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Short Communication

Performance evaluation of newly developed fluorescence immunoassay-based interferon-gamma release assay for the diagnosis of latent tuberculosis infection in healthcare workers

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Abstract The ichroma™ IGRA-TB (Boditech Med Inc., Chuncheon, Republic of Korea) is an automated fluorescent immunoassay-based point-of-care interferon-gamma release assay for detecting latent tuberculosis infection. We evaluated this assay with 408 health care workers, and demonstrated its acceptable performances comparing to QuantiFERON-TB Gold-Plus (QFT-Plus; Qiagen, Germantown, MD).

Introduction

Interferon-gamma (IFN-γ) release assays (IGRAs) are *in vitro* laboratory tests that measure the T-cell response after stimulation with *Mycobacterium tuberculosis* (MTB)-specific antigens.[1,2] In the Republic of Korea, which has an intermediate MTB burden,[3] and most of the population has been vaccinated with Bacillus Calmette—Guérin, the government uses IGRAs to operate a massive latent tuberculosis infection screening program in high-risk populations. One of the most widely used IGRAs is QuantiFERON technology (Qiagen, Germantown, MD), which uses an enzyme-linked immunosorbent assay (ELISA) to quantify the amount of IFN-γ. However, this technology has some disadvantages, such as being labor-intensive and time-consuming.[4]

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The ichroma™ IGRA-TB (Boditech Med Inc., Chuncheon, Republic of Korea) is a three-tube-based, fully automated point-of-care device using a fluorescent immunoassay (FIA) for IFN-γ quantification. It uses ESAT-6 and CFP-10 as MTB-specific antigens in one TB antigen tube (TB Ag). We evaluated the analytical performance of ichroma™ IGRA-TB, comparing it to that of QuantiFERON-TB Gold-Plus (QFT-Plus; Qiagen) for the detection of LTBI in health care workers (HCWs). Because IGRAs have two stages—blood incubation in tubes and IFN-γ quantification—we tested the two IGRAs in a cross-manner to investigate the performance of each stage separately.

Methods

Study participants

A total of 408 HCWs (329 women) were enrolled. The subjects who were suspected of having active TB based on a chest X-ray were excluded, and none of the subjects was undergoing treatment for TB or had a medical history of LTBI or active TB. The study protocol was approved by the Institutional Review Board (IRB) of Chung-Ang University Hospital (1922-002-361), and informed consent was obtained according to the IRB's policy.

ichroma™ IGRA-TB

For each participant, 1 mL of blood was obtained directly into each of the ichroma™ IGRA-TB tubes (Nil, TB Ag, and the mitogen tube). After collection, the tubes were shaken ten times firmly and incubated in a 37 °C incubator for 16—24 h. IFN-γ quantification was processed with the ichroma™-50 (Boditech med.), an automatic FIA device. The separated plasma samples from each tube, the cartridge, and the diluent were loaded on the ichroma™-50. From the sample loading on the cartridge to the reading of the fluorescence intensity and IFN-γ quantification, followed by the interpretation, the test is automatically performed and completed within 20 min. The results are interpreted according to the manufacturer's instructions.[5]

QuantiFERON-TB Gold-Plus (QFT-Plus)

The QFT-Plus assays were performed according to the manufacturer's instructions.[6] Blood sample collection and incubation were processed at the same time as the ichroma™ IGRA-TB. Interpretation criteria were the same as the ichroma™ IGRA-TB, however, the results were considered positive if either one or both of the TB1 and TB2 tubes are positive.

Standard E TB-Feron ELISA

For the subjects who showed discrepant results between ichroma™ IGRA™-TB and QFT-Plus, the Standard E TB-Feron ELISA (TBF, SD Biosensor, Gyeonggi-do, Republic of Korea) was additionally performed. It is 3-tubes and ELISA-based IGRA as the QFT. Its interpretation criteria are identical to other IGRAs.[7]

Cross-manner IGRAs

Plasma samples from each IGRAs were additionally analyzed using the ELISA or FIA from the other IGRAs (hereafter, "cross-manner" IGRAs). Cross-manner tests were conducted at the same time as the standard IGRAs.

Statistics

Qualitative comparisons were assessed using crosstab analysis. Because all of the quantitative data showed nonparametric distribution, the Mann—Whitney *U*-test or the Kruskal—Wallis test was used for quantitative comparison. Spearman's rank correlation and Deming regression were performed to investigate the correlation of the qualitative values. Statistical analyses were performed using SPSS v. 19 (IBM, Armonk, NY) and Microsoft Excel v. 2016 (Microsoft, Redmond, WA). $P \leq 0.05$ was considered statistically significant.

Results

Detection of LTBI using standard IGRAs and cross-manner IGRAs

The ichroma™ IGRA-TB showed 6.6% (27/408) positivity, which was lower than that of the QFT-Plus, 10.3% (42/408). Although indeterminate results were generated from 2.2% (9/408) of ichroma™ IGRA-TB tests, there were none from QFT-Plus tests. In the cross-manner IGRAs, the ichroma™ tube/QFT-Plus ELISA showed 2.2% (9/408) indeterminate results. Among nine subjects who showed indeterminate results with ichroma™ IGRA-TB, six (66.7%, 6/9) also showed indeterminate results for the ichroma™ tube/QFT-Plus ELISA. The IGRA results of the subjects who showed indeterminate results from one or more IGRAs are listed in Supplement 1.

Qualitative comparison among standard and cross-manner IGRAs

Qualitative comparisons between the IGRAS are listed in Table 1. The total agreement rate between the two IGRAs was 95.2% with a kappa value of 0.70 (strong agreement). A total of 28 subjects showed discordant results between the IGRAs, and their results from the TBF are listed in Supplement 3. Among 17 ichroma™ IGRA-TB-negative/QFT-Plus-positive subjects, 64.7% (11/17) showed positivity with the TBF. All the ichroma™ IGRA-TB-positive/QFT-Plus-negative subjects showed positive results for the TBF (2/2), whereas all the ichroma™ IGRA-TB-indeterminate/QFT-Plus-negative subjects showed negative results for the TBF (9/9).

In the qualitative comparisons among the standard and cross-manner IGRAs, higher kappa values were found in comparisons with IGRAs using the same tubes than in those using the same IFN-γ quantification methods. Thus, the results for the same-tube comparisons were 0.87 between standard ichroma™ IGRA-TB and ichroma™ tubes/QFT Plus ELISA and 0.86 between standard QFT-Plus and QFT-Plus tube/ichroma™ FIA. The results for comparisons between IGRAs using the same IFN-γ quantification methods were

Table 1 Qualitative comparisons between the ichroma™ IGRA-TB and the QuantiFERON-TB Gold Plus in a total of 408 health care workers.

		ichroma™ IGRA-TB				PPA (%)	NPA (%)	Total agreement (%)	Kappa
		Positive	Negative	Indeterminate	Total				
QFT-Plus	Positive	25 (6.1%)	17 (4.2%)	0 (0%)	42 (10.3%)	59.5	99.4	95.2	0.70
	Negative	2 (0.5%)	355 (87.0%)	9 (2.2%)	366 (89.7%)	(44.5—73.0)[b]	(97.8—100.0)	(92.5.-97.0)	(0.57—0.83)
	Indeterminate	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
	Total	27 (6.6%)	272 (91.2%)	9 (2.2%)	408 (100%)				

[a]Abbreviations: PPA, positive percentage agreement; NPA, negative percentage agreement; QFT-Plus, QuantiFERON-TB Gold Plus.
b 95% confidence interval.

0.73 between standard ichroma™ IGRA-TB and QFT-Plus tube/ichroma™ FIA and 0.75 between standard QFT-Plus and ichroma™ tubes/QFT-Plus ELISA.

Quantitative comparisons among the standard and cross-manner IGRAs

The scatter plots for the TB Ag minus nil values between ichroma™ IGRA-TB and QFT-Plus, including Deming regression fit lines, are illustrated in Fig. 1. The slopes and intercepts from the Deming regression were 0.922 and −0.056 for QFT-Plus TB1 and 0.894 and −0.045 for QFT-Plus TB2, with r_s values of 0.431 and 0.394, respectively.

The IFN-γ values generated from the standard and cross-manner IGRAs are listed in Supplement 3. For the TB Ag, nil, and mitogen tubes, the standard or cross-manner IGRAs using the ichroma™ FIA measured higher IFN-γ values than the values from the IGRAs using QFT-Plus ELISA ($P < 0.01$). However, the TB Ag minus nil values did not differ significantly among the IGRAs.

Supplement 4 shows the distribution of TB Ag minus nil values generated from the standard and cross-manner IGRAs for 19 samples that showed discordant results (excluding indeterminate results) from the two standard IGRAs.

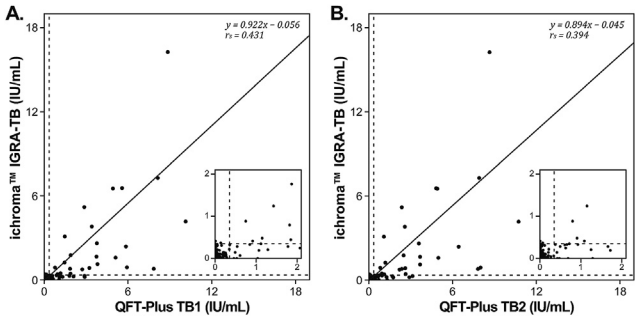

Figure 1. Scatter plot for the Tb antigen minus nil values of the 408 samples from health care workers from the ichroma™ IGRA-TB and the QuantiFERON-TB Gold Plus (A. TB1 and B. TB2). The dashed lines designate the cut-off values for the determination of positive results from the IGRAs (0.35 IU/mL). Deming regression fit lines and r_s from Spearman's rank tests are included.

Discussion and conclusion

In this study, the total agreement rate and kappa values between the ichroma™ IGRA-TB and QFT-Plus were 95.20% and 0.70, respectively. showing a strong degree of correlation. As a total of 28 subjects showed discordant results between the two IGRAs, TBF performed for those discordant results revealed that 20 (71.4%) and 7 (25.0%) subjects showed identical results to QFT-Plus and ichroma™ IGRA-TB, respectively. The IGRAs used differ with respect to the TB Ag and the IFN-γ quantification steps[5—7]; hence, the discordant results may have been affected by several factors.

One of the drawbacks of an IGRA is that the test result may be indeterminate.[8] In the present study, the ichroma™ IGRA-TB generated indeterminate results from 2.2% of the study samples, whereas QFT-Plus had no indeterminate results at all. Careful analysis suggests that these indeterminate results could be attributed to the tube factor of ichroma™ IGRA-TB. This is because i) the indeterminate samples showed negative results for TBF and QFT-Plus, ii) 66.7% of the indeterminate samples showed the same results in a cross-manner IGRA using the tubes of ichroma™ IGRA-TB, iii) all the study subjects were health care workers who receive annual health checkups, and all pre-analytical or analytical procedures of the IGRAs were conducted in the same manner by a well-trained technician. Qualitative comparisons among the standard and cross-manner IGRAs revealed higher kappa values in comparison with IGRAs using the same tubes than in comparisons between IGRAs using the same IFN-γ quantification methods. The performance of IFN-γ quantification was also different between the tested IGRAs. The FIA of ichroma™ IGRA-TB can generate higher IFN-γ values than the QFT-Plus ELISA even with the same plasma samples. Although those differences disappeared in the process of calculating the TB Ag minus nil value, the high nil values can be another cause of the high indeterminate frequency of the ichroma™ IGRA-TB. Because IGRA is a qualitative test based on a quantitative value, it is necessary to harmonize the performance of IFN-γ quantification, particularly when identical cut-off values are used. Otherwise, the cut-off values must be adjusted for each test method.

In conclusion, the ichroma™ IGRA- TB showed an acceptable performance compared to the QFT-plus, except

for the higher frequency of indeterminate results. The newly developed ichroma™ IGRA-TB has several advantages such as ease of use for a small number of subjects, rapid turn-around time, and simplified procedures. Because one of the challenging issues for LTBI diagnostics in resource-limited settings is achieving convenience without the need for technical skills, ichroma™ IGRA-TB may be a feasible point-of-care device in those areas.

Author contribution

Conception and design of study: MK Lee. Acquisition of data: OJ Kweon, YK Lim, HR Kim. Data analysis and/or interpretation: OJ Kweon, HR Kim, TH Kim. Drafting of manuscript and/or critical revision: OJ Kweon, MK Lee.

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Declaration of competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.05.007.