Challenges with QuantiFERON-TB Gold Assay for Large-Scale, Routine Screening of U.S. Healthcare Workers

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Rationale: North American occupational health programs that switched from the tuberculin skin test (TST) to IFN- γ release assays for latent tuberculosis screening are reporting challenges with interpretation of serial testing results in healthcare workers (HCWs). However, limited data exist on the reproducibility of serial IFN- γ release assay results in low-risk HCWs.

Objectives: To evaluate the short-term reproducibility of QuantiFERON-TB Gold In-Tube (QFT) in a large cohort of HCWs and to define a QFT cutoff yielding a conversion rate equivalent to historical TST rates.

Methods: We retrospectively evaluated the QFT results from HCWs with two or more QFT tests performed between June 2008 and July 2010 at an academic institution. Outcome measures were proportions of reproducibility, quantitative results, and conversion rates with alternate QFT cutoffs.

Measurements and Main Results: A total of 9,153 HCWs with two or more QFT tests were included in the analysis. Of 8,227 individuals with a negative result, 4.4% ($n = 361$) converted their QFT result over 2 years. A total of 261 (72.3%) of the HCWs with conversions underwent repeat short-term testing after the first positive result with 64.8% reverting ($n = 169$). An IFN- γ cutoff of 5.3 IU/ml or higher (manufacturer's cutoff is ≥ 0.35 IU/ml) yielded a conversion rate of 0.4%, equal to our institution's historical TST conversion rate.

Conclusions: The manufacturer's definition of QFT conversion results in an inflated conversion rate that is incompatible with our low-risk setting. A significantly higher QFT cutoff value is needed to match the historical TST conversion rate. Nonreproducible conversions in most converters suggested false-positive results.

Keywords: interferon- γ release assay; QuantiFERON; healthcare workers; reproducibility of results

Despite the all-time low incidence of tuberculosis (TB) in the United States, large numbers of U.S. healthcare workers (HCWs) are screened routinely for latent TB infection (LTBI) (1, 2). IFN- γ release assays (IGRAs), such as the QuantiFERON-TB Gold In-Tube (Qiagen/Cellestis, Carnegie, Australia) test (QFT), are

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Although IFN- γ release assays (IGRAs) are widely used for the screening of healthcare workers in North American institutions, there are limited data on the reproducibility of IGRA results in this population.

What This Study Adds to the Field

In the largest study of serial IGRA testing in healthcare workers to date, we found that most QuantiFERON-TB Gold In-Tube test result conversions were nonreproducible on repeat testing within 60 days. Using our historical tuberculin skin test conversion rate as a benchmark, the QuantiFERON-TB Gold In-Tube cutoff would need to be increased by more than 10-fold to yield an equivalent conversion rate. Conversions in this low-risk population should be interpreted with caution.

increasingly replacing the tuberculin skin test (TST) for occupational screening (3, 4). More than 70 U.S. medical institutions have adopted the QFT assay to serially test hundreds of thousands of hospital employees each year (5). In principle, the QFT has equivalent sensitivity compared with the TST, with improved specificity in bacille Calmette-Guérin vaccinated individuals and individuals with nontuberculous mycobacterial infection (6, 7). Additionally, the QFT improves the logistics and economics of TB screening because, unlike the TST, it does not require trained readers, a return visit, or baseline two-step testing for annual screening (7).

However, in practice, QFT results have proved more dynamic in serial testing than anticipated (8, 9). Early adopters of the QFT assay for occupational screening are now reporting major concerns with interpretation of the high conversion and reversion rates using the manufacturer's recommended cutoff of simple negative to positive change (10–13). This cutoff has not been rigorously validated in serial testing of HCWs.

The CDC published guidelines in 2005 indicating that the QFT may be used in all circumstances in which a TST is recommended, including serial screening in HCWs (14). The 2010 guideline update restates this recommendation but cautions against “a greater risk of test conversion due to false-positive IGRA results” in low-risk HCWs (2). This statement highlights the need for a more accurate definition of QFT conversion in serial testing.

In 2008, the Stanford University Medical Center (SUMC) replaced TST with QFT for annual TB screening of more than 10,000 employees. We retrospectively evaluated the rates of conversion and the reproducibility with short-term testing and determined the QFT cutoff yielding a conversion rate approximating our historical TST conversion rates.

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METHODS

Study Population and Site Characteristics

SUMC is a 611-bed hospital with a mean of 14 cases of pulmonary TB per year between 2006 and 2011. SUMC serves Santa Clara County with TB rates estimated between 10.7 and 10.9 cases per 100,000 people during the study period (15). SUMC screens all employees annually for TB infection. In 2006, the last year TST was used, the annual TST conversion rate was 0.4% consistent with rates reported by multiple U.S. hospitals located in both lower and higher TB incidence areas, all reporting TST conversion rates under 1.5% (range, <0.1–1.2%) (16–20).

SUMC replaced the TST for annual employee TB screening with the second-generation QuantiFERON-TB Gold assay (QFT-G) in December 2006 after an in-house cost analysis. The occupational health staff began noting that numerous individuals with no new TB risk factors were newly positive by the QFT-G. To avoid unnecessary LTBI treatment, the institutional policy was amended, prompting repeat testing within 6 weeks in newly positive individuals. In June 2008, the QFT-G was upgraded to the in-tube version (QFT). During the study period from June 1, 2008 to July 31, 2010, the clinic had an unusual policy of screening all employees annually with the QFT, regardless of history of prior LTBI treatment or of prior positive results with either the TST or QFT. The policy of retesting conversions within 6 weeks was continued. Only those with repeat positive testing were referred to their primary care provider for treatment.

We retrospectively analyzed qualitative and quantitative QFT results on 9,153 HCWs with greater than or equal to two QFT results between June 1, 2008 and July 31, 2010. The study received approval from the Stanford University Institutional Review Board.

Data Collection

We merged two clinical laboratory databases collecting results over the study period to yield a final database containing the age; sex; QFT results including the TB Antigen, Nil, and Mitogen; and the test run date. No information relating to TB exposure, TB risk factors, previous TB test results, country of birth, or bacille Calmette-Guérin status was available in the databases.

QFT Testing

The QFT was performed according to the manufacturer's instructions outlined in the package insert (21). Blood was drawn into three 1-ml

QFT tubes by phlebotomists trained in QFT collection and shaken vigorously for 5 seconds according to the package insert. The samples were incubated immediately for 16–24 hours at 37°C in an on-site incubator and then transported to the microbiology laboratory. The harvested plasma was separated by centrifugation and stored at 24°C. ELISA was performed within 24 hours according to manufacturer's instructions by an automated (robotic) ELISA system (DSX; Dynex Technologies, Chantilly, VA). Per manufacturer guidelines, the cutoff for a positive test result was defined as TB Antigen minus Nil IFN- γ concentration (TB response) greater than or equal to 0.35 IU/ml (after accounting for Nil and Mitogen control results). Because of imprecision in QFT ELISA measurements outside of the range of 10 IU/ml and to facilitate statistical analysis, all values less than 0 IU/ml or greater than 10 IU/ml were truncated at 0 and 10 IU/ml, respectively.

Definitions

We defined short-term testing as less than or equal to 60 days between sequential tests. We defined long-term testing as greater than 60 days between sequential tests. Conversions were initially defined, per manufacturer guidelines, as a change from negative QFT (IFN- γ TB response <0.35 IU/ml) to a positive QFT ($\geq$ 0.35 IU/ml). We also reanalyzed the data using an alternate dichotomous cutoff of greater than or equal to 1.0 IU/ml, a cutoff proposed by Fong and coworkers (10) based on results from a large cohort of U.S. HCWs. Because we expected no change in results with a short-term repeat test (no interval exposure or treatment), we defined short-term reproducibility as the concordance of QFT results when the test was repeated in the short-term. We used the TST conversion rate from 2006 as a benchmark for redefining a QFT cutoff resulting in an equivalent conversion rate. In the absence of a gold standard for LTBI, we used a proxy for "true-positives" (those who were persistently positive throughout their QFT testing history) to evaluate the approximated sensitivity with alternate cutoffs.

Data Analysis

All reported *P* values were two-tailed and calculated with statistical significance set at *P* less than 0.05. Independent group *t* tests were used to compare continuous variables; the *z* statistic for proportions was used to compare proportions; linear regression was used to compare continuous independent variables and categorical dependent variables. For results classified according to the manufacturer-recommended cutoff, we assessed agreement between the QFT assays with the kappa

Figure 1. QFT serial test results for all HCWs in the cohort. Conversions (Neg→Pos) = HCWs with a qualitative change from a negative to a positive QFT at any point in the individual's testing sequence; HCWs = healthcare workers; Indeterminates = HCWs with at least one indeterminate result; Persistent negatives (Neg→Neg) = HCWs with greater than or equal to two negative QFT results; Persistent positives (Pos→Pos) = HCWs with greater than or equal to two positive QFT results; QFT = QuantiFERON; Reversions (Pos→Neg) = HCWs with a qualitative change from a positive to a negative QFT at any point in the individual's testing sequence.

TABLE 1. CHARACTERISTICS OF HCWs AND QuantiFERON TESTING FREQUENCY/INTERVAL

Category	Number
HCWs with ≥ 2 QFT results	9,153
No. of females (%)	6,590 (72.0)
Mean age, yr ($\pm$ SD)	43 ($\pm$ 5D)
No. of tests per HCW (%)	
2 tests	7,710 (84.2)
3 tests	1,286 (14.1)
4 tests	140 (1.5)
5 tests	13 (0.1)
6 tests	3 (<0.1)
7 tests	1 (<0.1)
No. of repeats (%)	
Long-term repeats*	9,843 (92.6)
Short-term repeats*	788 (7.4)
Median no. of days between repeats	
Long-term repeats* (IQR)	360 (343–379)
Short-term repeats† (IQR)	13 (7–21)

Definition of abbreviations: HCW = healthcare worker; IQR = interquartile range; QFT = QuantiFERON-TB Gold In-Tube test.

* Long-term repeats greater than 60 days between tests.

† Short-term repeats less than or equal to 60 days between tests.

statistic of interrater agreement. Statistical analysis was conducted using Stata (Version 12.1; Stata Corp, College Station, TX).

RESULTS

QFT Results and Testing Patterns

There were 9,153 HCWs who were QFT tested two or more times over the 25-month study period (Figure 1, Table 1). A total of 6,590 (72.0%) were female and the mean age was 43 years (SD $\pm$ 12.5). Among all tests, 2,774 (13.9%) were positive; 17,094 (85.6%) were negative; and 113 (0.5%) were indeterminate. A total of 98 HCWs with at least one indeterminate were excluded from the analysis.

Of 1,223 individuals (13.4%) initially positive on QFT testing, 67.5% ($n = 828$) remained positive throughout testing (persistent positives) (Figure 1). Of 8,227 individuals with a negative QFT result, 4.4% ($n = 361$) converted (Conversions in Figure 1, Table 2). A significantly increased proportion of initial positive results fell between the cutoff value of 0.35 and 1.0 IU/ml in the reversions and conversions as compared with the persistent positives (73.9% vs. 62.4% vs. 27.8%, respectively; $P < 0.001$). Because of the policy of retesting new conversions to avoid unnecessary treatment for LTBI, 261 (72.3%) of the 361 HCWs with conversions underwent repeat short-term testing; 64.8% reverted ($n = 169$) (Figure 2). An additional 16.6% ($n = 60$) of the individuals who converted underwent repeat testing outside of the short-term retesting period of 60 days; 63.3% ($n = 38$) of these individuals reverted (median number of days to retest, 89.5; interquartile range, 72–166). A total of 11.1% ($n = 40$) did not return for repeat testing, because of staff turnover, refusal, or preference to be treated without repeat testing.

Of the 261 individuals with QFT conversions with repeat short-term testing, 246 had no prior positive QFT during the testing period; 15 had at least one prior positive. Reversion rates on repeat short-term testing for the two groups were 67.1% ($n = 165$) and 26.7% ($n = 4$), respectively.

Because of continued reluctance to believe the positive QFT result in low-risk HCWs, a second repeat short-term test was performed in 16 HCWs who were persistently positive on their first repeat test. Twelve (75%) of these reverted (Figure 2). A significantly larger proportion of HCWs who were persistently positive on short-term repeat testing underwent a second

short-term repeat test as compared with those that reverted (18.2% vs. 2.6%; $P < 0.001$). With a 7% annual staff turnover rate, the incidence rate of active TB among individuals with a QFT conversion was 0 cases per 1,299 person-years.

Investigating Alternative Cutoffs

By implementing greater than or equal to 1.0 IU/ml (10) as the cutoff for all testing (both short- and long-term repeats), we eliminated 67.0% of the discordant repeats, but failed to detect 33.7% of the persistent positives. By implementing greater than or equal to 1.0 IU/ml as the cutoff only in short-term testing, we improved reproducibility from 53.2 to 84.1% but failed to detect 52.2% of the persistent positives.

Deriving QFT Cutoff to Match TST Conversion Rates

We determined which cutoff for all testing (both short- and long-term) would produce conversion rates in the range of TST conversion rates in HCWs in North America (16–18). We found that a 1% conversion rate resulted from a QFT cutoff of 1.7 IU/ml, a 0.8% rate from 2.3 IU/ml, and our institution's historical reference of a 0.4% rate from 5.3 IU/ml (Figure 3). If the cutoff was adjusted to 5.3 IU/ml to yield a conversion rate of 0.4%, the proportion of HCWs with persistent positives (presumed to truly have LTBI) classified on initial QFT testing was reduced by 68.6% (Figure 4).

DISCUSSION

As data emerge describing North American healthcare institutions' experiences with serial QFT testing (10, 19, 22), challenges in interpreting results have arisen. Our institution is no exception. We found that of the 9,153 HCWs with repeat QFT testing, the conversion rate was 4.4% and the short-term reversion rate was 64.8%, fairly consistent with rates reported from other North American healthcare institutions using the QFT (4, 20). Our findings, based on the largest number of HCWs serially tested with QFT, raise serious questions about the reproducibility of this assay and the current definition of conversion in HCWs in North America. We also illustrate the tendency of occupational health staff to repeat the test until a negative was obtained, presumably to avoid LTBI treatment.

TABLE 2. QUALITATIVE RESULTS OF SERIAL QuantiFERON TESTS IN HCWs

Category	No. (%)	Mean Age ($\pm$ SD)*	Females No. (%)†
Negative tests	17,094 (85.6%)		
Positive tests	2,774 (13.9%)		
Indeterminate tests	113 (0.5%)		
HCWs persistently negative	7,496 (81.9%)	42 (12.7)	5,465 (72.9%)
HCWs persistently positive	828 (9.0%)	44 (11.7)	562 (67.9%)
HCWs with conversion	361 (4.4%)	43 (11.6)	260 (72.1%)
HCWs with reversion	613 (38.7%)	42 (12.0)	427 (69.6%)

Definition of abbreviation: HCWs = healthcare workers.

* Although differences in mean age among groups were statistically significant by linear regression (P value for $F < 0.001$), these differences do not seem clinically significant with mean ages of 42, 44, 43, and 42.

† The proportion of females was significantly increased in HCWs with reversion as compared with HCWs persistently positive ($P < 0.001$); HCWs with conversion as compared with reversion ($P < 0.001$); and HCWs persistently negative as compared with HCWs with conversion ($P < 0.001$). Proportions compared by z statistic for proportions.

Figure 2. QFT serial test results for healthcare workers (HCWs) who converted and underwent short-term repeat testing. Neg = negative QFT test result; Pos = positive QFT test result; QFT = QuantiFERON.

Our ability to interpret the meaning of QFT results is limited by the lack of a gold standard for diagnosing LTBI, the lack of TB risk factor data in our cohort, and the lack of long-term follow-up. However, evidence for the initial positive test being a false result includes the all-time low TB incidence in the United States, the low historical TST conversion rate of 0.4%, consistent with reported TST conversion rates at multiple other U.S. institutions (16–18), the rigorous infection prevention policies currently practiced, and the absence of progression to active TB. Therefore, the current definition of QFT conversion in this population is called into question. Given the high reversion rate, should HCWs in North America with new positive results be routinely retested before being offered LTBI treatment? More importantly, is the reproducibility of QFT good enough for routine use? Furthermore, we conclude that a large proportion

of the newly positive results are false, suggesting that in low-incidence settings, a positive result in an individual tested once may be false.

Our results support short-term retesting in new QFT conversions as a feasible option to reduce the erroneous high conversion rates in HCWs. Of the population of HCWs with no prior positive QFT during the study period that converted and underwent short-term repeat testing (the population with the lowest pretest probability of having LTBI) 67.1% ($n = 165$) reverted. If we consider this subgroup as false conversions, the overall conversion rate over the study period drops from 4.4 to 2.4%. Although this is closer to historical TST conversion rates, the rate is still very high for a low-risk setting. Also, a cost-effectiveness analysis would need to be conducted to thoroughly evaluate a policy of retesting new QFT conversions.

Figure 3. QFT conversion rate (CR) versus QFT cutoff. The historical tuberculin skin test conversion rate at our institution is 0.4%. The dashed reference line at 0.35 IU/ml is the QFT cutoff. Using this cutoff, the CR is 4.4%. CR = proportion of healthcare workers who converted from a negative to a positive QFT result; n = number of healthcare workers converting at varying conversion rates; QFT = QuantiFERON.

Figure 4. Percentage of persistently positive healthcare workers classified as positive on initial testing versus QFT cutoff. The dashed reference line at 0.35 IU/ml is the QFT cutoff. n = number of healthcare workers; QFT = QuantiFERON.

Alternatively, changing the definition of conversion has been proposed as a solution to the problem of the high conversion rates with QFTs in a low-risk population. Several studies have used their data to define new cutoffs, but none have been validated in longitudinal studies (4, 10, 12, 22). We reanalyzed our data with a TB response cutoff of 1.0 IU/ml (10). Assuming the short-term persistent positives truly have LTBI and the reversions do not, there would be a 70.0% reduction (363–109) in false-positives with a 52.2% increase (186 of 356) in false-negatives (see Figure E1 in the online supplement). Given that this approach improves QFT specificity at the cost of sensitivity, are we willing to accept missing over half of true-positives who potentially could progress to active TB? It has been shown that HCWs with QFT TB responses as low as 0.35–0.7 IU/ml can progress to active TB (12, 23).

Because TST screening practices have been effective in North American HCWs, another approach is to use TST conversion rates to derive cutoffs for IGRAs. We determined that the QFT cutoff would need to be increased by more than 10-fold to achieve a conversion rate equivalent to the historical TST rate. Although this approach improves specificity, our results are limited by the lack of concurrent TST data to determine whether the same individuals are identified as converters by both tests.

The high IGRA variability observed here and reported by others (10, 19) suggests the need to better characterize the sources of variability to (1) eliminate these sources when possible, (2) improve the standardization of the QFT assay, and (3) create a zone of uncertainty around the cutoff to account for QFT variability caused by uncontrollable sources. Sources of variability identified thus far include time to incubation (24), ELISA between-run variability (25), and manufacturing-related (26) and immunologic factors. One strength of this study is the standardized time to incubation, minimizing a known variation affecting reproducibility. However, future studies controlled for all sources of variability are needed to determine the extent to which QFT reproducibility can be improved. Only then can the question of whether QFT reproducibility is good enough for routine use be answered.

From a larger perspective, as the incidence of TB continues to decrease in North America, clinicians need to reevaluate the TB screening policy in HCWs; at the least, a policy of routinely screening only high-risk HCWs should be considered. If the

prevalence of LTBI acquisition in the hospital is low, the positive predictive value of the test for LTBI will also be extremely low, regardless of the test accuracy (27, 28). Furthermore, recent data have shown low rates (0.4–1.46 cases per 100 person-years) of progression to active TB in IGRA-positive individuals and converters (29, 30). Cost-effectiveness analysis as more outcome data emerge will be required to determine if serial QFT testing in HCWs in low-incidence settings is worthwhile in light of the low positive predictive value. In this context, it is noteworthy that the 2013 Canadian TB Standards explicitly discourages the use of serial IGRA testing in HCWs (31).

Strengths of our study include it being the largest cohort of HCWs undergoing routine serial QFT testing in North America. Additionally, we were able to describe the short-term variability of test results in a real-world setting. Limitations in this cohort include the lack of TB risk factors or treatment data that would help to interpret test results in the absence of a gold standard. Also, the intervals between tests were nonstandardized, so the threshold between testing intervals was chosen based on prior literature (4). A potential limitation with the analysis is the possibility of changing conversion rates over the 25-month study period suggesting changing laboratory practices. We examined this limitation by stratifying the data by 6-month blocks, finding consistent conversion rates.

In summary, as more institutions adopt serial QFT screening in the 18 million HCWs in the United States (5), it becomes increasingly important to understand test reproducibility and the optimal cutoff to distinguish new LTBI infection from test variability. Given the high rates of conversion with poor reproducibility seen in serial testing in HCWs, the current manufacturer recommended cutoff likely results in false-positive results and subsequent unnecessary diagnostics, medication toxicities, cost, and anxiety. These outcomes highlight (1) the need for caution in attributing a QFT conversion to true LTBI infection in low-risk individuals; (2) the need to reconsider, at an institutional level, QFT serial testing in low-risk HCWs guided by a cost-effectiveness analysis; and (3) the need for an urgent update of the existing CDC guidelines on IGRAs for HCW screening.

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