Original Article

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Microbiology Section

Comparison of Six Immunoassays for Assaying Levels of Immunoglobulin G against the Nucleocapsid and Spike Proteins of SARS-CoV-2

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ABSTRACT

Introduction: Assay kits for detection of Immunoglobulin G (IgG) against the nucleocapsid protein (anti-nucleocapsid IgG) and spike proteins (anti-spike IgG) of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) were commercially provided by several manufacturers. These assay kits should be verified by measuring the same sample.

Aim: To compare the diagnostic value of three Coronavirus Disease-2019 (COVID-19) kits in evaluating six immunoassays developed by three manufacturers (Abbott, Euglena, and Roche) to detect anti-nucleocapsid IgG and anti-spike IgG.

Materials and Methods: Present study was an observational cross-sectional study conducted from June 2020 to December 2020. Antibody titers for anti-nucleocapsid IgG and anti-spike IgG among 429 Healthcare Workers (HCWs) in a Tone Central Hospital, Japan where a nosocomial infection of the COVID-19 occurred were measured by six immunoassays with kits developed by three different manufacturers. The sensitivity and specificity of each kit was compared to real-time Reverse Transcription-Polymerase Chain Reaction (RT-qPCR).

Results: Six of the HCWs tested positive for SARS-CoV-2 via RT-qPCR, and the rest tested negative. The severity of COVID-19 among these six HCWs ranged from mild to moderate. The sensitivity and specificity values against RT-qPCR were, 100% and 99.5% for Abbott, 83.3% and 100% for Euglena, and 100% and 100% for Roche when using the nucleocapsid protein assay and 100% and 99.8% for Abbott, 100% and 100% for Euglena, and 100% and 100% for Roche when using the spike protein assay kit.

Conclusion: The commercial kits provided by three manufacturers reflected the immune status of individuals. There were no major differences in the performance of these test kits. Discordant results with the antibody titer for anti-nucleocapsid IgG and anti-spike IgG were detected by using assay kits provided by Abbott and Euglena. To evaluate the past history of COVID-19, it should be noted that the single measurement of anit-nucleocapsid IgG or anti-spike IgG could not exclude false negative or positives.

Keywords: Coronavirus disease-2019, Nucleocapsid protein, Severe acute respiratory syndrome corona virus

INTRODUCTION

As of April 2022, COVID-19 pandemic continues to profoundly affect countries worldwide. Serological testing has been a key strategy to evaluate the extent of COVID-19 infections in the community and to identify individuals who are immune and potentially protected from infection [1]. Several nationwide and regional serological surveys have shown that the dissemination of SARS-CoV-2 from higher to lower seroprevalence areas in Spain and United States (US) has a remarkable difference. The ongoing transmission is attributed to the infected individuals who were asymptomatic, as well as the symptomatic cases that remained untested [2,3]. The serological surveys in present setting revealed that, two HCWs were infected, but remained asymptomatic [4]. Serological surveys provide informative benchmarks for local disease prevalence and support public health decision-making during the COVID-19 pandemic [3]. Additionally, serological surveys can be utilised for COVID-19 diagnosis and characterisation of the course of the disease, identification of convalescent plasma donors, epidemiological investigations, lockdown exit decisions, and COVID-19 vaccine development [2,3,5,6]. Reports regarding commercial immunoassays for the measurement of immunoglobulin's against SARS-CoV-2 have also been reported [7,8]. However, only few compared the performance of assay kits from different manufacturers in detecting IgG against the nucleocapsid protein (anti-nucleocapsid IgG) and spike proteins (anti-spike IgG) of SARS-CoV-2. The clinical performance of commercial SARS-CoV-2 assays are comparable, and most can detect immunoglobulin's against SARS-CoV-2 in most patients 14 days after the onset of symptoms [7,8]. However, commercial immunoassays do not have sufficient clinical sensitivity before the 14th day from symptom onset to confirm acute infection [7]. Furthermore, asymptomatic patients with low levels of immunoglobulins against SARS-CoV-2 are likely to be seronegative; hence, serological surveys are required to determine the actual infection rate [9]. On the contrary, it is possible that false-positive results could lead to overestimation of seroprevalence and infections. Thus, it is reasonable to consider choosing assays with a high specificity based on serological surveys that have determined their performance characteristics [3,10]. Present study compared and evaluated six commercial kits to serologically assess the infection status of HCWs in a hospital that reported the nosocomial infection of COVID-19. The severe case of COVID-19 was hospitalized 48 hours after onset of symptoms, thus, IgG levels in the process of seroconversion could be investigated. All HCWs participating in this study had undergone RT-qPCR. Therefore, the sensitivity and specificity of the antibody titer could be evaluated using the RT-qPCR results as a gold standard.

MATERIALS AND METHODS

Present study was an observational cross-sectional study. It was a joint study between Gunma University and Tone Central Hospital

and was conducted from July 2020 to December 2020. The Gunma University Ethical Review Board for Medical Research involving human subjects approved the study protocol (protocol number; HS2020-23). All ethical and confidentiality considerations were handled in accordance with the Helsinki Declaration.

Inclusion criteria: All 531 HCWs over the age of 20 at Tone Central Hospital were included as of April 2020.

Exclusion criteria: 102 out of 531 HCWs who did not consent to participate in this study were excluded from the study.

Study Subjects

On April 17, 2020, a SARS-CoV-2 infection in HCWs of Tone Central Hospital, Gunma, Japan, was confirmed by RT-qPCR. The HCW was in charge of a patient with COVID-19. As a result, nosocomial infection of COVID-19 was suspected. In response to this fact, RT-qPCR was immediately performed on all of 531 HCWs. Seven of 531 HCW were tested positive. On April 20, 2020, a nosocomial infection of SARS-CoV-2 was confirmed at this hospital, resulting in the testing of all personnel using RT-qPCR (https://www.pref.gunma.jp/02/d29g_00338.html). A total of 429 out of 531 HCWs voluntarily participated in this study with written informed consent. Among 429 participants, six tested positive and the remaining 423 tested negative. Therefore, six of 429 participants were determined to have nosocomial infections with SARS-CoV-2 and the remaining 423 participants were not infected with SARS-CoV-2. Seven mL of blood from the 429 participants were sampled 12 weeks after the RT-qPCR. All of 429 participants were not vaccinated against SARS-CoV-2 at the time of blood sampling. No nosocomial infection of COVID-19 occurred at Gunma University during this study [4].

RT-qPCR

The RT-qPCR procedure was done as described in a previous report [11]. Briefly, RNA was extracted from pharyngeal swabs using the QIAamp Viral RNA Mini kit (QIAGEN, Valencia, VA, USA) according to the manufacturer's instructions. The primer set used for RT-qPCR targeted the specific SARS-CoV-2 gene encoding the N protein (N2). Control standard RNA was donated by the National Institute of Infectious Diseases [12]. RT-qPCR was performed with a 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) as follows: reverse transcription (50°C for 30 min) and denaturation at 95°C for 15 min to activate DNA polymerase, followed by 45 cycles of amplification with denaturation at 95°C for 15 sec, and annealing and extension at 60°C for 1 min using the QuantiTect Probe RT-PCR Kit (QIAGEN). Amplified data were collected and analysed using the 7500 fast System Software v2.0.6 (Thermo Fisher Scientific). The primers and probes used in RT-qPCR are described as the follows; Name, Sequence (5' to 3'), Nucleotide position, respectively, Primer F; NIID_2019-nCOV_N_F2, AAATTTTGGGGACCAGGAAC, 29142-29161, Primer R; NIID_2019-nCOV_N_R2, TGGCAGCTGTGTAGGTCAAC, 29299-29280, Probe; NIID_2019-nCOV_N_P2, FAM-ATGTCGCGCATTGGCATGGA-BHQ, 29239-29258. The results were validated by comparing the results to those from RT-qPCRs using synthesized RNA of an internal control [12].

Measurement of specific IgG against SARS-CoV-2

Six immunoassays were performed according to the manufacturers' instructions. IgG antibodies to the nucleocapsid protein (anti-nucleocapsid IgG) were measured using a Roche kit (Roche Diagnostics K.K., Tokyo, Japan; positive signals are reported at a cut-off index of ≥1.0 U/mL), an Abbott kit (Abbott Japan LLC, Tokyo, Japan; positive signals are reported at a cut-off index of ≥1.4 U/mL), and a Euglena kit (Euglena Co., Ltd, Tokyo, Japan, and Order-made Medical Research Inc., Tokyo, Japan; positive signals are reported at cut-off index of ≥2.0 U/mL). IgG antibodies to the S1 subunit of the spike protein (anti-spike IgG) were measured using the Euglena (positive signals are reported at a cut-off index of ≥1.0 U/mL) and Roche kits (positive signals are reported at a cut-off index of ≥0.8 U/mL) and the Abbott kit (positive signals are reported at a cut-off index of ≥50 U/mL). The antibody titer against SARS-CoV-2 was measured at Gunma University Hospital. Both anti-nucleocapsid IgG and anti-spike IgG positive indicated positive for COVID-19 disease and negative of either is false positive. The sensitivity and specificity of each kit was compared to against RT-qPCR.

STATISTICAL ANALYSIS

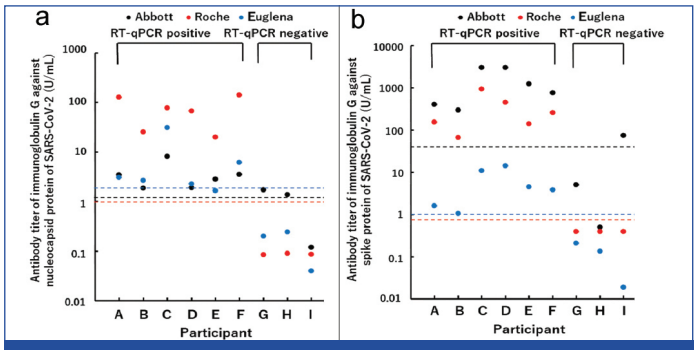
All statistical analysis was performed by Chi-square test using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics version 25.0 (Armonk, NY, USA). Statistical significance was set at p-value <0.05.

RESULTS

Characteristics of the 429 participants (HCWs of Tone Central Hospital) is shown in [Table/Fig-1]. Among the 429 participants, six were RT-qPCR positive [Table/Fig-2a,b] participants (A-F). The severity of COVID-19 among these six HCWs ranged from mild to moderate; therefore, mechanical ventilation was not needed. [Table/Fig-3] and [Table/Fig-4] showed the sensitivity, specificity, and positive and negative agreement rates against RT-qPCR among the three assay kits during measurements of anti-nucleocapsid IgG [Table/Fig-3] and anti-spike IgG [Table/Fig-4]. Anti-nucleocapsid IgG antibody titers measured using the Roche kit agreed perfectly with the RT-qPCR results [Table/Fig-3]. Two participants were positive for anti-nucleocapsid IgG as measured using the Abbott kit [Table/Fig-2a], participant G; 1.7 U/mL and H; 1.4 U/mL], but tested RT-qPCR negative. Both of these participants tested negative for the anti-nucleocapsid IgG as measured using the Roche and Euglena kits [Table/Fig-2a]. Additionally, both of these patients tested negative for the anti-spike IgG measured by assay kits provided by Abbott,

		Male	Female	Total
Age (years) 25%-75%		36 (29-45)	43 (33-51)	42 (32-50)
RT-qPCR	Clinical severity	Mild	Mild-Moderate	Mild-Moderate
	Positive (n)	1	5	6
	Negative (n)	89	334	423

[Table/Fig-1]: Clinical characteristics of 429 healthcare workers of Tone Central Hospital in which nosocomial infection of COVID-19 occurred.
RT-qPCR: real-time reverse transcription-polymerase chain reaction.



[Table/Fig-2]: Immunoassays for the detection of IgGs against the nucleocapsid; a) and spike proteins; b) of SARS-CoV-2 were performed according to manufacturers' instructions.
a) IgG antibodies to the nucleocapsid protein; positive signals are reported at a cut-off index of ≥1.0 U/mL (red dotted line) for Roche (red dot); at a cut-off index of ≥1.4 U/mL (black dotted line) for Abbott (black dot); and at a cut-off index of ≥2.0 U/mL (blue dotted line) for Euglena (blue dot). b) IgG antibodies to the S1 subunit of the spike protein; Positive signals were reported at a cut-off index of ≥0.8 U/mL (red dotted line) for Roche (red dot), ≥50 U/mL (black dotted line) for Abbott (black dot), and a cut-off of ≥1.0 U/mL (blue dotted baseline) for Euglena (blue dot). The alphabets on the horizontal axis indicate the participant. Participants A–F are healthcare workers with mild or moderate COVID-19 in Tone Central Hospital, and participants G–I are healthcare workers without COVID-19.
IgG; Immunoglobulin G

Euglena and Roche, repectivey. Therefore, the positive results of participant G and H as measured using the Abbott kit were false positives. One participant tested negative for anti-nucleocapsid IgG, but positive for anti-spike IgG as measured using the Euglena kit. The participant tested positive for anti-nucleocapsid IgG and anti-spike IgG as measured by the Abbott and Roche kit. The participant tested positive according to RT-qPCR [Table/Fig-2a-e]. In this case, the antibody titer for the anti-nucleocapsid IgG was 1.7 U/mL, and the cut-off value was 2.0 U/mL; thus, the negative Euglena kit results due to the cut-off value.

The antibody titers for the anti-spike IgG measured by using the Roche and Euglena kits agreed perfectly with the RT-qPCR results [Table/Fig-4]. As a result, no significant difference in sensitivity and specificity was observed between these Abbott, Euglena, and Roche kits [Table/Fig-3,4].

Kit used			RT-qPCR		Total
			Positive	Negative	
Abbott	Immunoglobulin G against nucleocapsid protein	Positive	6	2	8
		Negative	0	421	421
		Total	6	423	429
	Agreement rate (%)	Negative	100	Positive	75
	Sensitivity (%)	100	Specificity (%)		99.5
Euglena	Immunoglobulin G against nucleocapsid protein	Positive	5	0	5
		Negative	1	423	424
		Total	6	423	429
	Agreement rate (%)	Negative	99.8	Positive	100
	Sensitivity (%)	83.3	Specificity (%)		100
Roche	Immunoglobulin G against nucleocapsid protein	Positive	6	0	6
		Negative	0	423	423
		Total	6	423	429
	Agreement rate (%)	Negative	100	Positive	100
	Sensitivity (%)	100	Specificity (%)		100
Chi-square test	Abbott vs Euglena p = 0.360	Abbott vs Roche p = 0.980		Euglena vs Roche p = 0.386	

[Table/Fig-3]: Rate of concordance between RT-qPCR and assay kit provided by three manufacturers for detection of specific immunoglobulin G against nucleocapsid protein of SARS-CoV-2.
RT-qPCR: real-time reverse transcription-polymerase chain reaction.

Kit used			RT-qPCR		Total
			Positive	Negative	
Abbott	Immunoglobulin G against spike protein	Positive	6	1	7
		Negative	0	422	422
		Total	6	423	429
	Agreement rate (%)	Negative	100	Positive	85.7
	Sensitivity (%)	100	Specificity (%)		99.8
Euglena	Immunoglobulin G against spike protein	Positive	6	0	6
		Negative	0	423	423
		Total	6	423	429
	Agreement rate (%)	Negative	100	Positive	100
	Sensitivity (%)	100	Specificity (%)		100
Roche	Immunoglobulin G against spike protein	Positive	6	0	6
		Negative	0	423	423
		Total	6	423	429
	Agreement rate (%)	Negative	100	Positive	100
	Sensitivity (%)	100	Specificity (%)		100
Chi-square test	Abbott vs Euglena p=0.992	Abbott vs Roche p=0.992		Euglena vs Roche p=1.000	

[Table/Fig-4]: Rate of concordance between RT-qPCR and assay kit provided by three manufacturers for detection of specific immunoglobulin G against spike protein of SARS-CoV-2.
RT-qPCR: real-time reverse transcription-polymerase chain reaction.

Participant I tested positive for the anti-spike IgG (75 U/mL) as measured using the Abbott kit but tested negative using RT-qPCR [Table/Fig-2b]. Participant I tested negative for the anti-spike IgG and anti-nucleocapsid IgG as measured by using the Roche and Euglena kits. Therefore, the positive result by Abbott assay kit of participant I was false positive due to the cut-off value.

DISCUSSION

In this study, the diagnostic value of six commercial immunoassay kits was evaluated. The six kits were able to detect anti-nucleocapsid IgG or anti-spike IgG. The serological survey of the HCWs showed comparable sensitivity and specificity between all six kits. These kits could be used to assess the immune status of an individual. However, testing only for the presence of nucleocapsid antibodies may result in false positives. Additionally, the emergence of variants, such as Delta and Omicron, may have also affected the antigenicity of SARS-CoV-2.

The antibody titers for anti-nucleocapsid IgG and anti-spike IgG among HCWs of Tone Central Hospital (where a nosocomial infection of the SARS-CoV-2 occurred), as measured by the assay kits were compared with the RT-qPCR results. The results of the Roche kit for anti-nucleocapsid IgG and anti-spike IgG agreed completely with RT-qPCR results. However, an another laboratory reported the discordant results of anti-nucleocapsid IgG and anti-spike IgG measured by Roche kit [4,13]. In a previous study, one out of 769 HCWs showed discordant results, reporting that HCW was seropositive for anti-nucleocapsid IgG but seronegative for anti-spike IgG [4]. Therefore, a false positive was detected by Roche kit. In this study, there were discordant results between the anti-nucleocapsid IgG and anti-spike IgG when the results of the Abbott and Euglena kits were compared to those of the RT-qPCR for both positive and negative participants, and it was impossible to avoid false positives when detecting anti-nucleocapsid IgG. These results indicate the limitation of assessing only anti-nucleocapsid IgG. As in previous reports, false-positive results could lead us to overestimate seroprevalence and infections [3].

Simultaneously testing for anti-nucleocapsid IgG and anti-spike IgG helps detect active SARS-CoV-2 infection among asymptomatic and unvaccinated individuals [4], however, this situation is affected by the implementation of vaccination programs against SARS-CoV-2. Following the global outbreaks caused by the Delta and Omicron variants, booster vaccinations have been promoted. Most vaccinated individuals who were not infected with SARS-CoV-2 were seropositive for anti-spike IgG and seronegative for anti-nucleocapsid IgG [3]. Therefore, to confirm a history of SARS-CoV-2 infection, simultaneous testing for anti-nucleocapsid IgG and anti-spike IgG is necessary. Serological screening of anti-nucleocapsid IgG would contribute to the epidemiological study of SARS-CoV-2 [2,3].

Current study used three kits for six immunoassays to measure the changes in the levels anti-nucleocapsid IgG and anti-spike IgG in a severe case of COVID-19 reported by Yatomi M et al., [11] (data not shown). All three kits that measured the anti-spike IgG level yielded similar results. The initial appearance and peak levels of anti-spike IgG, in particular, were comparable for all three kits. Meanwhile, the Abbott and Roche kits also showed similar changes in the levels of anti-nucleocapsid IgG, but differed from the results of the Euglena assay, which showed early seroconversion. Additionally, the Euglena kit for anti-nucleocapsid IgG yielded one false-negative result.

The antibody titers of infected patients continued to increase 10 days after the onset of symptoms; therefore, collecting successive serum samples during the convalescent phase helps reveal the kinetics of immune memory during SARS-CoV-2 infections [14]. Previous reports have shown that seroconversion occurred 7-14 days after onset of symptoms [14,15]. Consistent with previous reports [14,15], the circulating levels of anti-nucleocapsid IgG and anti-spike IgG

in the patient with severe COVID-19 started to increase nine days after diagnosis and exceeded cut-off values at 9–11 days after diagnosis as determined by RT-qPCR. In all cases, the circulating levels of anti-nucleocapsid IgG and anti-spike IgG peaked at 17-19 days after diagnosis. As reported by previous reports, it takes ~14 days to detect anti-nucleocapsid IgG or anti-spike IgG [7,8]. Thus, measuring antibodies is not useful for diagnosing COVID-19 during the acute phase of infection [7,8].

The magnitude of the antibody response reflects the antibody production levels [16], which are correlated with the virus neutralisation titer [14]. Rapid seroconversion is related to decrease viral loads during the acute phase of COVID-19 [17]. The memory T and B cells produced in response to SARS-CoV-2 may limit SARS-CoV-2 dissemination and/or accumulation of viral load, resulting in a less severe course of disease [16]. Present data and investigations indicated that circulating antibody levels are not associated with clinical severity. On the contrary, the safety of hyperimmune intravenous immunoglobulin (hIVIG) depends on the endogenous neutralising antibodies of the recipient [18,19]. The fact indicated the contribution of serological screening of IgG against SARS-CoV-2. The anti-spike IgG concentrations as measured with the Roche assay correlate well with SARS-CoV-2 neutralisation activities [20], whereas the seroprevalence of anti-nucleocapsid IgG evaluated by the Abbott assay kit was suggested to be indicative of a productive and polyclonal humeral immune response that includes neutralising activity [21]. Thus, this study may contribute to the development of an appropriate treatment strategy against SARS-CoV-2 infection.

Limitation(s)

The sample size was small, and it consisted of participants from a single centre. For more conclusive findings, future studies should involve a large number of targeted patients, inpatients, and outpatients.

CONCLUSION(S)

Present study validated six immunoassays developed by Abbott, Euglena, and Roche for the determination of antibody titer for anti-nucleocapsid IgG and anti-spike IgG. There were no major differences among these kits. All of these assays can detect anti-nucleocapsid IgG or anti-spike IgG, but some assays yielded false positive and false negative results. Single measurement of anti-nucleocapsid IgG or anti-spike IgG could not exclude false positives to evaluate past history of SARS-CoV-2 infection.

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