial QFT-GIT was positive. No repeat testing was performed on HCWs with negative QFT-GIT results. The aim of the present study was to assess the performance and practicality of use, and the reversion rates for QFT-GIT testing among HCWs who tested positive in an employee screening program in the United States.

METHODS

The present study was a retrospective chart review conducted at the CAVHS in Little Rock, Arkansas. The study was initiated after approval by the CAVHS Institutional Review Board and Research and Development Committee. Electronic medical records of all HCWs were queried to identify those who had a positive QFT-GIT as a part of their employee screening between November 1, 2008 and October 31, 2009. Data regarding age, sex, previous TST results and all QFT-GIT results for those with positive QFT-GIT results were collected. All HCWs underwent screening for MTBI with QFT-GIT, irrespective of previous TST status, to establish baseline test results for comparison with future test results.

QFT-GIT was performed according to the manufacturer's instructions (5). Briefly, approximately 1 mL of blood is collected directly into each of three tubes (nil, TB antigen and mitogen control). The tubes are then shaken vigorously and transported to the clinical laboratory. Once the tubes reach the microbiology section, they are again mixed by shaking and then immediately incubated at a mean ($\pm$ SD) temperature of $37\pm1^\circ\text{C}$. The time from collection to incubation is approximately 1 h. After incubation in ambient air for 16 h to 24 h, the tubes are centrifuged for 15 min at 2500 rpm to separate cells from plasma. Freshly prepared conjugate, then plasma is added to an ELISA plate. The plate is placed on a microplate shaker to ensure mixing of the conjugate and plasma, covered, incubated at room temperature for 120 ± 5 min, then washed using a Columbus semi-automated ELISA plate washer (Tecan Group Ltd, Switzerland). Enzyme substrate is added to each well and the plates are mixed again, covered and incubated at room temperature for 30 min. Enzyme stop solution is added, and the optical density of each well is determined within 5 min of stopping the reaction using a Sunrise ELISA plate reader (Tecan Group Ltd, Switzerland).

The test results were reported positive if the IFN- γ value of TB antigen minus nil was ≥ 0.35 IU/mL and negative if < 0.35 IU/mL. Test results were considered to be indeterminate if nil concentrations were > 8.0 IU/mL and mitogen differences were < 0.5 IU/mL, as per

manufacturer's guidelines (5). In the present study, QFT-GIT reversion was defined as initial positive test results becoming negative on subsequent testing.

Statistical analysis

Statistical analysis was conducted using SAS version 9.2 (SAS Institute, USA). All reported P values were two-tailed and calculated with statistical significance set at $P\leq 0.05$. The χ^2 test was used to compare categorical variables and independent group *t* tests were used to compare continuous variables. For comparison of TB responses, values > 10 IU/mL were replaced with a value of 10 IU/mL.

RESULTS

A total of 3290 HCWs underwent a QFT-GIT test between November 1, 2008 and October 31, 2009. The initial QFT-GIT test was interpreted as positive for 129 (3.9%) HCWs, negative for 3155 (95.9%) HCWs, and indeterminate for six (0.2%) HCWs (Table 1). The characteristics of the 129 HCWs who tested positive on QFT-GIT testing are summarized in Table 2. The mean ($\pm$ SD) age of the 129 HCWs who tested positive with QFT-GIT was 49.9 ± 12.2 years. In this population, 70 (54.2%) were males. Among the 129 HCWs with positive QFT-GIT test results, 53 (41%) had a history of positive TST, 62 (48%) had a history of negative TST(s) and 14 (11%) had unknown TST status.

Testing with QFT-GIT was repeated (within two to 30 days) in 45 (34.9%) HCWs who were positive on initial testing. The repeat QFT-GIT results and TST status of these HCWs are shown in Figure 1. The test reverted to negative in 18 (40.0%) of the 45 HCWs who underwent repeat QFT-GIT. Among HCWs whose QFT-GIT reverted to negative, the mean and median TB response from the initial QFT-GIT were 1.44 IU/mL and 1.05 IU/mL, respectively. Changes in TB responses for these 18 HCWs are shown in Figure 2. The initial values were distributed over a range of 0.36 IU/mL to 2.1 IU/mL (with one outlier not shown in this figure with a value ≥ 10 IU/mL) whereas the TB responses obtained on repeat testing were clustered in a much smaller range (-0.1 to 0.1). While none of the subjects whose QFT-GIT reverted to negative had a previous positive TST, 13 (48%) of 27 subjects whose QFT-GIT remained positive had a previous positive TST. Of the HCWs with consistently positive QFT-GITs, 13 (48%) had a negative TST history, and the TST status was unknown for one individual (Figure 1). The IFN- γ values on repeat QFT-GIT testing increased in 10 of 13 HCWs who had previous positive TST, and decreased in 10 of 13 HCWs who had negative TST history.

TB response values were compared for the 18 HCWs who reverted on repeat testing with the values for the 27 who remained positive on repeat testing (Figure 3). The mean initial TB response in the group who reverted was 1.44 IU/mL (95% CI 0.36 IU/mL to 2.52 IU/mL), whereas the mean initial TB response in the group who remained positive was 3.47 IU/mL (95% CI 1.99 IU/mL to 4.95 IU/mL [$P=0.02$]). When limited to HCWs whose previous TST was negative (ie, those typically retest in employee screening programs), the difference in TB response values among those who reverted versus those who remained positive was not statistically significant ($P=0.84$) (Figure 4).

DISCUSSION

To our knowledge, the present study was the first to be presented and published as a scientific abstract (6) to assess the performance and practicality of the QFT-GIT test in a substantial number of United States HCWs undergoing screening for MTBI in a large tertiary centre. Although we found QFT-GIT testing feasible in a large tertiary health care setting, and logistically more convenient to perform than TST, we were faced with many unique challenges and clinical situations after replacing the TST with the QFT-GIT test. We had an unexpectedly high number of positive QFT-GIT test results (>20-fold higher than baseline positive TST results). This not only led to additional testing, including chest x-rays, TST and repeat QFT-GIT, but also lack of confidence in test results among our employees. Moreover,

TABLE 1
QFT-GIT* test results and tuberculin skin test (TST) status of 3290 health care workers who underwent QFT-GIT testing in a one-year study period

QFT-GIT	TST history			Total
	Positive	Negative	Unknown	
Positive	53	62	14	129
Negative	78	2474	603	3155
Indeterminate	0	6	0	6
Total	131	2542	617	3290

Data presented as n. *QuantiFERON-TB Gold In-Tube test (Cellestis Ltd, Australia)

TABLE 2
Characteristics of 129 health care workers (HCWs) who tested positive on initial QFT-GIT* test

Characteristic	HCWs
Sex	
Female	59 (46)
Male	70 (54)
Tuberculin skin test history	
Negative	62 (48)
Positive	53 (41)
Unknown	14 (11)
Age, years, mean ± SD	49.9±12.2

Data presented as n (%) unless otherwise indicated. *QuantiFERON-TB Gold In-Tube test (Cellestis Ltd, Australia)

Figure 1) The QuantiFERON-TB Gold In-Tube (QFT-GIT [Cellestis Ltd, Australia]) test results and history of tuberculin skin test (TST) status in 45 health care workers who underwent repeat testing

this resulted in a major dilemma when it came to clinical decision making in terms of offering LTBI treatment because many of these HCWs who tested positive on QFT-GIT testing had negative TST(s) in the preceding years. Interestingly, our results were very similar to another study recently published by Gandra et al (7), in which the authors raise a similar concern with serial QFT-GIT testing of HCWs.

Another concerning issue with serial QFT-GIT testing is short-term reproducibility, as demonstrated by the high reversion rate (40%) in our study population. The mean IFN- γ value for these HCWs who underwent repeat QFT-GIT testing was 1.44 IU/mL. Our results agree with previous studies questioning the short-term reproducibility of QFT-GIT (2,8-12), but our mean IFN- γ values were higher than those previously reported in literature. It has been proposed in these studies to increase the cut-off value for a positive test result to 0.70 IU/mL, but our results do not support this value for many reasons. First, our mean initial IFN- γ values for HCWs who reverted to negative was 1.44 IU/mL and is two times higher than the proposed 0.70 IU/mL. Hence, raising the cut-off value to 0.70 IU/mL will not solve the problem of these likely false-positive results. Second, a fixed cut-off in this population will continue to pose challenges because of test (QFT-GIT) variability

Figure 2) The initial and repeat interferon (IFN)-gamma values in 18 health care workers who reverted to negative on repeat QuantiFERON-TB Gold In-Tube (QFT-GIT [Cellestis Ltd, Australia]) testing. The dotted line represents the cut-off IFN-gamma value for positive tests (0.35 IU/mL). An outlier with IFN-gamma value ≥ 10 is not represented in this figure

Figure 3) Box-and-whisker plots showing distribution of initial interferon-gamma values in health care workers whose repeat QuantiFERON-TB Gold In-Tube test (QFT-GIT [Cellestis Ltd, Australia]) result reverted to negative (n=18) versus those who remained positive (n=27). On comparing interferon-gamma values, there was a significant difference (P=0.02) in the mean levels between the two groups

in serial testing, and it is not possible to distinguish false positives from true positives without having a gold standard for LTBI diagnosis. To address this problem, we suggest adding a borderline zone for interpretation of test results in serial testing of HCWs when IFN- γ values are between 0.35 IU/mL to 2.0 IU/mL. We also recommend cautious clinical interpretation of QFT-GIT test results in this borderline zone. Repeat testing with QFT-GIT and/or TST should be considered for HCWs whose IFN- γ values are in the above borderline range and TST status is negative. More longitudinal studies investigating positive predictive value and likelihood of developing active TB after a positive QFT-GIT test are needed to address this issue of optimal borderline zone in this subset of the population.

The reason(s) for the high reversion rates and, thus, poor short-term reproducibility is unclear. It may be due to variation in host immune responses, although this is perplexing when the QFT-GIT result changes from positive to negative within a few days. Another possibility is pretest variability as a result of the recommended manufacturer's test protocol. To investigate this, personnel collecting the specimens and those performing the test were closely observed during

testing of a few samples. We found that in several cases of positive test results (not reported as final results in the present study), pretest variability was a contributing factor for these likely false-positive results. According to the manufacturer's instructions, tubes should be shaken vigorously once blood is collected (5). We noted the force with which tubes were shaken varied among phlebotomists. Vigorous shaking was often associated with a false-positive result. Once this was recognized, phlebotomists were retrained, emphasizing proper (ie, not so vigorous) shaking. The number of false-positive results then declined but still occurred despite following the manufacturer's exact directions to perform the QFT-GIT test, suggesting that other factor(s) are involved. Further studies are needed, both in a controlled research setting and in the real world, to fully understand QFT-GIT variability and reproducibility. We would certainly recommend not shaking the test tubes too vigorously and would also suggest to the manufacturer to add a standardized shaking device to the test kit.

The comparison of mean IFN- γ values in HCWs who reverted to negative and those who remained positive on repeat QFT-GIT testing showed statistical significance. However, when these two groups were compared, including only the HCWs who had negative TST status (ie, those typically retested in employee screening programs), the comparison of mean IFN- γ values showed no statistical significance. This implies that negative TST status is a comparable (with QFT-GIT) predictor of absence of LTBI in serial testing. The diagnosis of LTBI among HCWs should be made using a combination of epidemiological and other diagnostic methods, and not based on TST results and/or QFT-GIT results alone.

We had another interesting observation in our results. The mean IFN- γ values on repeat QFT-GIT testing increased in HCWs with a history of positive TST, whereas they decreased in HCWs with negative TST history. It is yet to be determined as to what absolute difference in these IFN- γ values is actually clinically relevant in serial testing (13). It is, therefore, difficult to extrapolate anything conclusive; however, these trends are interesting to note and should be explored in future research. Nevertheless, we agree with Pai et al (13), who recommend that health professionals who interpret serial QFT-GIT should not merely rely on dichotomous (positive/negative) results, but learn to interpret trends in IFN- γ values over time.

The first and most recent systemic review of IGRAs in HCW by Zwerling et al (14) addressed many challenges involved in serial testing. The review concluded that the current guidelines and evidence available on the use of IGRAs needs an update to adequately address the challenges raised by serial testing.

Our study had several limitations. First, it was retrospective in nature. Repeat QFT-GIT testing was performed only in HCWs whose initial test results were positive. The negative tests were assumed to be true negatives despite 60% discordance among TST and QFT-GIT. Second, not all of the HCWs who had positive QFT-GIT test results underwent repeat QFT-GIT testing, which could have influenced reversion rates. Third, concurrent TSTs were not performed in all HCWs who underwent repeat QFT-GIT testing. Another limitation is that we did not have BCG data on all employees. However, being a federal facility, most of our employees are US-born citizens with a negative BCG history.

CONCLUSION

The QFT-GIT is not yet ready for 'prime time' to screen HCWs given the high number of positive test results and high reversion rates on repeat testing. The current CDC guidelines (15) supporting QFT-GIT test to screen HCWs for MTBI are based on evidence from controlled research studies and need an update in view of our current translational research. We hope that our large study and practical experience with QFT-GIT in the real world will help many health care organizations that are facing the same challenges that we encountered when TST was replaced by QFT-GIT to screen HCWs for LTBI. It will also guide the institutions who are in the process of implementing QFT-GIT. Finally, additional research studies are needed to assess biological

Figure 4) Box-and-whisker plots showing distribution of initial interferon-gamma values in health care workers who underwent repeat Quantiferon-TB Gold In-Tube testing (QFT-GIT [Cellestis Ltd, Australia]) testing and had 'negative' tuberculin skin test status. On comparing interferon-gamma values, there was no significant difference ($P=0.84$) in the mean levels among the health care workers whose repeat QFT-GIT test results reverted from positive to negative ($n=18$) versus those that remained positive ($n=13$)

variability, reproducibility and the likelihood of developing active TB after a positive QFT-GIT test in low-TB incidence countries.

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