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QuantiFERON-TB Gold In-Tube test in active tuberculosis patients and healthy adults

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ABSTRACT

Interferon-gamma (IFN-γ) release assays have improved latent tuberculosis (TB) detection and have been considered promising for the diagnosis of TB disease. However, diagnosis efficacy data is limited in high burden countries. The aim of this study was to determine the diagnostic potential of the QuantiFERON-TB Gold In-Tube (QFT-GIT) test for the diagnosis of active TB in an endemic setting for TB. A cross-sectional study was conducted in a group of 102 Thai patients with clinical symptoms and chest x-ray findings suggesting of active pulmonary TB and a group of 112 healthy adults. Testing was carried out using sputum microscopy, mycobacterial culture and QFT-GIT test. Of these patients, QFT-GIT was positive in 73 (71.57%), negative in 27 (26.47%), and undetermined in 2 (1.96%) cases. Among healthy controls, QFT-GIT was positive in 18 (16.07%), negative in 93 (83.04%), and undetermined in 1 (0.89%) person. Based on TB culture results, the sensitivity of QFT-GIT for diagnosing active TB was 84.21% (95% confidence interval (CI); 72.13-92.52). The positive and negative predictive values were 65.75% (95% CI; 59.26-71.70) and 66.67% (95% CI; 49.94-80.04), respectively. The median IFN-γ level in culture-confirmed TB patients was 3.91 compared to 0.03 IU/mL of the healthy group. QFT-GIT appears to be a useful indirect test for TB diagnosis in Thailand and its use is recommended in association with clinical and radiological assessments for identifying active or latent TB.

KEYWORDS: Active tuberculosis. Latent tuberculosis. Diagnosis. QuantiFERON. Interferon-gamma release assay. IGRA

INTRODUCTION

Tuberculosis (TB) remains a major global health threat. Despite significant efforts to control the disease, mortality and incidence rates remain extremely high[1]. An essential factor to control the spread of TB is the ability to diagnose the disease at the early stages. Smear microscopy is generally available but it has poor sensitivity. At least, 5,000 bacilli per mL of sputum are required for a smear-positive result. In addition, a positive finding is not specific only to *Mycobacterium tuberculosis*. On the other hand, mycobacterial culture, the gold standard, usually takes several weeks until final results. It also requires specific laboratory equipment, technicians with additional skills and appropriate bio-safety conditions[2]. Therefore, sensitive tools complementing conventional tests are needed to guide the initiation of therapy when the diagnosis of TB disease remains doubtful.

Interferon-gamma release assays (IGRAs) are immunodiagnostics tools in which interferon-gamma (IFN-γ) released by T-cells in response to *Mycobacterium*

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tuberculosis-specific antigen is measured[3]. During the past decade, two commercial *in vitro* IGRAs, including the QuantiFERON-TB Gold In-Tube (QFT-GIT) test (Cellestis, Ltd., Victoria, Australia) and T-SPOT.*TB* (Oxford Immunotec, UK), have been introduced for the early detection of *M. tuberculosis* infection. The QFT-GIT test detects IFN-γ production after the stimulation of whole blood samples with specific *M. tuberculosis* antigens, Early Secretory Antigenic Target-6 (ESAT-6), Culture Filtrate Protein-10 (CFP-10) and TB7.7 (Rv2654). These antigens are absent from all Bacille Calmette-Guérin (BCG) strains, as well as most common non-tuberculous mycobacteria. The QFT-GIT has been reported to have higher sensitivity, specificity and be more attractive than the tuberculin skin test (TST) which may result in interpretation errors, including the need of two clinic visits. However, the QFT-GIT performance in detecting active or latent tuberculosis infection (LTBI) can vary depending on the tested populations and regions[4].

The QFT-GIT test has been largely employed to evaluate the diagnosis of *M. tuberculosis* infection in developed countries[4]. In recent years, the studies of QFT-GIT performance have become more documented in non-developed countries such as Iran, India, Zambia, Brazil, etc.[5,6]. Currently available data suggest that the QFT-GIT test is less influenced by prior BCG vaccination and environmental mycobacteria[7]. Therefore, the QFT-GIT test has been increasingly used in locations where the coverage of BCG vaccination is high. Numerous studies have assessed the utility of this test in diagnosing latent TB infection (LTBI) in various clinical settings[8]. Regarding active TB, the use of IGRAs has been unclear particularly in high prevalence settings. Although the limitations of QFT-GIT in diagnosing TB disease have been recognized, it continues to be recommended by some investigators and used to aid in the diagnosis of tuberculosis[9,10]. Thailand is a TB-endemic country with an estimated prevalence of 172 per 100,000 inhabitants[1]. National guidelines have not yet been established using IGRA for LTBI screening or the diagnosis of TB, while the TST is the test of choice for identifying TB infection. Currently, the QFT-GIT test is available and has been used for the diagnosis of LTBI in Thailand. Nevertheless, it has been used in clinical practice for the rapid confirmation of TB disease or for ruling out active TB in many suspicious cases, especially when there are insufficient or non-sputum samples. Collecting blood samples may not frequently encounter difficulties compared with other specimens collection and QFT-GIT can provide rapid results, within two days. Despite the high cost, QFT-GIT may be a useful additional test for TB diagnosis especially when reference test results are not available. However, data on the utility and the efficacy of QFT-GIT for diagnosing active TB are limited in this setting. Here, we assessed the diagnostic potential of the QFT-GIT assay for detecting TB infection among the active TB patients in Thailand, with healthy adults as the controls.

MATERIALS AND METHODS

Study subjects

This study was reviewed and approved by the Ethics Committee of the Ministry of Public Health, Thailand and was conducted from September 2013 through October 2015. The participants were clinically diagnosed with pulmonary tuberculosis (PTB) and there was a group of healthy subjects. Informed consent was obtained from all participants before the enrollment in this study. Data concerning primary demographic characteristics and sputum smear results were collected.

Eligible patients included presumptive active TB cases (PTB) with sputum smear positive or negative and aged over 15 years. The presence of active TB was defined as the presence of abnormal images suggestive of TB in the chest x-ray or clinical manifestations such as a persistent cough for more than two weeks, night sweats, prolonged fever, weight loss or hemoptysis, with or without a history of TB exposure. As the disease could not be confirmed by bacteriological culture at the time of recruitment, the physicians determined whether the patients suffered from TB based on clinical, microbiological and radiological findings. For patients considered as suspected cases of PTB, if the sputum culture was positive they were diagnosed as certain TB patients. Subjects were recruited to this study consecutively. To avoid misinterpretation due to an immune response modulation, any patient who had previously received anti-tuberculous treatment, steroids, anti-TNF compounds or any other medication that might reduce the cellular immunity was excluded from the study. Patients with self-reported disease or presenting symptoms of diseases that compromise the immune system such as HIV, diabetes or cancer were not eligible. Clinical examination and screening for other underlying diseases were performed following routine guidelines.

The healthy subject group included adults in good physical conditions aged over 15 years, with no apparent TB symptoms, abnormal chest X-ray findings, history of TB or exposure to the disease. Their contact with physicians consisted primarily of annual medical check-ups.

Specimen collection and mycobacteriology

As part of a routine procedure for TB diagnosis, three

sputum samples, the first sputum, second early morning and the third sputum were collected from the patients, with all subjects taken for radiological examination. The collected sputum specimens were stained for acid-fast bacilli (AFB) microscopy by Ziehl-Neelsen method, and decontaminated samples were cultured in Lowenstein-Jensen (LJ), a conventional medium for the isolation and identification of *M. tuberculosis*[11]. Blood samples were drawn from all recruited study subjects for QFT-GIT at the time of standard microscopic examination, after which the QFT-GIT assay was carried out.

QFT-GIT assay

The whole-blood assay of QFT-GIT was performed according to the manufacturer's instructions (Cellestis, Ltd., Victoria, Australia). Briefly, a total of 3 mL of peripheral venous blood was taken from each subject and immediately transferred to 3 QFT-GIT tubes of 1 mL each (Nil control-no antigen for negative control, phytohaemagglutinin or mitogen for positive control and TB-specific antigens). Then, the tubes were homogenized by inversion for ten times and were subsequently incubated for 16-24 h (targeting 16-18 h incubation time in our study) at 37 °C. Plasma samples were recovered after centrifugation and stored at 4 °C until assayed. QFT-GIT enzyme-linked immunosorbent assay (ELISA) was carried out to measure IFN-γ within 2 weeks after blood collection. Raw optical densities were interpreted using specific software supplied by the manufacturer (QuantiFERON-TB Gold analysis software version 2.50.4). The results obtained by the Nil control were subtracted from the mitogen control and the antigen-stimulated samples. The cut-off point for the diagnosis was set according to the manufacturer's instructions. In brief, if the IFN-γ secretion in response to TB antigens after subtracting Nil control IFN-γ was 0.35 IU/mL, it was considered positive. If the value was less than 0.35 IU/mL, it was considered negative. The result of the test was considered undetermined if an antigen-stimulated sample was negative and the value of the positive control was less than 0.5 IU/mL. Subjects with IFN-γ secretion over 8.0 IU/mL in the Nil control samples were also considered undetermined for QFT-GIT.

Statistical analysis

Numbers, frequencies, percentages and medians were used to present data. The association between QFT-GIT results of the two groups was determined by the Chi-square test or the Fisher's exact test using SAS® University Edition. A *p*-value less than 0.05 was considered a statistically significant difference. The diagnostic performance of the QFT-GIT test was assessed using sensitivity and positive/negative predictive values. Sensitivity was calculated without undetermined results.

RESULTS

Demographic and microbiological characteristics of study subjects

A total of 214 subjects were enrolled in the study. Of these participants, 112 were healthy adults and 102 were new clinically diagnosed PTB patients. The demographic profile of TB patients and healthy controls is shown in Table 1. Patients and healthy controls had a median age of 37 (range, 15-68 years) and 42 (range, 15-76), respectively, and they were not age-matched. Sixty-nine of 102 (67.65%) patients and 76 of 112 (67.86%) healthy adults were men. Sputum smear microscopy was positive in 53 of 102 (51.96%) patients, while smear was negative in 49 of 102 (48.04%). Culture for *M. tuberculosis* was positive in 59 of 102, therefore, the culture positive rate in this study was 57.84%. Based on culture confirmation for *M. tuberculosis*, TB cases were definitively identified in a total of 59 cases, of which 48 and 11 were AFB smear positive and negative, respectively (Table 2).

Table 1 - Demographic and MTB microbiological characteristics of study subjects

Variables	Number of subjects	
	PTB patients (n=102)	Healthy adults (n=112)
Gender		
Female	33 (32.35)	36 (32.14)
Male	69 (67.65)	76 (67.86)
Age (min-max), median	(15-68), 37	(15-76),42
15-25	19 (18.63)	20 (17.86)
26-35	30 (29.41)	23 (20.54)
36-45	21 (20.59)	32 (28.57)
46-60	27 (26.47)	29 (25.89)
61-76	4 (3.92)	8 (7.14)
Unknown	1 (0.98)	-
Sputum smear status		
Smear positive	53 (51.96)	-
Smear negative	49 (48.04)	-
MTB Culture status		
Culture positive	59 (57.84)	-
Culture negative	43 (42.16)	-

PTB, pulmonary tuberculosis; MTB, *Mycobacterium tuberculosis*

Table 2 - QuantiFERON-TB Gold In-Tube (QFT-GIT) results in PTB patients and healthy subjects

Subjects	Number of subjects	Positive (%)	Negative (%)	Indeterminate (%)	*p*-value[a]
Suspected PTB	102	73(71.57)	27 (26.47)	2 (1.96)	<0.0001
S+, C+	48	40 (83.33)	6 (12.5)	2 (4.17)	<0.0001
S+, C-	5	3 (60.0)	2 (40.0)	-	0.04[b]
S-, C+	11	8 (72.73)	3 (27.27)	-	0.0002[b]
S-, C-	38	22 (57.89)	16 (42.11)	-	<0.0001
Healthy adults	112	18 (16.07)	93 (83.04)	1 (0.89)	

[a]QFT-GIT results in patients clinically diagnosed having PTB compared to those of healthy controls using Chi-square or Fisher's exact[b] test. PTB, pulmonary tuberculosis; S, acid-fast bacilli smear result; C, culture result. The patient group was stratified based on results of sputum microscopy and mycobacterial culture

Performance of QFT-GIT for TB diagnosis

Overall, 73 (71.57%), 27 (26.47%) and 2 (1.96%) of the cases had positive, negative and undetermined QFT-GIT results, respectively compared with 18 (16.07%), 93 (83.04%) and 1 (0.89%) of the controls respectively, with *p*-values < 0.0001 for positive and negative results. In subgroup analysis, the positive rate of QFT-GIT for the diagnosis of active PTB in the group of smear-positive and culture-positive cases was 40 of 48 (83.33%). The positive rate for QFT-GIT in detecting smear-negative and culture-positive PTB patients was 8 of 11 or 72.73%. In culture-negative patients, positive QFT-GIT was 60% and 57.89% in the group of AFB positive and negative, respectively. The number of positive results for each group of PTB patients obtained by the IFN-γ assay is shown in Table 2. The difference(s) in the percentages of QFT-GIT results for the IFN-γ test in each group of patients compared with those of the controls were significant (*p*-values < 0.05). A undetermined or uninterpretable result was found among the group of active PTB patients in two cases due to the low immune response to mitogen antigen in a positive control tube. Of the 112 healthy subjects, 18 (16.07%) had an IFN-γ level over 0.35 IU/mL, indicating probable *M. tuberculosis* infection or LTBI. One healthy adult had an IFN-γ level over 0.35 IU/mL, but less than 25% of the Nil value. The interpretation of this result was QFT-GIT negative. A undetermined result caused by the low immune response was also found in only one subject among the healthy controls (0.89%). Retesting of these samples has not been performed and all three undetermined results were excluded from further analysis. There was a significant difference of positive QFT-GIT results between the TB patient group and the asymptomatic or non-TB, healthy group, *p*-value <0.05. The sensitivity and predictive values for the diagnosis of active TB were calculated by excluding two culture positive cases presenting undetermined results for QFT-GIT. Among the remaining 57 culture-positive patients, 48 had a QFT-GIT positive result. Therefore, the sensitivity of QFT-GIT in culture-confirmed TB cases was 84.21% (95% CI; 72.13-92.52), whereas the sensitivity in the patient group with AFB smear-positive, regardless of the culture result, was 84.31% (95% CI; 71.41-92.98) (Table 3). Further analysis based on clinical symptoms, the smear microscopy result and radiological evaluation indicated that the sensitivity of QFT-GIT for the detection of active TB disease was 73.0% (95% CI 63.20-81.39). The positive predictive values (PPV) and the negative predictive value (NPV) are detailed in Table 3.

Level of IFN-γ between patient and control groups

The levels of IFN-γ in response to TB specific antigens in PTB patients and healthy adults are shown in Figure 1. There was an overlapping of IFN-γ levels between the groups of active TB disease and asymptomatic infected or LTBI. Four of LTBI showed very high levels of IFN-γ, over 10.0 IU/mL, thus the level of IFN-γ cannot be used alone to distinguish active from latent TB. Apparently, the analysis of IFN-γ levels in PTB patients revealed that most active TB cases (83.56%) in this study were positive to QFT-GIT with IFN-γ levels over 1.0 IU/mL. In culture confirmed PTB, the median IFN-γ level was 3.91 compared to 0.03 IU/mL of the healthy group. Among the healthy group, the median IFN-γ levels in LTBI and in non-LTBI were 1.44 IU/mL, and 0.02 IU/mL, respectively. The median IFN-γ levels in patients with smear positive and culture positive (S+C+), smear positive and culture negative (S+C-), smear negative and culture positive (S-C+), including smear negative and culture negative (S-C-), were 3.24, 0.52, 4.67, 0.71 IU/mL, respectively.

DISCUSSION

The measurement of IFN-γ in antigen-stimulated blood samples by QFT-GIT has been proposed as an *in vitro*

Table 3 - Diagnostic performance of the QuantiFERON-TB Gold In-Tube (QFT-GIT) test in active tuberculosis patients compared with microbiological results and clinical TB diagnosis

Number of subjects (102[a])		MTB culture		%sensitivity (95% CI)	%PPD (95% CI)	%NPD (95% CI)
		Positive	Negative			
QFT-GIT	Positive	48	25	84.21 (72.13-92.52)	65.75 (59.26-71.70)	66.67 (49.94-80.04)
	Negative	9	18			
Number of subjects (102[b])		AFB smear				
		Positive	Negative			
QFT-GIT	Positive	43	30	84.31 (71.41-92.98)	58.90 (52.69-64.85)	70.37 (53.44-83.09)
	Negative	8	19			
Number of subjects (214[c])		Clinical Dx TB				
		Suspected	Healthy			
QFT-GIT	Positive	73	18	73.00 (63.20-81.39)	80.22 (72.33-86.29)	77.50 (71.18-82.77)
	Negative	27	93			

[a]2 from MTB culture positive were indeterminate by QFT-GIT; [b]2 from AFB smear positive were indeterminate by QFT-GIT; [c]2 from suspected tuberculosis cases and one from healthy adults were indeterminate by QFT-GIT. MTB, *Mycobacterium tuberculosis*; CI, confidential interval; PPD, positive predictive value; NPV, negative predictive value; AFB, acid-fast bacilli; Dx, diagnosis

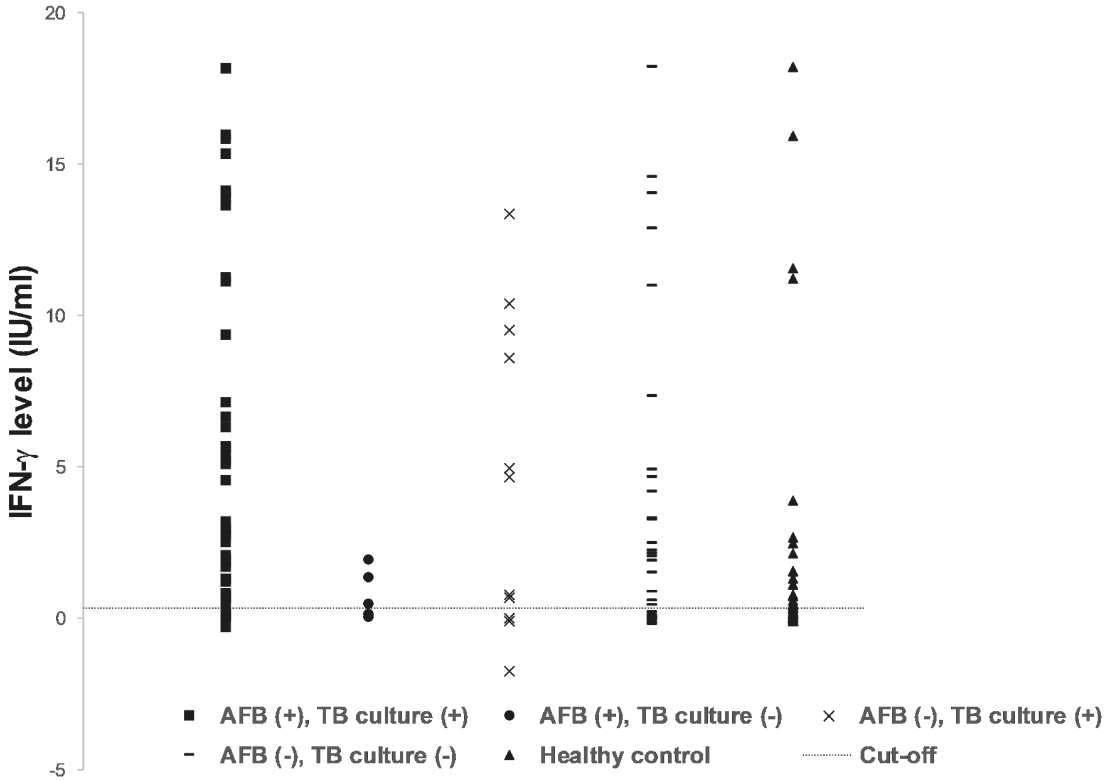

Figure 1 - Quantitative responses of the QuantiFERON-TB Gold In-Tube test in pulmonary tuberculosis patients in relation to acid-fast bacilli smear and culture results compared with the responses of healthy adults. Individual QFT-GIT results are plotted according to their diagnosis. The cut-off value for positive QFT-GIT is represented by the dotted line (0.35 IU/mL)

alternative method for latent TB diagnosis and has the potential to provide rapid and reliable results to diagnose active TB[12-14]. There are several published reports on the performance of the QFT-GIT test for TB diagnosis used in non-endemic countries[15] but few studies have been carried out in TB or TB-HIV endemic settings[16]. This study reported the use of the QFT-GIT assay in Thailand, where the burden of TB is high and BCG vaccination is commonly employed. In this study, the positive rate of QFT-GIT was 84.21% in culture-positive cases and 84.31% in AFB smear-

positive group, which was in the range described in some previous reports[17-20]. Previously published studies have also demonstrated the very high specificity of QFT-GIT, giving no reason to doubt the validity of QFT-GIT results for detecting MTB infection[21,22]. The high background of LTBI may result in poor specificity for active tuberculosis diagnosis in high-burden countries. In this study, we reported the percentage of LTBI to be 16.07%. It has been recommended that QFT-GIT could be used to rule out the suspicion of active TB disease among clinically suspected subjects[23]. In this condition, those suspected of having TB would show low levels of IFN-γ (< 0.35 IU/mL). It was proposed that QFT-GIT could be used as a confirmatory test for active TB disease at the moment of the clinical diagnosis, before culture results are available[17]. QFT-GIT is less influenced by prior BCG vaccination and environmental mycobacteria[3,7]. Therefore, high rates of BCG vaccination should not be a concern of use in this setting and elsewhere. QFT-GIT has considerably high sensitivity, comparable with or superior to that of TST. In addition, the sensitivity of QFT-GIT has been reported to be higher for detecting active TB disease in low endemic countries[24-26]. The positive rate of QFT-GIT in detecting active TB in Thailand reported in this study appeared to be similar to those of other countries. The slight differences in sensitivity found by our study and others may be explained by the difference in the number of analyzed patients and their individual characteristics. The false-negative results of QFT-GIT in active TB have been described previously[27-29]. Some hypotheses have been raised to explain the negative results in active TB patients. Immunocompromised patients with active TB at the beginning of the treatment were also reported and they were more likely to have negative QFT-GIT results. In immunocompetent patients with active TB but negative QFT-GIT, the explanation for this finding involves the release of anti-inflammatory cytokines by peripheral blood mononuclear cells and a temporary depression of the T-cell response. HIV is one of the major influencing factors for QFT-GIT diagnostic results[30]. Diabetes mellitus is another factor affecting the expression of Th1-related cytokines[31]. In addition, it was reported that QFT-GIT response could be diminished in cases of advanced TB[32]. Therefore, negative QFT-GIT test results should not be used alone to exclude active TB. For LTBI, as QFT-GIT is recommended for LTBI detection, a prevalence of LTBI should be further investigated and considered for appropriate management in endemic countries.

The limitations of this study included the small sample size, the lack of sociodemographic data, no molecular results to rapidly confirm the clinical diagnosis of tuberculosis and the absence of clinical information regarding the routine screening of other underlying diseases, as well as of the HIV status of study patients. We noted that the culture sensitivity in the present study was as low as 57.8%. The sensitivity of mycobacterial cultures can vary from 20-100%[33] and in a similar study, the sensitivity of MTB sputum culture was 60%[34]. Therefore, the culture sensitivity in our study was not out of this range. Another point that worths discussion is the role of culture as the gold standard, as it may sometimes result in false negative results due to poor sputum sample collection or paucibacillary sputum samples. Although the study had some limitations, the results demonstrated the usefulness of QFT-GIT when used in TB high-prevalence settings for diagnosis of *M. tuberculosis* infections. It is more useful in the case of smear-negative PTB patients and can help to rule out active pulmonary TB suspicions. Nevertheless, the QFT-GIT assay can result in false negative or undetermined results in active TB with low T-lymphocytes response. Therefore, QFT-GIT should not be used alone or as the final test for the identification of active TB patients. Culture results are still required for conclusive TB diagnosis. We have also shown that QFT-GIT could not discriminate active TB disease from latent TB infection alone. Undetermined results were found in a small number of patients in this study. It was reported that QFT-GIT undetermined results increase with age and are associated with the severity of other underlying diseases in patients with active TB disease. Because of the small number of elderly patients, the study had insufficient data to address this issue. For further studies, the testing in environmental mycobacteriosis and in HIV-infected patients in Thailand is proposed.

CONCLUSIONS

In general, there is concern about the usefulness of QFT-GIT for active TB diagnosis in TB-endemic countries. Based on our results, we conclude that QFT-GIT is useful as a complementary test for diagnosing active TB cases. It appeared to be useful for detecting latent tuberculosis in a high prevalence setting country as Thailand. IFN-γ levels could not distinguish between active and latent TB infection. Therefore, QFT-GIT cannot be used separately without considering clinical and radiological data for identifying active or latent TB. There is still much to learn on the QFT-IT assay and its performance in different clinical settings, meaning that the subject deserves further study.

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