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Evaluation of the efficacy of four anti-SARS-CoV-2 antibodies after vaccination using kits from two manufacturers: A prospective, longitudinal, cohort study at 11 serial time points within 160 days

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ABSTRACT

Purpose: The accuracy of level of anti- severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies is a great concern. We aimed to compare the efficacy of anti-SARS-CoV-2 antibody detection kits from two manufacturers in evaluating the efficacy of SARS-CoV-2 vaccines.

Methods: The immune responses and consistency of four anti-SARS-CoV-2 antibodies were evaluated using two manufacturers' antibody kits (A and B) in 61 subjects within 160 days after vaccination with the CoronaVac vaccine.

Results: The total seropositivity rates of neutralizing antibodies and IgM antibodies detected by kit A were higher than those detected by kit B ($P = 0.003$ and $P < 0.001$, respectively). Conversely, the total seropositivity rates of total antibodies and IgG antibodies were higher in kit B than kit A ($P < 0.001$ and $P < 0.001$, respectively). The consistency rates showed less than 90% agreement between the kits for the detection of the four antibodies, and the κ score showed moderate or substantial consistency. The half-lives of neutralizing antibodies, total antibodies, and IgG antibodies within 160 days after vaccination, detected by kit A were 63.88 days, 80.50 days, and 63.70 days, respectively and by kit B were 97.06 days, 65.41 days, and 77.99 days, respectively.

Conclusion: The efficacy of antibody detection differed between the two commercial anti-SARS-CoV-2 antibody kits, although there was moderate consistency, which may affect the clinical application and formulation of the vaccine strategy.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is spreading worldwide, leading to extremely high morbidity and mortality [1,2]. Vaccination is one of the most effective interventions to substantially reduce severe disease and death due to SARS-CoV-2 infection [3]. A large-scale vaccine inoculation program against SARS-CoV-2 was implemented globally [4]. The level of anti-SARS-CoV-2 antibodies is a critical parameter for evaluating the effects of vaccination and SARS-CoV-2 infection. Careful screening of antibodies is of great significance for the development of COVID-19 therapies and guidance of vaccine strategies [5,6]. Several anti-SARS-CoV-2 antibody test kits have been approved for emergency use to provide tools for large-scale clinical and epidemiological screenings. Recently, a large number of serological assays were validated in a restricted number of samples [7], and several serological tests were not recommended for COVID-19 population surveillance due to poor performance and low sensitivity [8,9]. The accuracy of these methods is an ongoing concern. Here, we compared anti-SARS-CoV-2 antibody detection kits from two manufacturers that use the receptor-binding domain (RBD) and spike epitopes as target antigens to evaluate the efficacy of SARS-CoV-2 vaccines. The immune responses, seropositivity rates, and attenuation half-lives of four anti-SARS-CoV-2 antibodies (a neutralizing antibody, a total antibody, an IgG antibody,

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and an IgM antibody) were evaluated using antibody test kits from two manufacturers in 61 CoronaVac vaccine recipients within 160 days after vaccination, and the consistency of the test results of both kits was analyzed. By comparing the kits, we aimed to provide a basis for the clinical and epidemiological application of anti-SARS-COV-2-specific antibody kits, which will contribute to the authorities' quality monitoring and quality control protocols.

2. Methods

2.1. Study design and participants

The vaccination cohort was designed as described in our previous report [10]. The participants received the first standard dose (0.5 mL/dose) of inactivated SARS-CoV-2 vaccine (Sinovac Biotech Ltd., Beijing, China) on January 24, 2021, and the second dose 28 days later. Serum samples were then collected by centrifugation from the 61 participants at 11 time points over 160 days, and the tests on the serum were completed within six hours. This study was approved by the Institutional Ethics Committee of Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University and was in compliance with national legislation and the Declaration of Helsinki guidelines. All the participants provided written informed consent.

2.2. Detection of anti-SARS-CoV-2 antibodies

For both kit A (Anto Biological Pharmacy Enterprise Co., Ltd., Zhengzhou, China) and kit B (Xiamen Innovax Biotech Co., Ltd., Xiamen, China), anti-SARS-CoV-2 antibody tests were performed using a chemiluminescent microparticle immunoassay on a compatible instrument according to the manufacturers' instructions. Antigen epitope and antibody detection methodologies differed between the two manufacturers' kits (Table 1). The neutralizing antibody assay for kit A was based on the competition method. When SARS-CoV-2-neutralizing antibodies were present in the sample, they bound to the horseradish peroxidase (HRP)-labeled RBD antigen and blocked (neutralized) the binding of angiotensin-converting enzyme 2 (ACE2) (coated on microparticles) to the RBD antigen. The HRP-labeled RBD antigen that was not neutralized by SARS-CoV-2-neutralizing antibodies formed a complex with ACE2 on the microparticles. Thus, the relative light units (RLUs) were inversely proportional to the amount of SARS-CoV-2-specific neutralizing antibodies in the sample. The neutralizing antibody assay for kit B was also based on a competition assay, in which neutralizing antibodies in the sample competed with biotinylated anti-SARS-CoV-2 antibodies for binding to the acridine ester-labeled spike protein to form "biotinylated SARS-CoV-2 antibody–acridine ester spike protein" complexes. The concentration of competing neutralizing antibodies in the sample was inversely proportional to the RLU detected. The neutralizing antibody titer was calibrated to the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin and was recorded in IU/mL [11]. A neutralizing antibody concentration $\geq$ 54 IU/mL was defined as positive [12].

For the total antibody, both kits A and B used the same RBD of the spike protein as the coating antigen. Kit A detected the total antibody using a double-antigen sandwich method: the microparticle coated with the RBD of the SARS-CoV-2 spike protein was combined with the sample and detected by an anti-human immunoglobulin antibody labeled with an enzyme, and a total antibody concentration $\geq$ 8 AU/mL was defined as positive. Kit B measured the total antibody using a double-antigen sandwich method, and the total antibody was recorded as signal/cut-off (S/CO) (RLU of samples to be tested/cut off). S/CO $\geq$ 1.00 was defined as positive. Both kits A and B used an indirect method to detect the level of IgG antibodies, but kit A used the SARS-CoV-2 spike protein for coating and kit B used the RBD. IgM antibody levels were detected using a capture method. The enzyme conjugate was HRP-labeled spike protein in kit A, and acridine ester-labeled RBD was used as the antigen in kit B. Both IgG and IgM antibody titers were recorded as S/CO. S/CO $<$ 1.00 was considered negative and S/CO $\geq$ 1.00 was considered positive (Table 1).

2.3. Statistical analysis

Data were analyzed using IBM SPSS Statistics version 26 (SPSS, Inc., Chicago, IL, USA). A McNemar's test was used to compare test rates. The agreement rate and Cohen's kappa coefficient (κ) were used to analyze the consistency. The consistency analysis of the two manufacturers' kits was classified as nearly perfect (0.81–1.0), substantial (0.61–0.8), moderate (0.41–0.6), fair (0.21–0.4), mild (0–0.2) or poor ($<$0) according to the consistency of the κ value [13,14]. Power law models were used to analyze antibody waning [15]. A summary independent-samples *t*-test was used to analyze the differences in half-life between the two kits. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Seropositive rates of four anti-SARS-CoV-2 antibodies determined by kits from two manufacturers

The results of the dynamic evaluation of the seropositivity rates of each antibody detected by the kits from the two manufacturers at each time point are shown in Table 2. The seropositivity rate of neutralizing antibodies increased slowly from day 0 to day 28 after vaccination. On day 28, the seropositive rate of neutralizing antibodies detected by kit A was 4.92%, and that detected by kit B was 8.20%. The seropositivity rate of neutralizing antibodies determined by the kits from the two manufacturers began to increase rapidly after the second dose, and the seropositivity rates were 52.46% and 68.65%, respectively. There were significant differences between the seropositive rates detected by the kits from the two manufacturers from the 42nd day to the 56th day, and the seropositivity rates of neutralizing antibodies detected by kit A were higher than those detected by kit B ($P < 0.001$). On days 130 and 160, the seropositivity rate of neutralizing antibodies decreased significantly. On day 160, the seropositivity rates determined by the kits from the two manufacturers were 19.67% and 18.03%, respectively, and there was no significant difference between the two kits ($P > 0.05$). The total seropositivity rate of neutralizing antibodies detected using kit A was higher

Table 1
The coated antigen types and detection methods of the kits from the two manufacturers.

	Methodology		Antigen epitope		Cut-off value		Quantitative/ Qualitative	
	Kit A	Kit B	Kit A	Kit B	Kit A	Kit B	Kit A	Kit B
Neutralizing antibody	Competitive method	Competitive method	RBD	Spike protein	≥54 IU/mL	≥54 IU/mL	Quantitative	Quantitative
Total antibody	Double-antigen sandwich method	Double-antigen Sandwich method	RBD	RBD	≥8AU/mL	S/CO ≥ 1.00	Quantitative	Qualitative
IgG antibody	Indirect method	Indirect method	Spike protein	RBD	S/CO ≥ 1.00	S/CO ≥ 1.00	Qualitative	Qualitative
IgM antibody	Capture method	Capture method	Spike protein	RBD	S/CO ≥ 1.00	S/CO ≥ 1.00	Qualitative	Qualitative

RBD: receptor-binding domain of the spike protein; S/CO: RLU of samples to be tested/cut off.

Table 2
Antibody seropositivity rates detected by kits from two manufacturer at 11 serial time points within 160 days after vaccination (%).

Antibody type	Kit	D0	D7	D14	D21	D28	D35	D42	D49	D56	D130	D160	P_a
Neutralizing antibody	**Kit A**	0	0	1.64	3.28	4.92	52.46	95.08	95.08	91.80	22.95	19.67	0.003
	Kit B	0	4.92	6.56	6.56	8.20	68.65	68.85	63.93	55.74	26.23	18.03	
P_b		ns	ns	ns	ns	ns	ns	<0.001	<0.001	<0.001	ns	ns	
Total antibody	**Kit A**	0	0	26.23	34.43	27.87	83.61	100.00	100.00	100.00	83.61	54.10	<0.001
	Kit B	0	1.60	42.60	62.30	72.10	100.00	100.00	100.00	100.00	90.20	90.20	
P_c		ns	ns	0.013	<0.001	<0.001	ns	ns	ns	ns	ns	<0.001	
IgG antibody	**Kit A**	0	0	4.92	9.84	21.31	77.05	100.00	100.00	100.00	78.69	50.82	<0.001
	Kit B	0	9.80	18.00	44.30	75.40	100.00	100.00	100.00	100.00	91.80	73.77	
P_d		ns	ns	0.008	<0.001	<0.001	ns	ns	ns	ns	0.039	<0.001	
IgM antibody	**Kit A**	0	0	1.64	1.64	3.28	34.43	59.02	42.62	24.59	3.28	3.28	<0.001
	Kit B	0	0	0	0	4.90	34.40	26.20	24.60	9.80	0	0	
P_e		ns	ns	ns	ns	ns	ns	<0.001	<0.001	0.012	ns	ns	

ns: not significant. McNemar's test was used to assess the differences between the two manufacturer's kits. P_a: Compared to the total positivity rate of the two kits at 160 days. $P_{b\text{-}e}$: Compared to the positivity rate of the two kits at each time point.

than that detected using kit B ($P = 0.003$).

The seropositivity rate of total antibodies was low, and there was no difference between the kits from the two manufacturers on days 0 and 7 ($P > 0.05$). However, the seropositivity rate of total antibodies detected by kit B increased rapidly from the 14th day to the 28th day, which was higher than that detected by kit A ($P = 0.013$–$P < 0.001$). Subsequently, the seropositivity rate of total antibodies determined with both kits reached 100% after the 42nd day and remained unchanged until the 56th day. The seropositivity rate of total antibodies determined with both kits remained relatively high on day 130, but on day 160, the seropositivity rate of total antibodies detected by kit A was lower than that detected by kit B ($P < 0.001$). The total seropositivity rate of anti-SARS-CoV-2 total antibody determined using kit A was lower than that determined using kit B ($P < 0.001$).

The seropositivity rates of IgG antibodies were similar to those of the total antibodies, as determined by the two manufacturer's kits. From days 14 to 28 and after day 130, the seropositivity rates determined using kit B were higher than those determined using kit A ($P = 0.039$–0.001). The total seropositivity rate of anti-SARS-CoV-2 IgG antibodies determined using kit A was lower than that determined using kit B ($P < 0.001$).

The seropositivity rates of IgM antibodies detected by both kits remained low at most time points. From day 42 to 56, the seropositivity rate of IgM antibodies detected by kit A was higher than that detected by kit B ($P = 0.012$–$P < 0.001$). The total seropositivity rate of anti-SARS-CoV-2 IgM antibodies determined using kit A was higher than that determined using kit B ($P < 0.001$).

3.2. Consistency of detection of four anti-SARS-CoV-2 antibodies by kits from two manufacturers

The consistency rate and kappa value were calculated to perform consistency analysis on the seropositivity rates of the four anti-SARS-CoV-2 antibodies detected by the kits from the two manufacturers. The consistency rates of neutralizing, total, IgG, and IgM antibodies detected by the kits from the two manufacturers were 79.73%, 83.75%, 82.71%, and 88.38%, respectively, and all were less than 90%. Kappa values were 0.539, 0.661, 0.656, and 0.454, respectively (Table 3). The results indicated that the kits from the two manufacturers for neutralizing antibodies and IgM antibodies showed moderate consistency, and total antibody and IgG antibody detection demonstrated substantial consistency.

3.3. Decay of anti-SARS-CoV-2 antibodies after vaccination determined by the kits from the two manufacturers

To measure anti-SARS-CoV-2 antibody waning after vaccination, the power law model was fitted, and half-life analyses were performed based on antibody levels at 11 serial time points after vaccination. For kit A, the half-lives of neutralizing antibodies, total antibodies, and IgG antibodies were 63.88 (95% CI, 57.20–72.79) days, 80.50 (95% CI, 68.79–96.89) days, and 63.70 (95% CI, 56.36–73.21) days, respectively, within 160 days after vaccination. For kit B, the half-lives of neutralizing antibodies, total antibodies, and IgG antibodies were 97.06 (95% CI, 77.38–129.64) days, 65.41 (95% CI, 55.68–79.19) days, and 77.99 (95% CI, 72.32–84.61) days, respectively (Fig. 1A-C). Summary independent-samples *t*-test results showed that the half-lives of neutralizing antibodies, total antibodies and IgG antibodies were significantly different between the two kits ($P < 0.001$). Owing to the small amount of data, the fitting curves and decay half-lives of IgM antibodies failed to fit the model, and the decay half-lives could not be estimated. These results showed that the estimated half-lives of the four antibodies differed between the two commercial kits.

Table 3
Consistency analysis of four anti-SARS-CoV-2 antibodies determined by kits from two manufacturers.

		Kit B			
Antibody type	Kit A	+	-	Consistency rate	Kappa
Neutralizing antibody	+	300	172	79.73%	0.539
	–	100	770		
Total antibody	+	726	18	83.75%	0.661
	–	200	398		
IgG antibody	+	650	12	82.71%	0.656
	–	220	460		
IgM antibody	+	82	130	88.38%	0.454
	–	26	1104		

"+": positive; "-": negative. The agreement rate and Cohen's kappa coefficient (κ) were used to analyze the consistency.

4. Discussion

Extensive and reliable antibody tests have contributed to the serological diagnosis of COVID-19 and to vaccine serological studies. Increased accuracy and consistency of antibody test kits provides a reliable guarantee for evaluating the effects of vaccination and SARS-CoV-2 infection. In our study, the same vaccinated population showed different antibody responses when using the different kits. This conclusion was supported by the finding that the total seropositivity rates of the four anti-SARS-CoV-2 antibodies (neutralizing, total, IgG, and IgM antibodies) detected by the two commercial kits were different at 11 serial time points after vaccination. Consistency analysis also demonstrated that the four antibodies showed only moderate or substantial consistency (consistency rate from 79.73% to 88.38%, kappa values from 0.454 to 0.661). Further, the antibody decay using the power-law model showed different half-lives for the four antibodies determined by the kits from the two manufacturers.

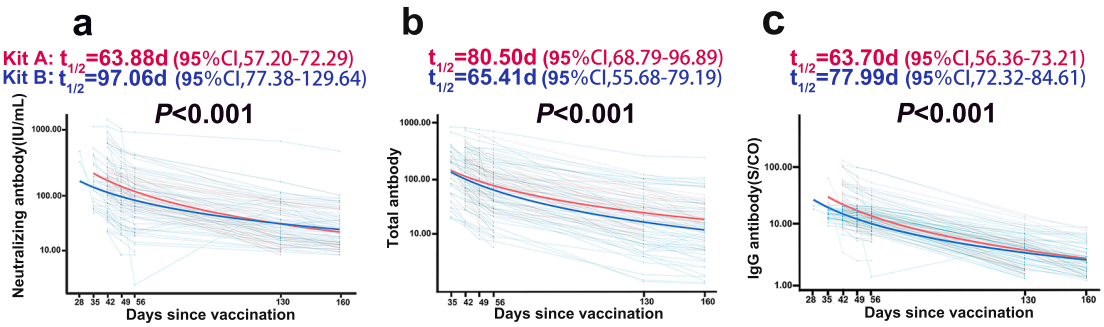

Fig. 1. Power law model for the decay half-life of four antibodies. a. Neutralizing antibody; b. total antibody; c. IgG antibody. Antibody decay curves and half-lives at day 120 estimated by kit A are shown in red, and those by kit B are shown in blue. A summary independent-samples *t*-test was used to analyze the differences in half-lives between two kits. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Establishing immune memory is essential for the defense against SARS-CoV-2 infection. The serological positivity rate and half-life of antibodies, especially neutralizing antibodies and IgG antibodies, are important indicators for evaluating vaccine efficacy. Neutralizing antibodies block the ability of SARS-CoV-2 to infect host cells by inhibiting the binding of the SARS-CoV-2 spike protein to host cell ACE2 [16,17] and are an important predictor of vaccine efficacy. Our previous study showed that neutralizing antibody and anti-RBD IgG antibody levels were strongly correlated. Thus, anti-RBD IgG antibody levels can be used for the assessment of immunity following SARS-CoV-2 infection or vaccination [18]. The results of this study confirmed that the vaccinated population had a high positive rate of neutralizing and IgG antibodies after the second dose of the vaccine, which could have a protective effect against SARS-CoV-2 infection. However, the total positivity rates of neutralizing and IgG antibodies were different when using kits from different manufacturers ($P < 0.001$). Similarly, the positivity rates of total antibodies and IgM antibodies determined by the kits from the two manufacturers were also shown to be different in the vaccinated population. Furthermore, our results showed that the half-lives of the four antibodies estimated using the power law model were different between the two commercial kits. Notably, the serological positivity rate and half-life of antibodies might not be sufficient to reflect a robust immune response. One opinion is that SARS-CoV-2-specific memory T and B cells are important for long-term protection [18]. Due to the lack of data on long-term protection among the vaccinated population, we found only different serological positive rates and half-lives of antibodies and could not determine which reagent was more suitable for antibody surveillance in the vaccinated population.

An understanding of the degree of immune protection conferred by a vaccine is urgently required to assist in the future deployment of vaccines. The selection of a high-performance antibody detection kit to assess the degree of immune response to a vaccine is a common concern in developing vaccine strategies. In this study, four antibody responses to vaccine administration were detected using kits from two manufacturers in parallel. Two interrater reliability measures, the percentage of agreement and the κ score were used to compare the consistency of antibody measurements using kits from two manufacturers. The overall results showed less than 90% agreement in the detection of the four antibodies between the kits from the two manufacturers, and the κ score showed moderate or substantial consistency. Neutralizing antibodies and IgG antibodies showed moderate agreement, which may be caused by the different target sites of neutralizing antibodies and the different antibody detection methods. Although the same target antigen was used for total antibody detection in kits from the different companies, the κ score showed only substantial and imperfect consistency, which may be due to the nonconformity of the recombinant antigen from different companies. Therefore, to improve the consistency of antibody testing, it is important to standardize the process, and the quality control system should carry out external quality control led by health authorities to improve the consistency of different products. A clinical evaluation should be performed before selecting a certain reagent, and a traceability system should be established.

To the best of our knowledge, this is the first prospective cohort study of four anti-SARS-CoV-2 antibodies to compare serum antibody detection kits for evaluating the efficacy of SARS-CoV-2 vaccines after vaccination. However, this study had several limitations that need to be addressed. First, only dynamic changes in antibody levels were detected in vaccinees, and the role of anti-SARS-CoV-2 antibodies was not evaluated in COVID-19 patients. Second, the results of neutralizing antibody tests were not verified by the gold standard neutralization test. Only the consistency of the kits from the two manufacturers was compared, so no diagnostic performance evaluation was performed to judge the strengths or weaknesses of the kits from the two manufacturers.

In conclusion, the immune responses, seropositivity rates, and attenuation half-lives of the four anti-SARS-CoV-2 antibodies were different between the two manufacturer's commercial anti-SARS-CoV-2 antibody test kits, and the consistency between the kits was moderate, which may affect clinical application and the decision-making process for subsequent disease prevention and control and formulation of vaccine strategies.

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Author statement

Tian-Ci Yang and Jian-Jun Niu contributed to the experimental design. Lin Xie and Jian-Hang Xue was involved in the analysis and interpretation of the data. Xin-Qi Zheng collected the samples. Lin Xie and Qiu-Yan Xu wrote the first draft of the manuscript. Qiu-Yan Xu participated in the drawing of the half-life diagram. Tian-Ci Yang and Lin Xie reviewed the manuscript before submission for accuracy and intellectual content. All authors contributed to the article and approved the submitted version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

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