Clin. Lab. 2026;72:XXX-XXX

ORIGINAL ARTICLE

Performance of a Fluorescence Immunoassay-Based Interferon-Gamma Release Assay for Latent Tuberculosis Infection

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ABSTRACT

Background: **Latent tuberculosis infection (LTBI) diagnosis is crucial for controlling tuberculosis (TB). Interferon-gamma release assay (IGRA) diagnoses LTBI by measuring interferon-gamma produced by TB antigen-stimulated T-cells. In this study, we evaluated the clinical performance of AFIAS IGRA-TB (Boditech Med, Inc.).**
Methods: **A total of 200 subjects, including those without known risk factors for TB infection, those with known TB contact, and those with active TB, were analyzed using both AFIAS IGRA-TB and QuantiFERON-TB Gold In-Tube (QFT-GIT; Qiagen) according to the manufacturers' instructions.**
Results: **The positive, negative, and overall percent agreements between AFIAS IGRA-TB and QFT-GIT were 88.2% (95% confidence interval [CI]: 76.6 - 94.5%), 95.4% (95% CI: 90.4 - 97.9%), and 93.4% (95% CI: 88.8 - 96.2%), respectively. Cohen's kappa coefficient was 0.84, indicating excellent agreement.**
Conclusions: **AFIAS IGRA-TB demonstrated equivalent clinical performance to QFT-GIT. Given its ease of use, minimal reliance on trained personnel or complex equipment, and rapid turnaround time, AFIAS IGRA-TB has the potential to serve as an alternative test for diagnosing LTBI and active TB in resource-limited settings.**
(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250937)

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KEYWORDS

latent tuberculosis, diagnosis, IGRA

Manuscript accepted September 22, 2025

INTRODUCTION

Tuberculosis (TB) is a major health threat worldwide. It is estimated that approximately 10.8 million patients suffered from TB disease and 1.25 million patients died due to TB disease in 2023 [1]. Once infected, most patients remain in a state of immunologic stimulation by the *Mycobacterium tuberculosis* antigen without showing signs of active TB disease, known as latent TB infection (LTBI), and 5 - 10% of these patients progress to the active TB disease through reactivation of *M. tuberculosis* during their lifetime [2,3]. The risk of reactivation is highest during the first two years, with approximately half (5% of the total infected population) progressing to active TB disease [4,5]. A recent study showed that one quarter of the world's population has LTBI [6,7].

Programmatic management of LTBI is an important part of the End TB Strategy led by the World Health Organization (WHO) [2]. The WHO has identified those who can benefit from TB preventive treatment, such as people living with HIV/AIDS, household contacts of active TB, patients initiating anti-TNF agents or preparing for transplantation, patients on dialysis, patients with silicosis, healthcare workers or prisoners [2, 8]. However, there is no gold standard test for LTBI diagnosis. The WHO endorses the tuberculin skin test (TST) and interferon-gamma release assay (IGRA) as tools for LTBI identification. However, the trend is shifting towards increased utilization of IGRAs, especially in high-risk populations and countries with widespread BCG vaccination. This shift is driven by IGRA's specificity in BCG-vaccinated individuals, comparable or potentially superior sensitivity and a better predictive value for progression to active TB [8].

In 2011, the WHO recommended IGRA for LTBI diagnosis, including QuantiFERON-Gold (Qiagen, Venlo, Netherlands), QuantiFERON-TB Gold In-Tube (QFT-GIT; Qiagen) and T-SPOT.TB (Oxford Immunotec, Abingdon, United Kingdom). As numerous new and alternative IGRAs have been developed, WHO Technical Advisory Group on TB Diagnostics and Laboratory Strengthening (TAG) examined these novel assays, adding QuantiFERON-TB Gold Plus (Qiagen) and Wantai TB-IGRA (Wantai BioPharm, Beijing, China) as valid IGRAs for LTBI diagnosis in 2022 [9]. The WHO TAG performance evaluation indices could be applied to evaluate the novel IGRA.

AFIAS IGRA-TB (Boditech Med, Inc., Chuncheon, Republic of Korea) is a newly developed IGRA for LTBI diagnosis using advanced fluorescence immunoassay (FIA). This assay uses early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) for the stimulation of TB-sensitized T-cells. By adopting FIA, this assay has a rapid analytical time with relatively simple methods, offering a promising point-of-care solution. However, to date, the clinical performance of AFIAS IGRA-TB remains unknown. This study aims to evaluate the clinical performance of AFIAS IGRA-TB by comparing it with the established IGRA, QFT-GIT.

MATERIALS AND METHODS

Subjects

In this cross-sectional study, the subjects were sequentially recruited between July 2023 and January 2024 at the National Institute of Tuberculosis and Respiratory Diseases (NITRD; New Delhi, India). The following subjects were excluded from the study: 1) patients currently on anti-TB or TB preventive therapy for more than two weeks, 2) immunocompromised patients such as patients with HIV/AIDS or those receiving anti-TNF therapy, and 3) patients aged younger than 18 years or older than 80 years. A total of 200 subjects were enrolled in the study. To assess the clinical performance of AFIAS IGRA-TB based on the risk of TB infection, subjects were further stratified into three groups: 1) Group 1: subjects without known risk factors for TB infection (88/200, 44.0%), 2) Group 2: subjects with known TB contact, including close contact (more than 15 minutes within 6-foot distance) and household contact (56/200, 28.0%) and 3) Group 3: active TB patients confirmed by acid-fast bacilli (AFB) smear (56/200, 28.0%). Given that NITRD is a tertiary healthcare facility focusing on TB and other respiratory diseases, most subjects in Group 1 reported nonspecific respiratory symptoms. QFT-GIT was positive in 51 subjects (25.5%) and 23 (26.1%) among total subjects and Group 1, respectively. The detailed characteristics of subjects are provided in Table 1. This study was approved by the Ethical Committee of National Institute of Tuberculosis and Respiratory Diseases (NITRD/EC/2023/649). Written informed consent was obtained from each participant prior to study enrollment.

Sample preparation and IGRA assays

One mL of whole blood was collected into three different tubes from AFIAS IGRA-TB tubes (Boditech Med, Inc.) and three tubes from QuantiFERON-TB Gold blood collection tubes in the following order: nil, TB antigen (TB Ag), and mitogen tubes. Each tube was incubated at 37℃ for 20 hours. After incubation, each tube was centrifuged at 3,000 x g for 15 minutes. IFN-γ was measured in the separated plasma using each assay platform according to the manufacturer's instructions. For the measurement using AFIAS IGRA-TB, 100 μL of plasma was transferred into the cartridge and loaded on the AFIAS 6 analyzer (Boditech Med, Inc.). For the measurement using QFT-GIT, 50 μL of plasma and standards were transferred into each microplate well with enzyme-conjugated solution and incubated for two hours. After incubation, each well was washed six times. Following the washing, an enzyme substrate solution was added to each well and incubated for an additional 30 minutes. After incubation, a stopping solution was added, and the microplate was loaded onto the

Alere Easy Reader (Abbott Laboratories, Chicago, IL, United States). For interpretation of IGRA assays, manufacturer-provided criteria were adopted. Both AFIAS IGRA-TB and QFT-GIT used the same criteria: 1) positive: if TB Ag - nil ≥ 0.35 IU/mL and ≥ 25% of nil, 2) negative: if TB Ag - nil ≥ 0.35 IU/mL and < 25% of nil, or TB Ag - nil < 0.35 IU/mL, and 3) indeterminate: if nil > 8.0 or mitogen - nil < 0.5 IU/mL and not meeting positive criteria.

Clinical performance evaluation

The clinical performance of AFIAS IGRA-TB was evaluated against QFT-GIT in terms of positive, negative, and overall percent agreements, as well as Cohen's kappa coefficients. These performance metrics were calculated for both the entire study population and the subgroups of subjects. Indeterminate results from either AFIAS IGRA-TB or QFT-GIT were excluded from the agreement analysis. For reproducibility evaluation, the AFIAS IGRA-TB was repeated using three different test lots with the same samples.

Statistical analysis

The χ^2 test and Kruskal-Wallis test were performed to compare baseline characteristics among groups. For quantitative analysis, Spearman's correlation, Deming regression, and Bland-Altman analysis were performed to compare between AFIAS IGRA-TB and QFT-GIT. Statistical analysis was performed using R version 4.4.2.

RESULTS

Qualitative comparison of AFIAS IGRA-TB and QFT-GIT

Both AFIAS IGRA-TB and QFT-GIT exhibited low indeterminate rates (AFIAS IGRA-TB: 4.0% and QFT-GIT: 5.5%) (Table 2). The positive, negative, and overall percent agreements across all subjects between AFIAS IGRA-TB and QFT-GIT were 88.2% (95% CI: 76.6 - 94.5%), 95.4% (95% CI: 90.4 - 97.9%) and 93.4% (95% CI: 88.8 - 96.2%), respectively, with excellent kappa statistics showing almost perfect agreement (κ = 0.84). While Group 3 showed the highest agreement, with overall percent agreement reaching 96.1% and almost perfect agreement (κ = 0.91), Group 1 demonstrated relatively poor agreement compared to other groups, though it was still substantial (κ = 0.76). Additionally, the positive, negative, and overall percent agreements among Group 1 were nearly 90% (87.0%, 90.6% and 89.5%, respectively). Thus, AFIAS IGRA-TB demonstrated excellent agreement across all subgroups. For reproducibility evaluation, a total of 153 samples (38 positive and 115 negative samples) were tested by repeating AFIAS IGRA-TB with three different lots (Table 3). All the samples demonstrated identical results across test lots, indicating no lot-to-lot difference.

Quantitative comparison of AFIAS IGRA-TB and QFT-GIT

For quantitative results of TB Ag - nil data, AFIAS IGRA-TB and QFT-GIT showed a moderate correlation (Spearman's ρ = 0.69, $p < 0.001$) (Figure 1A). The slope and intercept of the Deming regression fit line were 1.18 and -0.31, respectively. The mean difference between AFIAS IGRA-TB and QFT-GIT was -0.16 ± 1.85 IU/mL (Figure 1B).

DISCUSSION

To the best of our knowledge, this is the first study to assess the clinical performance of AFIAS IGRA-TB in comparison with the WHO-endorsed IGRA test, QFT-GIT. AFIAS IGRA-TB demonstrated excellent agreement with QFT-GIT in both the overall study population and the subgroups of subjects. AFIAS IGRA-TB exhibited a minimal negative bias compared to QFT-GIT, with greater bias observed in samples with higher IFN-γ results. Given the differences in TB antigens between AFIAS IGRA-TB (ESAT-6 and CFP-10) and QFT-GIT (ESAT-6, CFP-10 and TB7.7), the direct comparison of IFN-γ levels is of limited value. Overall, AFIAS IGRA-TB demonstrated comparable clinical performance to QFT-GIT TB across all groups with varying TB infection risk.

Our study demonstrated that AFIAS IGRA-TB showed overall high agreement with QFT-GIT; however, subjects without known risk factors for TB infection (Group 1) exhibited more discordant results than higher-risk groups. This lower agreement is likely attributable to a higher proportion of low-risk subjects with IGRA levels near the assay cutoff, increasing discordance. Therefore, in populations without known risk factors for TB infection, more stringent criteria for positivity should be applied, or LTBI testing should be avoided [10]. Additionally, among subjects considered to have active TB (Group 3), notable findings were observed. These subjects were considered infected with TB, as AFB were detected on sputum smear. However, only 32.1% were positive in both AFIAS IGRA-TB and QFT-GIT. In contrast, most studies reported the sensitivity of QFT-GIT to range between 80% and 100%, with the lowest reported sensitivity being 62.1% [9,11]. False-negative IGRA results, associated with advanced age and reduced peripheral lymphocyte counts [12], have also been related to poor patient prognosis [13]. In our cohort, IGRA-negative Group 3 subjects were not particularly older than the others and were not immunocompromised. Given that a recent study reported the prevalence of non-tuberculous mycobacteria in India to be approximately 30% [14], a considerable proportion of negative IGRA results in Group 3 may indicate non-tuberculous AFB infection.

In the era of increasing biologic agents, especially anti-TNF agents, and advances in transplantation, the need for LTBI diagnosis is expected to grow further, not only

Table 1. Characteristics of study subjects.

Parameter, n (%)	Total	Group 1	Group 2	Group 3	p-value
Number	200 (100.0)	88 (44.0)	56 (28.0)	56 (28.0)	
Gender					
Male	142 (71.0)	62 (70.5)	36 (64.3)	44 (78.6)	0.247
Female	58 (29.0)	26 (29.5)	20 (35.7)	12 (21.4)	
Age, year median (IQR)	29.0 (23.8 - 41.0)	30 (24.0 - 44.3)	29.5 (25.5 - 41.0)	28.0 (21.8 - 40.3)	< 0.001
Clinical symptoms					
Fever	110 (55.0)	58 (65.9)	6 (10.7)	46 (82.1)	< 0.001
Cough	133 (66.5)	73 (83.0)	9 (16.1)	51 (91.1)	< 0.001
Weight loss	75 (37.5)	27 (30.7)	2 (3.6)	46 (82.1)	< 0.001
Night sweat	1 (0.5)	1 (1.1)	0 (0.0)	0 (0.0)	NA
Breathlessness	74 (37.0)	40 (45.5)	2 (3.6)	32 (57.1)	< 0.001
Hemoptysis	50 (25.0)	20 (22.7)	3 (5.4)	27 (48.2)	< 0.001
AFB smear	142 (72.0)	77 (87.5)	9 (16.1)	56 (100.0)	
Positive	56 (39.4)	0 (0.0)	0 (0.0)	56 (100.0)	NA
QFT-GIT					
Positive	51 (25.5)	23 (26.1)	10 (17.9)	18 (32.1)	0.122 [a]
Negative	138 (69.0)	59 (67.0)	46 (82.1)	33 (58.9)	
Indeterminate	11 (5.5)	6 (6.8)	0 (0.0)	5 (8.9)	

AFB: acid-fast bacilli, IQR: interquartile range, NA: not applicable, TB: tuberculosis, QFT-GIT: QuantiFERON-TB Gold In-Tube.
[a] Indeterminate results in QFT-GIT were not included.

Table 2. Comparison of AFIAS IGRA-TB and QFT-GIT results.

Group	QFT-GIT	AFIAS IGRA-TB, n (%)				Percent agreement (95% CI)			Kappa
		Positive	Negative	Indeter-minate	Total	Positive	Negative	Overall	
Total	positive	45 (22.5)	6 (3.0)	0 (0.0)	51 (25.5)	88.2 (76.6 - 94.5)	95.4 (90.4 - 97.9)	93.4 (88.8 - 96.2)	0.84 (0.75 - 0.93)
	negative	6 (3.0)	125 (62.5)	7 (3.5)	138 (69.0)				
	indeter-minate	0 (0.0)	10 (5.0)	1 (0.5)	11 (5.5)				
	total	51 (25.5)	141 (70.5)	8 (4.0)	200 (100.0)				
Group 1	positive	20 (22.7)	3 (3.4)	0 (0.0)	23 (26.1)	87.0 (67.9 - 95.5)	90.6 (79.7 - 95.9)	89.5 (80.6 - 94.6)	0.76 (0.60 - 0.92)
	negative	5 (5.7)	48 (54.5)	6 (6.8)	59 (67.0)				
	indeter-minate	0 (0.0)	6 (6.8)	0 (0.0)	6 (6.8)				
	total	25 (28.4)	57 (64.8)	6 (6.8)	88 (100.0)				
Group 2	positive	8 (14.3)	2 (3.6)	0 (0.0)	10 (17.9)	80.0 (49.0 - 94.3)	100.0 (92.1 - 100.0)	96.4 (87.7 - 99.0)	0.87 (0.69 - 1.00)
	negative	0 (0.0)	45 (80.4)	1 (1.8)	46 (82.1)				
	indeter-minate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				
	total	8 (14.3)	47 (83.9)	1 (1.8)	56 (100.0)				
Group 3	positive	17 (30.4)	1 (1.8)	0 (0.0)	18 (32.1)	94.4 (74.2 - 99.0)	97.0 (84.7 - 99.5)	96.1 (86.8 - 98.9)	0.91 (0.80 - 1.00)
	negative	1 (1.8)	32 (57.1)	0 (0.0)	33 (58.9)				
	indeter-minate	0 (0.0)	4 (7.1)	1 (1.8)	5 (8.9)				
	total	18 (32.1)	37 (66.1)	1 (1.8)	56 (100.0)				

CI: confidence interval, QFT-GIT: QuantiFERON-TB Gold In-Tube.
[a] Indeterminate results in either AFIAS IGRA-TB or QFT-GIT were not included in the calculation of percent agreement or kappa coefficient.

Table 3. AFIAS IGRA-TB results across different test lots.

		Lot 1				Lot 2				Lot 3	
		Positive	Negative			Positive	Negative			Positive	Negative
Lot 2	positive	38	0	Lot 3	positive	38	0	Lot 1	positive	38	0
	negative	0	115		negative	0	115		negative	0	115

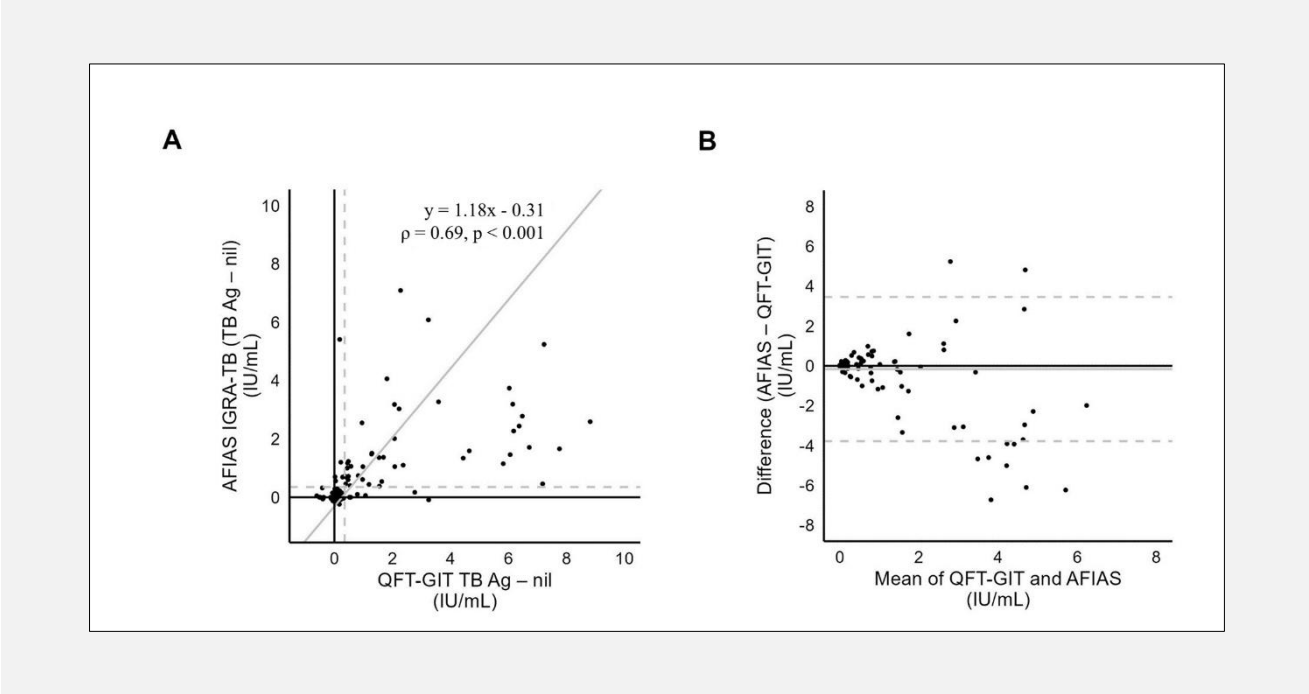

Figure 1. Comparison of AFIAS IGRA-TB and QFT-GIT Assays.

A: Scatter plot comparing TB Ag - nil values of AFIAS IGRA-TB and QFT-GIT. The grey solid line represents Deming regression line, while grey dashed lines mark 0.35 IU/mL, the cutoff for both tests. B: Bland-Altman plot of AFIAS IGRA-TB and QFT-GIT. Grey solid line indicates mean difference (-0.16 IU/mL) and the grey dashed lines represent the upper and lower 95% confidence intervals (standard deviation: 1.85 IU/mL).
QFT-GIT: QuantiFERON-TB Gold In-Tube, TB Ag: tuberculosis antigen.

in countries with a high TB burden but also in countries with a low TB burden. A major limitation of conventional enzyme-linked immunosorbent assay-based IGRA is dependency on trained personnel and costly, complex equipment [8]. To address these challenges, novel IGRAs, such as AFIAS IGRA-TB, utilizing lateral flow assay and chemiluminescence immunoassay have recently been developed [15]. AFIAS IGRA-TB offers simple testing procedures and minimal manual handling without requiring an automated platform. In addition, it does not require trained personnel and the analytical run time is much shorter (15 minutes) than that of conventional IGRA. The cartridge-based closed system also reduces the risk of cross-contamination among samples.

This study has some limitations. First, TB confirmatory test relied exclusively on the AFB smear, which can produce false positives in patients with non-tuberculous AFB infections. In addition, the sensitivity of the AFB smear is estimated to be approximately 70% [16]. Therefore, additional diagnostic methods, such as nucleic acid amplification test or TB culture, are required for more reliable confirmation of active TB. Second, the study design did not support evaluating the predictive value of AFIAS IGRA-TB. As LTBI diagnosis relies on immune reaction-based indirect methods, large-scale cohort studies are required to assess the association between positive results and TB reactivation risk. However, due to the significant time and financial resources required for such studies, the WHO TAG has declared

that IGRA tests demonstrating comparable sensitivity, specificity, and a high level of agreement with established IGRA tests can be considered acceptable [9]. Given the high agreement of AFIAS IGRA-TB with QFT-GIT, an established IGRA test, AFIAS IGRA-TB may therefore be considered an acceptable assay for LTBI diagnosis.

In conclusion, AFIAS IGRA-TB demonstrated comparable clinical performance compared to QFT-GIT across all groups with varying TB infection risk. Given its user-friendly platform, minimal dependency on trained personnel or complex equipment, and rapid turnaround time, AFIAS IGRA-TB represents a promising alternative for the diagnosis of LTBI and active TB in resource-limited settings.

Source of Support:

This study was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: RS-2024-00332244).

Declaration of Interest:

J.J.L., H.R., M.N. received financial compensation from Boditech Med, Inc. for manuscript preparation using data provided by the company. The company had no role in the interpretation of the data, the conclusions drawn, or the decision to submit the manuscript for publication.

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