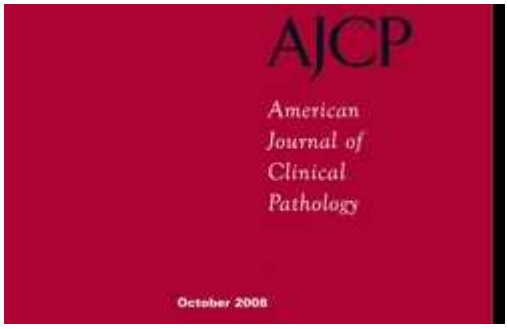

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Is the QuantiFERON-TB Blood Assay a Good Replacement for the Tuberculin Skin Test in Tuberculosis Screening?

A Pilot Study at Berkshire Medical Center

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Abstract and Introduction

Abstract

The QuantiFERON-TB Gold In-Tube method (QFT-GIT; Cellestis, Carnegie, Australia) is a recently US Food and Drug Administration–approved interferon-γ release assay (IGRA) for the detection of tuberculosis infection, which has been screened for by the tuberculin skin test (TST) for nearly a century. We report a pilot study comparing the QFT-GIT and TST results for screening health care workers (HCWs) at Berkshire Medical Center (BMC; Pittsfield, MA), the second hospital in Massachusetts to use QFT-GIT. For the study, 40 BMC HCWs, 20 TST+ and 20 TST−, were screened with the QFT-GIT test.

All 20 TST− subjects were also QFT-GIT−, while only 10 of 20 TST+ subjects were QFT-GIT+. The overall agreement between the QFT-GIT and TST results was 75% (κ = 0.5; 95% confidence interval, 0.268–0.732). The suboptimal agreement was partially due to a higher specificity of QFT-GIT. Confounding factors (eg, bacille Calmette-Guérin vaccination status and birthplace) are discussed, and literature regarding IGRAs and their comparison with TST is reviewed.

Introduction

Tuberculosis (TB) is caused by the intracellular pathogen *Mycobacterium tuberculosis* (Mtb). Despite ongoing advances in medical prevention, detection, and treatment, the global prevalence of TB remains high, with an incidence at approximately 9.2 million cases in 2006.[1] TB is still the leading cause of death from a single infectious disease, recently accounting for approximately 1.7 million deaths per year worldwide. The current treatment strategy for active TB is not adequate for disease elimination, partially owing to latent TB infection (LTBI), which may not be identified initially but can develop into an active disease after a certain period of latency. It has been estimated that one third of the world population has LTBI. People with LTBI can serve as potential reservoirs for future acute infections if the host immune system is compromised due to aging, corticosteroid medications, and/or immunodeficiency conditions such as HIV infection. Therefore, identifying and treating people with LTBI is crucial for TB control, especially in low endemic geographic areas (eg, the United States) and high-risk populations (eg, HCWs).

HCWs are especially vulnerable to TB exposure and infection in developing and developed countries.[1] Therefore, TB screening of HCWs or any person with a potential for exposure to people with active TB is critical for infection control. The purpose of screening programs has been widened from detecting early active TB to identifying LTBI and providing subsequent preventive chemotherapy.[2]

The tuberculin skin test (TST) has been used for TB screening for nearly a century, since first described by Mantoux in 1912. Until recently, it was the only available diagnostic method to screen for LTBI. Although traditionally widely accepted, TST is not an ideal diagnostic tool. Because purified protein derivative (PPD) used in the TST is a mixture of more than 200 Mtb antigens, most of which are highly homologous to the antigens in bacillus Calmette-Guérin (BCG) vaccines and non–tuberculosis mycobacterium (NTM) species, TST response has been known to be affected by prior BCG vaccination and/or NTM infection.

To overcome the relatively low specificity associated with TST, antigens encoded in the region of difference (RD1) of the Mtb genome were studied to develop T lymphocyte–based interferon (IFN)-γ release assays (IGRAs), which became commercially available in 2005. With superior specificity and better correlation with the exposure gradient, IGRAs could be a good supplement or even a total replacement of the TST in some circumstances.[3]

IGRAs measure the cellular immune responses to a few Mtb-specific antigens, including early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) of RD1, in comparison with the mixed nonspecific antigens used in TST.[3] Two IGRA systems using RD1-encoded antigens are currently commercially available for TB detection. One system includes QuantiFERON-TB Gold (QFT-G) and its variant QuantiFERON-TB Gold In-Tube (QFT-GIT) (Cellestis, Carnegie, Australia), which uses whole blood specimens to measure IFN-γ released by antigen-activated T lymphocytes. In those QFT assays, enzyme-linked immunosorbent assay (ELISA) is used to measure the concentration of IFN-γ released into plasma supernatant. The other system, T-SPOT. TB (Oxford Immunotec, Oxford, England), uses the ELISpot method to measure INF-γ-secreting T cell counts ("spots") on stimulation by Mtb-specific antigens in microplate wells.

From the approval by the US Food and Drug Administration (FDA) of QFT-G in May 2005, the US Centers for Disease Control and Prevention recommend that "QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of recent immigrants, and sequential testing" with warnings and limitations.[4] In 2007, the FDA approved the QFT-GIT assay, which is assumed to be even more specific, with the addition of TB7.7 as the third antigen, and more efficient, with the coating of stimulating antigens in the blood collection tube. In 2008, the FDA approved the premarket application for T-SPOT.TB.

In July 2008, the Department of Occupational Health at Berkshire Medical Center (BMC; Pittsfield, MA) adopted QFT-GIT as a screening test for LTBI in BMC employees. That was the result of an intense collaboration among the BMC Department of Occupational Health, the Infection Control Committee, and the Department of Pathology and Laboratory Medicine. To the best of our knowledge, BMC is the second hospital in Massachusetts to use an IGRA as a single screening assay for TB infection in HCWs. Whenever a new method or test is introduced by a laboratory, the laboratory is required by the College of American Pathologists to perform a verification process in which the new method or test is compared with the standard method or test used for the same purpose. We present the results of the laboratory verification process comparing the new method (QFT-GIT) with the conventional one (TST) in screening HCWs at BMC for LTBI.

Materials and Methods

Study Design and Subjects

To evaluate the accessibility and effectiveness of QFT-GIT as an alternative to TST in the screening for LTBI, a small-scale, cross-sectional study was conducted from March to May 2008 at BMC. It was a pilot study before a final decision was made to implement QFT-GIT as a single TB screening test. This study, part of the laboratory's verification process, was designed to recruit 40 (20 historically known to be TST+ and 20 TST−) BMC HCWs, including laboratory staff, nurses, and attending physicians, on a voluntary basis to be tested with QFT-GIT. Exclusion criteria were age younger than 18 years, pregnancy, recent exposure to Mtb, and immunocompromised status.

QFT-GIT Blood Assay

In total 3 mL of whole blood was collected from each subject, with 1 mL directly collected into each of 3 tubes in the strict order of gray- (negative control, "nil"), red- (test tube), and purple-cap (positive control; mitogen-coated) tubes. The test tube is a specifically designed blood collection tube coated with Mtb-specific antigens (ESAT-6, CFP-10, and a portion of TB 7.7 [p4]). All 3 tubes were tested at the same time. They were incubated upright at 37℃ for 16 to 24 hours before centrifugation at 3, 000*g* for 15 minutes. The INF-γ release was measured by ELISA according to the manufacturer's protocol, with an 8-point standard curve set up for each microplate. The results were read at 450 nm by a BioTek ELx800 Absorbance Microplate Reader (BioTek Instruments, Winooski, VT). All assays performed met the manufacturer's quality control standards. A positive result was defined as a difference in the IFN-γ levels between the test tube and negative control equal to or greater than 0.35 IU/mL and no less than 25% of the nil value. Laboratory staff conducting the QFT-GIT assays were blinded to the subjects' TST status.

Statistical Analysis

For each subject, historic TST status was recorded as positive or negative. Each QFT-GIT assay was also recorded as positive or negative based on the IFN-γ concentration cutoff value of 0.35 IU/mL. The overall results of this study were expressed as percentages for qualitative variables. Agreement between the tests was quantified by using κ statistics.

Results

TST Status, BCG Vaccination Status, and Other Biographic Data

Historic TST status was determined for each subject based on in-house medical records, accompanied by a standard TB screening questionnaire. BCG status was recorded based on records of vaccination or memory recall, confirmed by examining the presence of deltoid scarring. Other biographic data such as age, sex, birthplace/immigration origin, and work history were collected to the maximum possible level. Owing to strict laboratory adherence to the confidentiality of the collected data, biographic information was not available for all subjects at the time of data analysis, making further stratification of the subject population impossible at the current stage.

QFT-GIT Results

According to the result interpretation described in the "Materials and Methods," 10 (25%) of 40 subjects had positive results. All were from the TST+ group.

Agreement between the TST Status and QFT-GIT Results

Among the 20 TST+ subjects, 10 had a positive QFT-GIT result (categorical agreement, 50%), whereas all 20 TST− participants showed negative QFT-GIT results (categorical agreement, 100%). The overall percentage agreement was 75%. The overall κ value was 0.5, with a 95% confidence interval between 0.268 and 0.732 (Table 1).

Table 1. Agreement Between TST Status and QFT-GIT Results

	QFT-GIT+	QFT-GIT−	Agreement (%)
TST+	10	10	50
TST−	0	20	100
Overall	10	30	75

QFT-GIT, QuantiFERON-TB Gold In-Tube; TST, tuberculin skin test.
*κ = 0.5 (95% confidence interval, 0.268–0.732).

Discussion

Observed Discordance between the QFT-GIT and TST Results

This study supported the finding in several previous studies that the percentage of concordant results between IGRAs (including QFT-GIT in our case) and TST was not high (75% in this study). The Cohen κ coefficient, which is assumed to test whether observed concordance exceeds pure chance, was used to further assess the interrater agreement between QFT-GIT and TST. The κ value at 0.5 indicated moderate agreement. Caution is needed, however, in the interpretation of the κ statistic because it is not a strict chance-corrected measurement of agreement, especially in this study in which the size of subject population (N = 40) was not large and the sampling (recruitment of subjects) was not strictly randomized. Previous studies by other researchers showed that the concordance between QFT-GIT and TST ranged from 53% to 94% in immunocompetent adults who were screened for LTBI or evaluated in contact investigations.[3,5]

The discordance between IGRA and TST results has been frequently observed. In a meta-analysis of studies in healthy populations with varying risk for LTBI, discordant results were found in 21% (T-SPOT.TB by ELISpot) or 29% (QFT by ELISA) of participants.[6] Among the discordant results, including those from this study, the majority showed the TST-positive and IGRA-negative (TST+/IGRA−) combination.

Difference in Test Specificities as a Possible Cause of Result Discordance

Theoretically, a lower rate of positivity in the IGRA (eg, QFT-GIT in our study) could be explained by a higher specificity of IGRA than TST. Historic data showed that, at most, 1 of 10 people with positive TST results would eventually have active TB. In a German contact-tracing study, only subjects with a positive IGRA result had disease that progressed toward active TB, whereas none of TST+/IGRA− subjects developed TB during the 2 years following close contact with active TB.[7] The low specificity of TST is associated with a high number-needed-to-treat value, ie, many TST+ people must receive chemical prophylaxis to prevent 1 new case of active disease.

To evaluate a new screening tool like the QFT-GIT, multiple possible confounding factors need to be considered. Such factors usually include the biographic constitution of the subject population, eg, age, sex, work and medical history, among which the BCG vaccination history has been considered a major factor to affect the specificity of TB screening tests. In a recent meta-analysis, for people at very low risk for LTBI, the specificity values of both QFT-G and QFT-GIT assays were 0.99 among nonvaccinated people and 0.96 among BCG-vaccinated people, whereas the pooled specificity of the T-SPOT.TB was 0.93. In comparison, the pooled specificity of TST was 0.97 among nonvaccinated people but only 0.59 among people who had received the BCG vaccination.[8]

The superior specificity of IGRA over TST could come from the intrinsic difference in the methods of the 2 tests. IGRAs use antigens (ESAT-6 and CFP-10 of the RD1) that are highly specific to Mtb and to only a limited number of NTM, such as *Mycobacterium kansasii, Mycobacterium szulgai,* and *Mycobacterium marinum*, in contrast with the tuberculin used in TST, which represents a mixture of more than 200 nonspecific antigens shared with NTM and with the strains developed from *Mycobacterium bovis* used for BCG vaccination.[9]

Other confounding factors, such as birthplace, could also be a risk factor for TST+/IGRA− discordance. In a US Navy study, it was observed that NTM (eg, *Mycobacterium avium*) infection was 5 times more frequent in foreign-born recruits.[6] It is currently well accepted that most of the TST+/QFT− discordant results can be explained by BCG vaccination or birth in a foreign country, which might, in turn, be associated with a BCG vaccination history and/or indicate NTM infection. In a pooled analysis of 2 recent studies conducted in Germany, being foreign-born and BCG-vaccinated explained 95.7% of the TST+/QFT− results, whereas 85.5% of all TST+/QFT− results in BCG-vaccinated participants could be attributable to the vaccination itself.[10]

BCG vaccination and being born in a country with high TB incidence can be strongly correlated.[11] Most BCG-vaccinated people received the vaccine while residing in a country with high TB prevalence, making it rather difficult to determine whether the positive TST result is due to a real infection or to the BCG vaccination. The TB incidence in the United States peaked in 1992 and then began to decline. More than 50% of the cases of active TB in the United States occur in foreign-born people, most of whom have received the BCG vaccine. In addition, a fairly high percentage of HCWs in the United States are foreign-born. Thus, a more specific screening test like an IGRA is desirable to reduce the number of false-positive results in case finding, contact investigation, and mandatory HCW and new employee screening programs.

It might be worth noting that the interval between the BCG vaccination and the TST test could significantly affect test specificity[12] because BCG may have only a minimal effect on TST results several years after the vaccination, especially if it is administered only during infancy. In a meta-analysis conducted in 2006, it was estimated that depending on the interval between vaccination and testing (fewer or more than 10 years), BCG vaccination accounted for about 21% to 41% of positive TST results (diameter >10 mm) in people vaccinated after the first birthday. For people who receive BCG in primary school or during adolescence, about 15% to 25% would have positive TST reactions up to 20 years later.[13] This finding was supported by other similar studies. For example, in rural India where the BCG vaccination is provided only for newborns and the prevalence of TB is high, the TST and IGRA results showed strong agreement.[3] In comparison, in Germany where BCG vaccination was only performed in newborns but the TB prevalence is low, high discordance was observed.[10] In Japan where the BCG vaccination is widely applied and repeated, the TST and IGRA results had poor agreement.[14]

Difference in Test Sensitivities as a Possible Cause of Result Discordance

Theoretically, it is also possible that the TST+/IGRA− discordance is due to a lower sensitivity of QFT than of TST, especially considering that the ESAT-6 and CFP-10 antigens used in IGRAs do not represent the whole spectrum of Mtb antigenicity. In addition, the IGRAs might reflect more recent, rather than remote, TB infections because once the antigen is cleared, the activated memory T lymphocytes that produce IFN-γ persist for a limited time in the peripheral circulation.[15] Thus, in cases of remote infection, the plasma IFN-γ level might not increase significantly within the short period of antigen stimulation in the ex vivo IGRA.

The sensitivity of IGRAs has been examined in several studies. Until now, it seems that both IGRA systems are at least as sensitive as TST, if not more. In a meta-analysis on patients with active TB infection, sensitivities were 0.78 for QFT-G, 0.70 for QFT-GIT, and 0.90 for T-SPOT.TB.[8] The pooled sensitivity of TST was 0.77, not significantly different from that for QFTs for active TB. Sensitivity of the newer ELISpot+ for active TB is even higher.[16] Higher sensitivity is associated with a higher negative predictive value, ie, it is more reliable to use a negative result to rule out the disease.

In addition to detecting active infection, the IGRAs also showed good sensitivity in screening for LTBI, partially indicated by the high progression rate in IGRA+ people. In a study conducted in Germany with a 2-year follow-up, the progression rate for the TST+ group (N = 219) was 2.3%, whereas it was 14.6% for the QFT-GIT+ group (N = 41).[7] Another study conducted in Turkey showed that T-SPOT.TB positivity predicted a much lower number of people needed to be treated to prevent a new case (ie, lower number-needed-to-treat value), allowing more focused and efficient prevention.[17]

For LTBI in people at a higher risk for progression to active disease, IGRAs have demonstrated even higher sensitivity than TST. In children younger than 2 years old and in immunocompromised adults (eg, HIV-infected or taking immunosuppressive medications), the risk of progression to full-blown TB is significantly higher than that (5% risk) of immunocompetent adults. In patients with CD4 cell counts lower than 100/μL, the test sensitivity was significantly decreased for TST but not for T-SPOT.TB.[18]

It is interesting to note that the observed sensitivity values of IGRAs and TST were not consistent across the evaluation studies. This may be at least partially explained by the variety in severity of TB cases examined or the background TB prevalence across countries. Anergy due to advanced disease, malnutrition, and HIV-associated immunosuppression may lower the sensitivity of IGRAs.

The Impact of IGRA Implementation on Clinical Laboratories

Because IGRAs are performed by medical technologists, the adoption of IGRAs would increase the importance of laboratories in TB control. The corresponding features of IGRAs, in turn, are facilitating their acceptance into clinical laboratories. With highly instrumented procedures, the interpretation of IGRA results is simple and objective: it is usually a yes-or-no answer for every subject based on 1 fixed diagnostic cutoff value. Accompanying analytic software programs perform all calculations. The assays have been claimed by the

manufacturers to be highly precise (reproducible). For example, the claimed intra-assay and interassay coefficients of variance are both less than 15% for the QFT-GIT.

IGRAs are, in general, more convenient than the TST for subjects and for the HCWs who run the tests. The QFT assays could be even easier than the T-SPOT.TB to be conducted in clinical laboratories because QFT assays use whole blood specimens with no need for the T-cell fractionation and counting steps in the T-SPOT.TB. The ELISA measurements for QFT assays could be performed with existing facilities common to most clinical laboratories. The QFT-GIT, in which reactions occur in the same tubes for blood collection, is currently considered the easiest to use.

There are, however, some specific technical details that a laboratory manager and/or operator may want to consider. Blood specimens need to be delivered to the laboratory within a relatively short time after being obtained because both IGRA systems require viable T lymphocytes. Delivery time is 8 hours for the T-SPOT.TB, 12 hours for the QFT-G, and 16 hours for the QFT-GIT (which is currently used at BMC). In addition, the tubes used in the QFT-GIT contain antigens in dry form, thus requiring adequate shaking to dissolve the antigens once the blood is collected. Such manual vortex of the blood collection tubes before overnight incubation can help significantly reduce the number of indeterminate QFT-GIT results.[19] However, it could be counterintuitive to phlebotomists to vigorously shake the tubes, thus introducing possible variability in processing by handlers.

Regarding the test cost for laboratories administering the QFT-GIT, a few factors merit consideration. University laboratories, nonprofit hospitals, long-term valued customers, and wholesale buyers may acquire discounts from the test kit distributors, thus reducing purchase costs. In the use of the test kits, because tests are run on 8-well strips, each microplate holder accommodates no more than 12 strips, and each run needs an 8-point (1-strip) standard curve, batched specimens would share the same standard curve and, thus, reduce the cost per run. On the other hand, a larger batch is not always associated with a lower cost per patient. Because 3 wells are used for each patient (1 well for each tube: test negative and positive controls), if specimens are not batched to a multiple of 8, some wells in the last strip of each run will be wasted rather than all used. Table 2 shows the cost per run and per patient, given certain numbers of patient specimens per run. Some laboratories are willing to try a 4-point (half-strip), rather than 8-point, standard curve to further decease the cost per run; this application, however, is not encouraged per the Clinical Laboratory Improvement Amendments. Compared with the 8-point standard curve, a 4-point curve would be less robust to "outlier" data points and, thus, less reliably represent the "true" reference curve. In addition, a 4-point standard curve may, but does not necessarily, reduce the cost, if considering the aforementioned to-be-wasted wells in the last test strip. Our laboratory would like to continue using the 8-point standard curve in all of our QFT-GIT assays, unless suggested otherwise by official inspection organizations.

Table 2. Laboratory Cost of QFT-GIT Assays per Test Run and per Patient (Order) Based on the Number of Patients per Run*

No. of Patients per Test Run	No. of Wells per Run		No. of 8-Well Strips Used per Run	No. of Patients "Testable" per Kit (72 Strips)	Cost per Run ($)	Cost per Patient ($)
	Used	Wasted				
1	11	5	2	36	113.89	113.89
2	14	2	2	72	113.89	56.94
3	17	7	3	72	170.83	56.94
4	20	4	3	96	170.83	42.71
5	23	1	3	120	170.83	34.17
6	26	6	4	108	227.78	37.96
7	29	3	4	126	227.78	32.54
8	32	0	4	144	227.78	28.47
9	35	5	5	126	292.86	32.54

10	38	2	5	140	292.86	29.29
11	41	7	6	132	341.67	31.06
12	44	4	6	144	341.67	28.47
13	47	1	6	156	341.67	26.28
14	50	6	7	140	410.00	29.29
15	53	3	7	150	410.00	27.33
16	56	0	7	160	410.00	25.63
17	59	5	8	153	455.56	26.80
18	62	2	8	162	455.56	25.31
19	65	7	9	152	512.50	26.97
20	68	4	9	160	512.50	25.63
21	71	1	9	168	512.50	24.40
22	74	6	10	154	585.71	26.62
23	77	3	10	161	585.71	25.47
24	80	0	10	168	585.71	24.40
25	83	5	11	150	683.33	27.33
26	86	2	11	156	683.33	26.28
27	89	7	12	162	683.33	25.31
28	92	4	12	168	683.33	24.40
29	95	1	12	174	683.33	23.56

QFT-GIT, QuantiFERON-TB Gold In-Tube.
*For each test run, regardless of the number of patients, an 8-well strip is used to establish the 8-point standard curve. For each patient, 3 wells are used, 1 well for each tube: negative control (nil), test (Mtb antigens), and positive control (mitogen). Calculations are based on the inventory information at the authors' institute: QFT-GIT kit, $4,100 each, including 600 tubes and six 96-well microplates, each of which can be separated into twelve 8-well strips.

The Application of IGRAs in TB Screening for the General Population

As discussed, IGRAs have demonstrated superior specificity to that of TST. They are minimally affected by BCG vaccination and nearly all NTM. IGRAs also have high sensitivity, which is a preferable feature for a screening test. They are laboratory-based tests with established parallel positive and negative controls for normalization. In contrast with the reading of TST results by various clinicians, the result reading of IGRAs is instrumented and, thus, objective, minimizing subjective interpretation and operator bias.

From the patient's viewpoint, IGRAs are more convenient, requiring only a single visit for a blood draw, compared with the mandatory 2 visits within a narrow time window (48–72 hours) for TST and 4 visits if a 2-step TST protocol is followed (per recommendation for TB screening in health care settings). IGRAs are also rapid, with results usually available within 24 hours. Because they are completed in vitro instead of in vivo as in TST, IGRAs are claimed to be safe with no risk of allergic reactions in hypersensitive people.

However, one of the common concerns regarding IGRAs is their cost. In regard to the initial expenditure on reagents, the assays are several times more expensive than TST, especially in the case of the T-SPOT.TB. Quite a few health economic analyses, however, have shown that IGRAs are more cost-effective in the medium term. Unlike IGRAs that are primarily performed in laboratories, TST is administered and read by nurse practitioners, who are compensated more highly than technologists. The improved sensitivity of IGRAs in vulnerable populations

can better identify people with LTBI, which is, if progressed to active TB, associated with potentially more expense for treatment.

Furthermore, the higher specificity of the IGRAs discussed herein helps minimize unnecessary treatment in false-positive cases, particularly for BCG-vaccinated people, and, thus, avoid extra cost and side effects (eg, hepatotoxicity) from such treatment. The more false-positive cases that IGRAs help tease out, the more the cost reduction for the whole package. At BMC, each TST costs approximately $30, taking into account the expense of PPD reagents and necessary laboratory supplies, plus the time consumption and hourly pay of the front desk staff (case registration and tracking) and nurse practitioners (test administration and reading); the other expense on the patient side (eg, the gasoline costs and excuse-from-work time for 2 visits) and the unreimbursed cost on the "lost" cases ("no shows" in the second visit) are not included in this calculation. If the TST reading is positive, the subsequent cost of the chest radiographic examination, the TB clinic follow-up visits, and the isoniazid and vitamin B_6 for a 9-month treatment course approximates $600, which makes the total cost approaching $630 per TST+ case. In comparison, each QFT-GIT assay at BMC costs approximately $100, which includes the expense of the test kit components and supplementary laboratory supplies and the prorated payment for the laboratory staff work.

In a simplified calculation of the cost comparison among different TB screening methods for the general population, let us assume that the TST and QFT-GIT identify all true-positive TB cases, whereas only the TST shows false-positives. Thus, the total cost for screening the population is determined by the size of the population (P), the percentage of false-positive TSTs (F), and the percentage of true-positives (Tr). If we apply the preceding approximates ($30 per TST, $100 per QFT-GIT, and $600 for treatment and follow-up), the total cost of each TB screening method in a given population would be as follows:

TST only: Cost = [30 + 600 (Tr + F)] * P
QFT-GIT only: Cost = (100 + 600Tr) * P
TST followed by QFT-GIT confirmation of TST+ cases:
Cost = [30 + 100 (Tr + F) + 600Tr] * P

Table 3 shows the cost-per-case comparison among the 3 strategies, given different percentages of true- and false-positives. As expected, TST would be the most cost-efficient method when F (false-positive) approaches 0; the higher the F value, the more prominent the economic advantage of IGRA.

Table 3. Cost-per-Case Comparison Among Different Methods for Tuberculosis Screening*

True-Positive Results (%)	False-Positive TST Results (%)†																				
	0			5			10			15			20			25			30		
	T	Q	T-Q	T	Q	T-Q	T	Q	T-Q	T	Q	T-Q	T	Q	T-Q	T	Q	T-Q	T	Q	T-Q
0	30	100	30	60	100	35	90	100	40	120	100	45	150	100	50	180	100	55	210	100	60
5	60	130	65	90	130	70	120	130	75	150	130	80	180	130	85	210	130	90	240	130	95
10	90	160	100	120	160	105	150	160	110	180	160	115	210	160	120	240	160	125	270	160	130
15	120	190	135	150	190	140	180	190	145	210	190	150	240	190	155	270	190	160	300	190	165
20	150	220	170	180	220	175	210	220	180	240	220	185	270	220	190	300	220	195	330	220	200

Q, QuantiFERON-TB Gold In-Tube assay; T, tuberculin skin test; T-Q, T followed by Q confirmation of T+ results.
* At the authors' institute, the costs of each tuberculosis (TB) screening case approximate as follows: $30 for T, $100 for Q, and $600 for 9-month treatment and follow-up of each positive case. In a simplified calculation, it is assumed that both T and Q identified all true-positive TB cases, while only T shows false-positives. Data are given

in US dollars.
†Associated with bacillus Calmette-Guérin vaccination history, etc.
‡Prevalence under the preceding assumption.

Application of IGRA in TB Screening of HCWs

Mandatory TB screening of employees is based on the work by Heimbeck in Norway in the late 1920s, in which all initially TST− nursing students eventually became infected during their 3 years of training. This further led to the mandate that all HCWs who have the potential for close contact with patients be annually screened for TB. TST screening has had a critical role not only in identifying HCWs who contract TB from patients but also in preventing the transmission of TB from infected HCWs to hospitalized patients.

The low specificity and associated low positive predictive value of TST results, however, have been well appreciated by HCWs and significantly interfere with their decisions about preventive care and treatments. For example, the Cleveland Clinic (Cleveland, OH) hires about 2,500 HCWs annually, of whom approximately 80% are foreign-born and 78% have a history of BCG vaccination (60% as infants). A survey in the summer of 2007 showed that nearly none of the HCWs believed that they were at risk of developing TB and 68% believed that BCG accounted for their positive TST results, with only 11% TST+ HCWs agreeing to undertake the chemotherapy. In comparison, 53% of the HCWs who later tested positive for LTBI according to on-site QFT assays agreed to take the medications. This finding suggested that the implementation of IGRAs in lieu of TSTs to identify TB infection in HCWs at the time of employment would increase their acceptance of preventive treatment for LTBI.

Nowadays, an important subset of the HCW population in the United States is foreign-born, many coming from countries with high prevalence of TB. That fact introduces the dilemma when interpreting the positive TB screening test results in those HCWs. Some of the HCWs will have true TB infection (LTBI or active TB) because of a high TB prevalence in their birthplaces, whereas others may have false-positive TST results due to BCG vaccination. More than 60% of the HCWs who would be candidates for preventive chemotherapy for LTBI according to positive TST results eventually showed a negative IGRA result.[20] Using the more specific IGRA instead of TST would save HCWs and other TB contacts from unnecessary follow-up and potential adverse effects of chemotherapy.

While IGRAs are being incorporated into many public health programs, hospitals also are adopting them in employee screening programs. They are especially beneficial for institutions in which many HCWs are immigrants from endemic areas. For occupational health departments, IGRAs may seem to be too expensive at first glance. However, taking into account the expense of follow-up examinations owing to false-positive TST results, particularly if radiology is included in the TB workup, the overall cost associated with IGRA screening is fairly acceptable. Furthermore, the new employees would not start work until they are "cleared" from the mandatory TB screening, thus the more efficient IGRAs are desirable.

Since July 2008, the BMC Department of Occupational Health has used QFT-GIT for LTBI screening in new employee preplacement physical examinations, cooperating with the BMC Department of Pathology and Laboratory Medicine. To the best of our knowledge, this makes BMC the second hospital in Massachusetts to use IGRA for LTBI screening. Taking into consideration the time frame of optimal specimen processing for QFT-GIT assays and the usual need to batch specimens, our laboratory offers the QFT-GIT every 3 business days. As our practice continues to progress, more data on the advantages and limitations of QFT-GIT application in our community setting will be available for more comprehensive analysis.

Current Limitations and Concerns Associated with IGRAs

Like other diagnostic tools, IGRA is not perfect, and there is always room for improvement. It is generally considered that IGRAs, like TSTs, do not discriminate between active TB infections and LTBI. The reproducibility of IGRAs is also uncertain, although it is claimed to be superior by the manufacturers. The rather dynamic feature of IGRAs increases the likelihood of conversions and reversions over time. In contrast with TST, in which a definition of conversion is available (eg, an increase in diameter of 10 mm in the United States and 6 mm in

Canada), there is no validated definition of the rate for IGRAs. Furthermore, IGRA data on high-risk populations, such as children and immunocompromised persons, are limited.

Currently, a big source of concern is the use of IGRAs in serial testing after TST. Some recent guidelines for the diagnosis of LTBI recommend a 2-step process using TST followed by an IGRA (eg, QFT-GIT) in TST+ cases.[21] However, because the ESAT-6 and CFP-10 antigens used in IGRAs are also contained in the tuberculin PPD, prior exposure to the tuberculin in TST has the potential to boost the IGRAs. Although no significant boosting effect was observed for the T-SPOT.TB,[22] application of the TST within a certain period before subsequent QFT-G tests resulted in an increase in the IFN-γ levels, and, in some cases, it even converted previous negative QFT-G results to positive in the absence of new infection.[20] The maximum boosting effects of the TST are found to be within an interval of 1 to 5 weeks. Boosting effects are much less frequent if the interval is only 48 hours or more than 60 days, although it has been detected more than 1 year after the first negative TST result. As described earlier, the most recently developed QFT-GIT contains a third Mtb antigen, TB7.7, that is absent in PPD. QFT-GIT is thus assumed safer to be used following a TST. However, caution is needed to exclude a possible "leaky" boosting effect from a prior TST on any IGRA.

Our study was designed to be a small-scale cross-sectional pilot study before the formal decision was made to use QFT-GIT as a single TB screening test at BMC. Owing to the design and objective limitations, a few facts of this study need to be taken into account before any definitive conclusion can be drawn and applied to our clinical practice. First, more subjects need to be included to better represent our local population. The relatively small number of study subjects and non-randomized recruitment raised our concern of selection bias. Biographic information was not available from all subjects, making it impossible to further stratify the subject population at the current stage. More detailed information on subjects' medical and work histories, eg, age at BCG vaccination, BCG revaccination, and/or exposure to NTM, would be helpful for more extensive future studies.

Acknowledging the limitations, our results suggest that QFT-GIT has a superior specificity over TST and can be the test of choice in populations in which BCG vaccination status raises a concern of false-positive results in TB screening.

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