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Comparison of Lateral-flow Nanoparticle Fluorescence Assay and ELISA Method for Interferon-γ Release Assay Test

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ABSTRACT

The detection of latent tuberculosis (TB) infection to prevent progression to active TB disease is an essential part of the WHO's end-TB strategy. Diagnosis of latent TB infection is based on detecting immune responses to *Mycobacterium tuberculosis* antigens. Interferon Gamma Release Assays (IGRA) are superior to Tuberculin Skin Tests (TST) for detecting latent infection; however, the performance of IGRA is limited in resource-limited settings. This study evaluated the sensitivity, specificity, and agreement of the lateral-flow nanoparticle fluorescence assay (QIAreach QFT) compared with the ELISA method (QFT-Plus) as a reference test. This cross-sectional study was carried out in the laboratory department of Siloam Hospitals in Lippo Village, Banten, Indonesia, between January and June 2023. A total of 60 samples consisting of both males and females of all age groups were tested for QFT-Plus and were involved in the study using consecutive samples. Sensitivity, specificity, Negative Predictive Value (NPV), and Positive Predictive Value (PPV) of QIAreach QFT were 100% (95% CI 86.28-100), 70.96% (95% CI 51.96-85.78), 100%, (95% CI 84.56-100) and 73.53% (95% CI 55.64-87.12), respectively. The agreement calculation using Cohen's kappa coefficient, excluding indeterminate data, showed a kappa value 0.68 (95% CI 0.507-0.864). QIAreach QFT, with its superiority, could support the expansion of IGRA testing, particularly in remote areas, thereby helping the eradication attempt of TB infection.

Keywords: Tuberculosis, IGRA, ELISA, lateral-flow immunoassay

INTRODUCTION

Tuberculosis (TB) represents one of the significant causes of death worldwide. Approximately 45% and 9.2% of its latest cases were reported in Southeast Asia and Indonesia in 2021, respectively.[1] Latent tuberculosis infection is estimated to affect 25% of the global population and 5-10% of those individuals are predicted to develop active tuberculosis disease, particularly five years after the initial infection.[2,3] Therefore, detection of latent tuberculosis infection is an essential part of the WHO end TB strategy to prevent progression to active TB disease, especially in susceptible individuals.[2]

Unlike active TB infection, it is impossible to detect the pathogen in latent TB infection; therefore, the diagnosis of latent TB infection is based on detecting immune responses to *Mycobacterium tuberculosis* antigens.[4] Identification of TB latent infection according to WHO recommendations can use a Tuberculin Skin Test (TST) or Interferon-γ Release Assay (IGRA).[2] Both tests measure the cellular immune response to tuberculosis. The minimum interference by the Bacille Calmette-Guérin (BCG) vaccine and its more suitability for children due to one-time sample collection and no side effects are advantages of IGRA over TST.[5,6] One of the WHO-endorsed IGRA tests is QuantiFERON®-TB Gold Plus (QFT-Plus), which can assess the amount of interferon-γ produced by CD4 and CD8 cells in response to the antigen associated with the *M.tuberculosis* complex, CFP-10, and ESAT-6 protein.[7,8] Although QFT-Plus has advantages over TST, this test uses the 4th generation of Enzyme-Linked Immunosorbent Assay (ELISA) method that requires four tubes (nil, TB1, TB2, mitogen; each tube requires 1 mL of sample from blood), complicated laboratory infrastructure, qualified human resources, and a long running time, thereby limiting their use in resource-limited settings.[7]

A new diagnostic test is available for the detection of latent TB infection, QIAreach QuantiFERON-TB (QIA reach QFT), measures the IFN-γ level as a response to the same antigen as QFT-Plus but using a lateral-flow nanoparticle fluorescence assay. This method is portable, less time-consuming, easy to perform without specialized laboratory equipment and human resources, and only requires 1 mL of blood for a TB2 tube. Therefore, this test is ideal for point-of-care testing and can be performed remotely.[7,9]

This study aimed to evaluate the sensitivity, specificity, and agreement of QIAreach QFT compared with QFT-Plus as a reference method for samples obtained in clinical settings to diagnose latent TB infection.

METHODS

This cross-sectional study was carried out in the laboratory department of Siloam Hospitals in Lippo Village, Banten, Indonesia, between January and June 2023. Calculation of sample size based on regulation by Clinical and Laboratory Standard Institute (CLSI) EP09-A3 showed a requirement of a minimum of 40 samples for comparison of the two methods.[10] A total of 60 samples of males and females of all age groups in this study were tested for QFT-Plus and were grouped into positive, negative, and indeterminate QFT-Plus using consecutive sampling. The remaining samples from the TB2 tube of QFT-Plus samples were analyzed for QIAreach QFT. The Medical Research Ethics Commission approved the protocol used in this research, Medical Faculty of Pelita Harapan University (No. 122/K-LJK/ETIK/III/2023).

QFT-Plus assay was performed as instructed by the QFT-Plus package inserts. Four blood collection tubes were used to collect venous blood (nil, TB1, TB2, and mitogen tubes); each consisted of 1 mL of blood. The tubes were transferred to 37^{0}C incubator within 16 hours of collection and incubated for 16 to 24 hours. The tubes were centrifuged at 3000 g for 15 minutes after incubation. Plasma supernatants were collected to perform ELISA testing. IFN-y levels were quantified, and the results were reported as positive, negative, and indeterminate (Table 1).[11]

QIAreach QFT was performed as instructed by the QIAreach QFT insert package. Plasma samples from TB2 tube QFT-Plus testing were applied for QIAreach QFT and performed on the same day. The average time from the inclusion of the sample to the test result was 20 minutes. QIAreach QFT raw data were analyzed on the eStick firmware and interpreted as a positive or negative result according to the internal algorithm of the IFN-y level. There was no indication of a clinically significant prozone effect at the IFN-y level up to 1000 IU/mL, whereas the limit detection of QIAreach QFT was 0.3 IU/mL.[12]

Data were statistically analyzed using SPSS version 24 and provided as percentage (%) or median (interquartile range). The sensitivity, specificity, and agreement of the QIAreach QFT were calculated using QFT-Plus as the reference method. Calculations were made to determine the agreement between QIAreach QFT and QFT using Cohen's kappa coefficient with an interpretation of kappa values >0.9 for almost perfect, 0.8-0.9 for strong, 0.6-0.79 for moderate, 0.4-0.59 for weak, 0.21-0.39 for minimum, and <0.2 for no agreement.[13]

RESULTS AND DISCUSSIONS

QIAreach QFT and QFT-Plus were performed in 60 samples, with a median age of 26 years (2-80), and 26 of the total 60 (43.3%) subjects were male (Table 2). Out of 60 samples tested for QFT-Plus, 25 were positive, 31 were negative, and 4 were indeterminate. The median white blood cell count was 7590/µL (2940-24820), and the lymphocyte count was 1911/µL (621-7208). Lymphocyte counts <1500/µL were found in 7 samples (3 positive, 0 negative, and four indeterminate for QFT-Plus).

All 25 samples that were tested positive with QFT-Plus were also tested positive for QIAreach QFT, whereas 22 of 31 samples tested negative using QFT-Plus were also tested negative for QIAreach QFT, resulting in sensitivity, specificity, Negative Predictive Value (NPV), and Positive Predictive Value (PPV) of QIAreach QFT of 100% (95% CI 86.28-100), 70.96% (95% CI 51.96-85.78), 100%, (95% CI 84.56-100) and 73.53% (95% CI 55.64-87.12), respectively (Table 3). The agreement calculation using Cohen's kappa coefficient with the exclusion of indeterminate data showed a moderate agreement with a kappa value of 0.68 (95% CI 0.507 - 0.864).

Of the 13 samples with contradictory results, nine samples of negative QFT-Plus became positive in the QIAreach QFT test, and four samples of indeterminate QFT-Plus became negative in the QIAreach QFT test. Even though QIAreach QFT is a qualitative test, it was discovered that the detection limit of QIAreach QFT was 0.3 IU/mL.[12] Therefore, 7 of 9 negative QFT-Plus with TB2 value >0.3 IU/mL were detected positive for QIAreach QFT, and 4 indeterminate QFT-Plus were detected negative for QIAreach QFT as their TB2 tube

Table 1. Interpretation of QFT-Plus[11]

Nil (IU/mL)	TB Antigen Minus Nil (IU/mL)	QFT Result*	Interpretation
	<0.35	(-)	Infection with *M.tuberculosis* is not likely
<8.0	≥0.35 and <25% of nil value	(-)	Infection with *M.tuberculosis* is not likely
	≥0.35 and ≥25% of nil value	(+)	*M.tuberculosis* infection is likely
>8.0	Any	(i)	Indeterminate TB -antigen responsiveness

*(-): Negative, (+): Positive, (i): Indeterminate

Table 2. Characteristics of samples

Variable	Unit	N	Percentage (%)	Median (IQR)
Gender				
Male		26	43.3	
Female		34	56.7	
Age group				
<18	Year	23	38.3	23.5 (2-80)
≥18		37	61.7	
White blood cell count				
<5000	/µL	3	5.0	7590 (2940-24820)
5000-10000		43	71.7	
>10000		14	23.3	
Lymphocyte count				
<1500	/µL	7	11.7	1911 (621-7208)
≥1500		53	88.3	

IQR: interquartile range, n: number

Table 3. Performance of QIAreach QFT's diagnostics using QFT-Plus as a reference

IGRA TB		Quantiferon QFT-Plus			
		Positive	Negative	Indeterminate	Total
QIAreach QFT	Positive	25	9	0	34
	Negative	0	22	4	26
	Total	25	31	4	60

value <0.3 IU/mL regardless nil value because QIAreach QFT only used 1 tube (TB2 tube) and the nil value in the determination of the result was not considered.

Another sample with contradicting results was diagnosed as an autoimmune disease. Immunity imbalance in autoimmune disease might disrupt cytokine production and T cell modulation, which leads to T helper-1 signaling inhibition and low Interferon-y.[14] In addition, it was possible that autoantibodies also interfered with antigen-antibody binding, which might lead to false positive or false negative results both in lateral flow immunoassays and ELISA. Therefore, to establish the diagnosis of latent TB infection in autoimmune disease, a combination of IGRA and radiology examinations such as chest X-ray and chest CT can increase the sensitivity.[15]

The remaining sample with a contradicting result had a high triglyceride level (>300 mg/dL), which interfered with lateral flow function by affecting the speed of the sample solution of the lateral flow immunoassay due to its high viscosity on the nitrocellulose membrane.[16,17]

The indeterminate result of QFT-Plus might be related to immune status, such as an insufficient lymphocyte count or a lymphocyte inability to produce IFN-y.[11] All indeterminate samples in this study had a lymphocyte count <1500/µL, which decreased the response to mitogen-positive control. Technical factors such as longer delay between sample collection and incubation at 37^0C (more than 16 hours) that reduce lymphocyte activity, improper temperature of sample storage, or insufficient mixing of blood collection tubes might also cause indeterminate results.[11,18,19] Nevertheless, technical factors can be excluded as causes of discrepancies because the same sample of blood in a TB2 tube was used in the QIAreach QFT test exactly like QFT Plus. In addition, both tests were performed on the same day. Previous studies demonstrated high sensitivity, specificity, and excellent agreement of QIAreach QFT compared to QFT-Plus in the USA, Japan, and Malaysia.[16,17,20] The sensitivity of QIAreach QFT reported in this study was comparable to previous studies, although specificity and agreement were lower because of the small sample size. The samples used in this study had various ages and clinical backgrounds, which might interfere with the test results.

The sensitivity, specificity, and agreement of QIAreach QFT in this study indicated that QIAreach can still be used to test latent tuberculosis infection like QFT-Plus. However, further investigations, such as clinical judgment or radiology tests, are still needed to establish tuberculosis infection and initiate anti-tuberculosis treatment.

CONCLUSIONS AND SUGGESTIONS

QIAreach QFT has advantages as a portable device that is rapid, easy to perform without complicated laboratory equipment, and requires a smaller blood sample. It could support the expansion of IGRA testing, particularly in remote areas, thereby helping to achieve the goal of ending TB infection. To further assess the potentiality of this assay, it was recommended that more extensive studies and more specific sample settings be performed, particularly in susceptible individuals for active tuberculosis disease.

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