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Development and Evaluation of a Rapid Neutralizing Antibody Assay for COVID-19 Vaccination

Heshan Yu, Huan Liu, Yongju Yang, and Xuefeng Guan*

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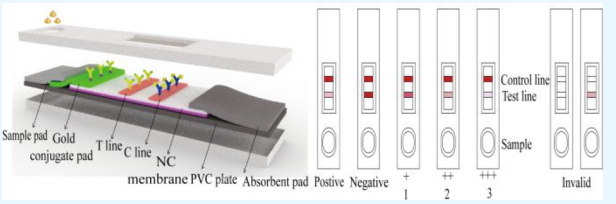
ABSTRACT: SARS-CoV-2 neutralizing antibodies have excellent application prospects in the prevention and treatment of COVID-19. This study established a competitive colloidal gold immunochromatography assay (GICA) to detect neutralizing antibodies against the receptor-binding domain (RBD) of SARS-CoV-2 in postvaccination serum. The sensitivity, stability, and specificity of GICA were evaluated using neutralizing antibody solution reference material and positive serum. The consistency and correlation between GICA, pseudovirus neutralization (PN) assay, and ELISA were compared. Consistency analysis of serum neutralizing antibody and specific IgG antibody titers was conducted, and changes in neutralizing antibodies and specific IgG antibodies in serum after inoculation with the homologous booster inactivated vaccine and recombinant vaccine were noted. The sensitivity of the reagent was 20.66 IU/L, and the specificity was 100%. There was a strong consistency and correlation between GICA and PN (κ = 0.886, n = 165; r = 0.918, P < 0.001). The correlation coefficient of serum anti-RBD antibody and specific IgG antibody titers was 0.5253 (P < 0.001). The specific IgG antibody titers in serum after (W4) inoculation with homologous booster inactivated vaccine were 10.80 (S/CO).The anti-RBD antibody titers were 28.33. The anti-RBD omicron variant (B.1.1.529) antibody titers were 11.67. After inoculation with the recombinant vaccine, the specific IgG antibody titers in the serum of W4 were 10.68. The serum anti-RBD antibody titers of W4 were 103.30. The serum anti-RBD omicron variant (B.1.1.529) antibody titers of W4 were 56.67. Therefore, vaccination of the third dose of the homologous booster inactivated vaccine and recombinant vaccine can enhance the level of neutralizing antibodies against the omicron variant (B.1.1.529). This study demonstrates that a GICA kit for neutralizing antibodies against the RBD of SARS-CoV-2 can be used for COVID-19 vaccine evaluation. Changes in titers enable long-term monitoring of a population's immunity and guide interventions when their immunity declines.



1. INTRODUCTION

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 has rapidly become an international public health crisis, causing significant damage to the lives and health of people and the global economy.[1,2] Respiratory droplets and contact are the main transmission routes, and rapid screening and isolation of infected people is key to the prevention and control of the pandemic.[3] Timely vaccination against coronavirus disease 2019 (COVID-19) is the most effective means of prevention and control. As of February 19, 2022, a total of 3,080,788 million doses of the COVID-19 vaccine have been reported.[4] With the application of the COVID-19 vaccine, the production of antibodies after vaccination has become a concern. SARS-CoV-2 belongs to the β-coronavirus, which includes four structural proteins: spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). In clinical studies of different types of COVID-19 vaccines, the detection of specific IgG and neutralizing antibody titers are the main indicators for evaluating vaccine immunogenicity.[5−7] Among them, SARS-CoV-2-specific IgG detects targets mainly against S and N proteins,[8] and the receptor-binding domain (RBD) in S proteins is thought to produce highly effective neutralizing antibodies.[9] Neutralizing antibodies are the key to establishing protective immunity. Therefore, immunogenicity analysis of vaccines, especially the analysis of neutralizing antibodies, is very important for the evaluation of vaccine efficacy.[10] Research shows that whether it is a natural infection or vaccine immunity, the body's immune response to SARS-CoV-2 generally declines within 6−12 months after initial infection or vaccination, so it is essential to detect neutralizing antibodies.[11−13] Currently, the wild virus plaque reduction neutralization test is the gold standard for the detection of neutralizing antibodies. However, the detection of SARS-CoV-2 neutralizing antibodies has low detection throughput, takes a long time, and needs to be completed in a BSL-3 laboratory. This method has high requirements for experimental conditions and personnel, and it is difficult to evaluate the

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production of antibodies after vaccination as a routine detection method in clinical laboratories. There is an urgent need to develop a rapid, easy-to-use screening method. Therefore, the colloidal gold test has the advantage of being widely used. It can detect whole blood, serum, and plasma with a simple operation (no professional equipment and professional technicians are required), fast detection speed (10−15 min in the whole process), and low cost. To date, blood-based diagnostic tests, such as rapid detection of antiviral antibodies or viral antigens, have been widely used in many clinical laboratories.[14−16] Considering all the advantages of this new test, it has the potential to become an auxiliary diagnostic method for the treatment of COVID-19 and has excellent potential in containing the global pandemic.

In this research, a rapid detection kit for SARS-CoV-2 neutralizing antibodies was designed based on competitive colloidal gold immunochromatography assay (GICA, Figure 1A), which was established using recombinant RBD proteins

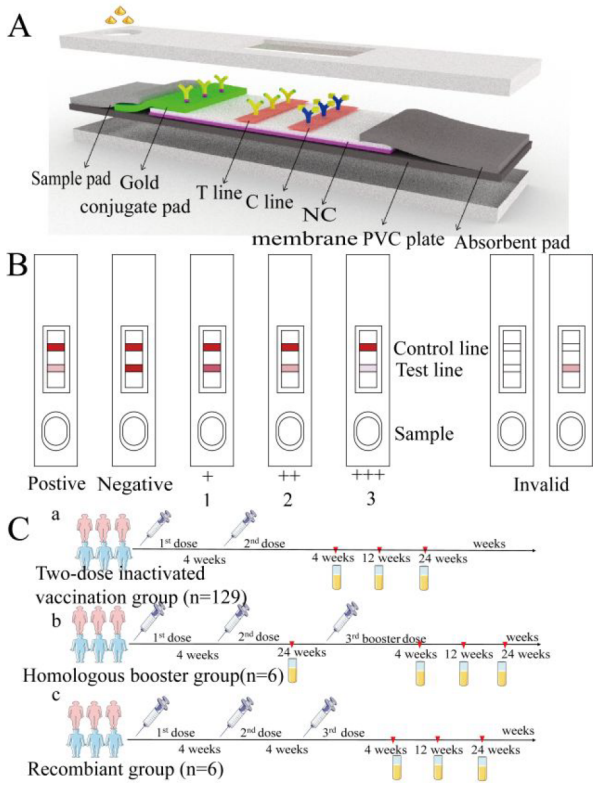

Figure 1. (A) Schematic illustration of a competitive GICA strip for neutralizing antibodies against the RBD of SARS-CoV-2. (B) Diagram of the SARS-CoV-2 neutralizing antibody test kit and its positive, negative, and invalid results. The appearance of one line indicates a positive result, whereas a valid negative test produces only the control line. Intensity of antibody: 0 = negative, 1 = +, 2 = + +, 3 = ++ +. (C) The details of the vaccine groups and serum samples collected in our study.

that can be used for detection in serum samples. The neutralizing antibodies in the samples will compete with angiotensin-converting enzyme 2 (ACE2) for GICA-RBD binding. ACE2 was used as the test line (T line), and goat antirabbit IgG was used as the control line (C line). If the sample solution contains neutralizing antibody, when the sample solution flows through the colloidal gold binding pad by capillary action, the neutralizing antibody binds to the colloidal gold-labeled RBD protein and continues to flow to the nitrocellulose membrane (NC membrane) ACE2 (T line). Since the RBD protein binding site is occupied by the neutralizing antibody and cannot bind to ACE2 protein, there is no reaction line at the T line position. Conversely, when there is no neutralizing antibody in the sample (or the content is low), the sample solution flows and drives the colloidal gold RBD protein to flow through the position of the T line, and the colloidal gold RBD protein is captured by the ACE2 protein, forming a red reaction line. The interpretation method of the test results of the colloidal gold SARS-CoV-2 neutralizing antibody rapid detection kit is shown in Figure 1B. A T line lighter than the reference line of the standard colorimetric card is considered positive, while the opposite is considered negative. The colloidal gold SARS-CoV-2 neutralizing antibody rapid test kit test results and standard colorimetric cards are detailed in Supporting Information Figure S1.

The sensitivity, specificity, and accuracy of the reagents were evaluated by detecting the solution reference material and positive serum. The neutralizing antibody titers against RBD of SARS-CoV-2 and the Omicron variant (B.1.1.529) in serum after inoculation with different types of vaccines were compared.

2. MATERIALS AND METHODS

2.1. Study Participants. SARS-CoV-2 immunoreactivity of serum samples from 141 healthy vaccinated individuals was studied. All study participants provided written informed consent. To protect the identity of respondents, personal identification was removed and was replaced with barcodes. We collected a total of 129 two-dose inactivated vaccine donors, 41 4-week serum samples, 40 12-week serum samples, and 48 24-week serum samples; 6 booster inactivated vaccine donors, 6 4-week serum samples, 6 12-week serum samples, 6 24-week serum samples; 6 recombination vaccine donors, 6 4-week serum samples, 6 12-week serum samples, and 6 24-week serum samples. Participants were split into two-dose inactivated vaccine groups (a, n = 129), booster inactivated vaccine groups (b, n = 6), and recombinant vaccine groups (c, n = 6) depending on COVID-19 vaccine type and inoculation time (4, 12, 24 weeks) (Figure 1C). This study was approved by the ethical committees of Affiliated Hospital of Liaoning University of Traditional Chinese Medicine (2022023CS-(KT)-017-01).

2.2. Proteins and Reagents. *2.2.1. Consumables for GICA Kit.* SARS-CoV-2 spike RBD protein was purchased from Huamei Biological Company (Wuhan, China). Neutralizing antibody solution reference material against the receptor-binding domain (RBD) of SARS-CoV-2 was obtained from the Chinese Institute of Metrology (Beijing, China). GICA nitrocellulose (NC) membranes, goat antirabbit IgG polyclonal antibody, and gold colloidal solution (size 40 nm) were obtained from FANTIBOY Biological Technology Company (Chongqing, China). Human ACE2 protein, Anti-SARS-CoV-2 Neutralizing Antibody Titer Serologic Assay Kit (Spike RBD), and Anti-SARS-CoV-2 Omicron variant (B.1.1.529) Neutralizing Antibody Serologic Assay Kit (Spike RBD) were purchased from ACROBiosystems (Beijing, China). Anti-SARS-CoV-2-specific IgM and IgG assays (chemiluminescence immunoassay) were obtained from Mike Biology Co., Ltd. (Chongqing, China).

2.2.2. Anti-SARS-CoV-2 Neutralizing Antibody Titer Assay (ELISA). A total of 50 μL of the diluted sample was added to

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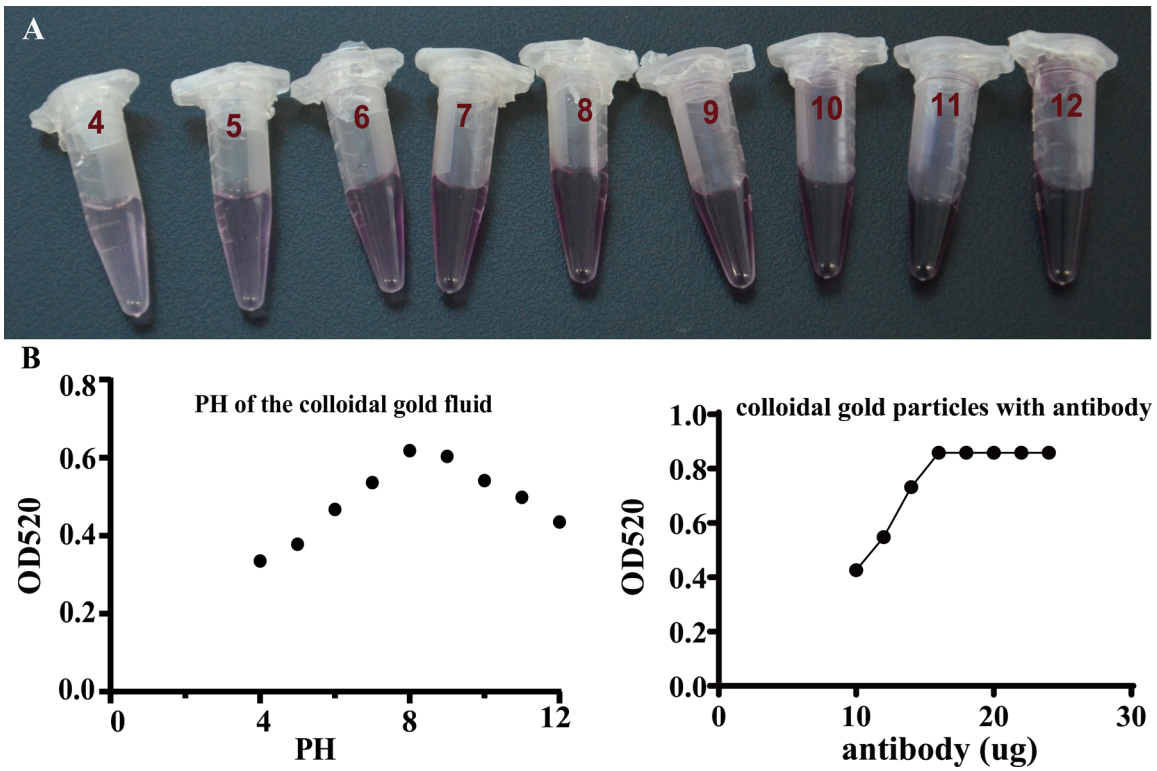

Figure 2. (A) Color renderings of colloidal gold solutions with different pH values, from left to right: pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0. (B) 520 nm OD value of colloidal gold solution at different pH values. (C) Minimum amount of colloidal gold-labeled RBD antigen protein.

the corresponding wells, and 50 μL of HRP-SARS-CoV-2 Spike RBD working fluid was added to each well. The sample was shaken gently to mix and incubated at 37 °C for 1.0 h. The solution was removed, 300 μL washing buffer was added to each well, and any remaining washing buffer was removed by aspiration or decanting. The plate was inverted and blotted with paper towels. The steps above were repeated three times.

To each well, 100 μL substrate solution was added. The plate was sealed with microplate sealing film and incubated at 37 °C for 20 min. Stop solution (50 μL) was added to each well and shaken gently to mix. The absorbance was read at 450 and 630 nm using a microplate reader.

For qualitative detection of antibodies: Positive reading (percentage inhibition of sample ≥ cutoff value meant anti-SARS-CoV-2 neutralizing antibodies were detected). Negative reading: (percent inhibition of sample < cut-off value meant Anti-SARS-CoV-2 neutralizing antibodies were not detected). For determination of antibody titer, the positive sample was diluted with a gradient, and the antibody titer of the sample corresponded to the highest dilution factor with a positive reading. The cutoff value = 20% signal inhibition, and the percent inhibition = (1 − OD value of sample/OD value of negative control) × 100%.

2.2.3. Anti-SARS-CoV-2-Specific IgM and IgG Assay (Chemiluminescence Immunoassay). The standard for the determination of the results of the SARS-CoV-2-specific IgG and IgM detection kits was as follows: When S/CO ≥ 1.0, the antibody test result was judged to be positive, and when S/CO < 1.0, the antibody test result was judged to be negative.

2.2.4. Pseudovirus Neutralizing Antibody Assay. The samples were diluted with complete DMEM in a 96-well white flat bottom plate to reach a volume of 75 μL per well, and then 25 μL pseudovirus suspension was added to each well to reach a final volume of 100 μL per well. The plate was gently shaken to mix well and incubated in a 5% CO_2 incubator at 37 °C for 60 min. HEK293/human ACE2 overexpression stable cells were digested and resuspended in complete DMEM. A total of 100 μL of the cell suspension was seeded per well in a 96-well plate. The plate was shaken gently to mix well and incubated in a 5% CO_2 incubator at 37 °C for 48 h. The 96-well plate was removed, 100 μL medium was discarded per well, and 100 μL detection reagent was added. After mixing, the plate was incubated for 2 min at room temperature. The luminescence values (RLU) of the wells were read with a luminescence meter.

2.3. Experimental Method. *2.3.1. Optimization of the Colloidal Gold Test Strip. 2.3.1.1. The Most Suitable pH for the SARS-COV-2 RBD Antigen in Colloidal Gold Labeling Solution.* We took eight 1 mL measures of colloidal gold solution, added 12, 14, 16, 18, 20, 22, 24, 26, and 28 μL of 0.2 mol/L K_2C0_3 solutions, mixed well, and adjusted the pH of the colloidal gold solution to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0. SARS-COV-2 RBD antigen protein was added and mixed well, and the solutions were maintained at 37 °C for 10 min. Then, 100 μL of 10% NaCl solution was added, and the mixture was left at 37 °C for 10 min. The lowest pH that consistently remained red was observed and recorded at pH 8.0 (Figure 2A). The colloidal gold solution with different pH concentrations was centrifuged at 3500 rpm for 30 min, the supernatant was removed, and the OD value was measured at a wavelength of 520 nm for each pH gradient. The most suitable pH was 8.0 (Figure 2B). Then, 20 μL of 0.2 mol/L K_2C0_3 solution was added to each 1 mL of colloidal gold solution, consistent with the previously reported theory.

2.3.1.2. The Most Suitable SARS-COV-2 RBD Antigen Labeled with Colloidal Gold. We took eight copies of 1 mL

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colloidal gold solution, added 20 μL of 0.2 mol/L K_2C0_3 solution to adjust to the optimum pH, and mixed well. The amount of antigen was 10, 12, 14, 16, 18, 20, 22, and 24 μg. The samples were maintained at 37 °C for 1 h, and the OD value at 520 nm was measured (Figure 2. C). The maximum OD value corresponded to the minimum protein amount of 16 μg, and the appropriate protein amount was 1.2 times the minimum protein amount, so the most suitable protein amount per 1 mL of the colloidal gold solution was 20 μg.

2.3.2. Verification of the Colloidal Gold Method Test Strip. *2.3.2.1. Specificity of the Colloidal Gold Test Strip.* To assess the analytical specificity of the rapid assay, we tested 18 prepandemic serum samples from the biospecimen bank of Liaoning University of Traditional Chinese Medicine. Positive serum for rubellavirus antibody (A-RV), cytomegalovirus antibody (A-CMV), herpes simplex virus antibody (A-HSV), rheumatoid factor (RF), hepatitis B surface antibody (anti-HBs), hepatitis E virus antibody (A-HEV), hepatitis C virus antibody (A-HCV), HIV antibody (A-HIV) and syphilis antibody (TP) were used to evaluate the specificity of the reagent (Figure 3A). All results were negative, so the kit was 100% specific. Different concentrations of serum from 18 samples are detailed in Supporting Information Table S1.

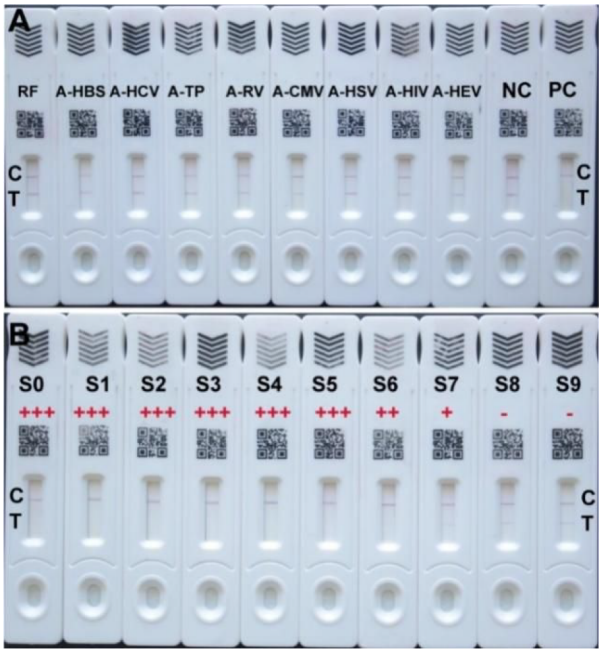

Figure 3. Specificity (A) and sensitivity (B) of the GICA test strip against the RBD of SARS-CoV-2.

2.3.2.2. Sensitivity of the Colloidal Gold Test Strip. Neutralizing Antibody Solution Reference Material against the receptor-binding domain (RBD) of SARS-CoV-2 S_0 (330.5 IU/L) diluted with deionized water into nine concentration gradient solutions [S_1 (165.25 IU/L), S_2 (82.63 IU/L), S_3 (41.31 IU/L), S_4 (33.05 IU/L), S_5 (27.54 IU/L), S_6 (23.61 IU/L), S_7 (20.66 IU/L), S_8 (18.36 IU/L), S_9 (16.53 IU/L)]. Each concentration gradient was repeated 20 times, and the detection rate was ≥95% of the lowest concentration for GICA reagent sensitivity (minimum detection limit). The S_0−S_7 detection rate was 100%, the S_8 detection rate was 65%, and the S_9 detection rate was 20%. Among them, S_7 was positive (1+), and the concentration of 20.66 IU/L was the sensitivity of the reagent (Figure 3B).

2.3.2.3. Stability of GICA Strips in Whole Blood and Serum. Three people's serum and whole blood samples were used to evaluate the stability of GICA test strips, and the tested test strips were stored in a cool, dry, airtight, contamination-free environment for 20, 40 min, 1 h, and 1 week. Neutralizing antibody test strips can obtain the same results as serum and whole blood samples. Negative, weakly positive, and positive results can all be determined by the intensity of the T line, which can easily be observed with the naked eye (Figure 4).

2.3.2.4. Correlation Analysis between GICA, ELISA, and PN. The 165 cases were divided into three groups depending on the weeks of vaccination (Table 1). After routine vaccination (4 weeks), the GICA test presented a detection rate (92.45%, 49/53) similar to that of the PN test (96.25%, 51/53) and ELISA test (94.34%, 50/53). However, the GICA test presented a lower detection rate, decreasing to 19.23% (10/52) and up to 6.67% (4/60) for vaccination in the middle and late phases (>4 weeks). The PN and ELISA tests also showed a decreasing trend of neutralizing antibody positivity with increasing time.

The result was supposed to be reasonable because SARS-CoV-2 neutralizing antibodies were commonly produced in humans after approximately 7−10 days of infection and could be maintained for several months. The colloidal gold method has good consistency with the detection results of the pseudovirus neutralization (PN) assay (κ = 0.886, p = 0.18) (Supporting Information Table S2.3.4).

2.3.2.5. Correlation Analysis between GICA, PN, and ELISA Titer Test. There was a strong correlation between the GICA and ELISA testing anti-RBD neutralizing antibody titer results (Pearson correlation coefficient r = 0.9977, P < 0.001, Figure 5A). There was a strong correlation between the GICA and PN testing anti-RBD neutralizing antibody titer results (Pearson correlation coefficient r = 0.918, P < 0.001, Figure 5C).Consistency analysis of serum anti-RBD antibody and specific IgG antibody titers showed that the correlation coefficient was 0.5253 (P < 0.001, Figure 5B).

2.4. Statistical Analysis. A database was established using Excel, and GraphPad Prism 9 software was used to analyze and process the data. The measurement data were first tested for normality and are expressed as (X ± s) when they obeyed a normal distribution. Two independent samples t tests were used to compare two groups, and one-way ANOVA was used to compare multiple groups. Enumeration data were expressed as the number of cases (%). P < 0.05 was considered to be statistically significant.

3. RESULTS

3.1. Changes in Specific IgG and Neutralizing Antibodies in Serum after Two Doses of Inactivated Vaccine. At 4 weeks (W4, n = 41), 12 weeks (W12, n = 40), and 24 weeks (W24, n = 48), the anti-SARS-CoV-2-specific IgG antibody titers in the serum were 6.19, 3.21, and 0.24 (S/CO), respectively. W4 was 1.93 and 25.79 times that of W12 and W24, respectively, and W12 was 13.38 times that of W24 (Figure 6A). The positive rates of anti-RBD neutralizing antibodies in serum were 38/41 (W4), 0/40 (W12), and 0/48 (W24), and neutralizing antibody titers were 12.44 in W4 (Figure 6C). The positive rates of anti-RBD omicron variant (B.1.1.529) neutralizing antibodies were 8/41 (W4), 0/40 (W12), and 0/48 (W24), and neutralizing antibody titers were

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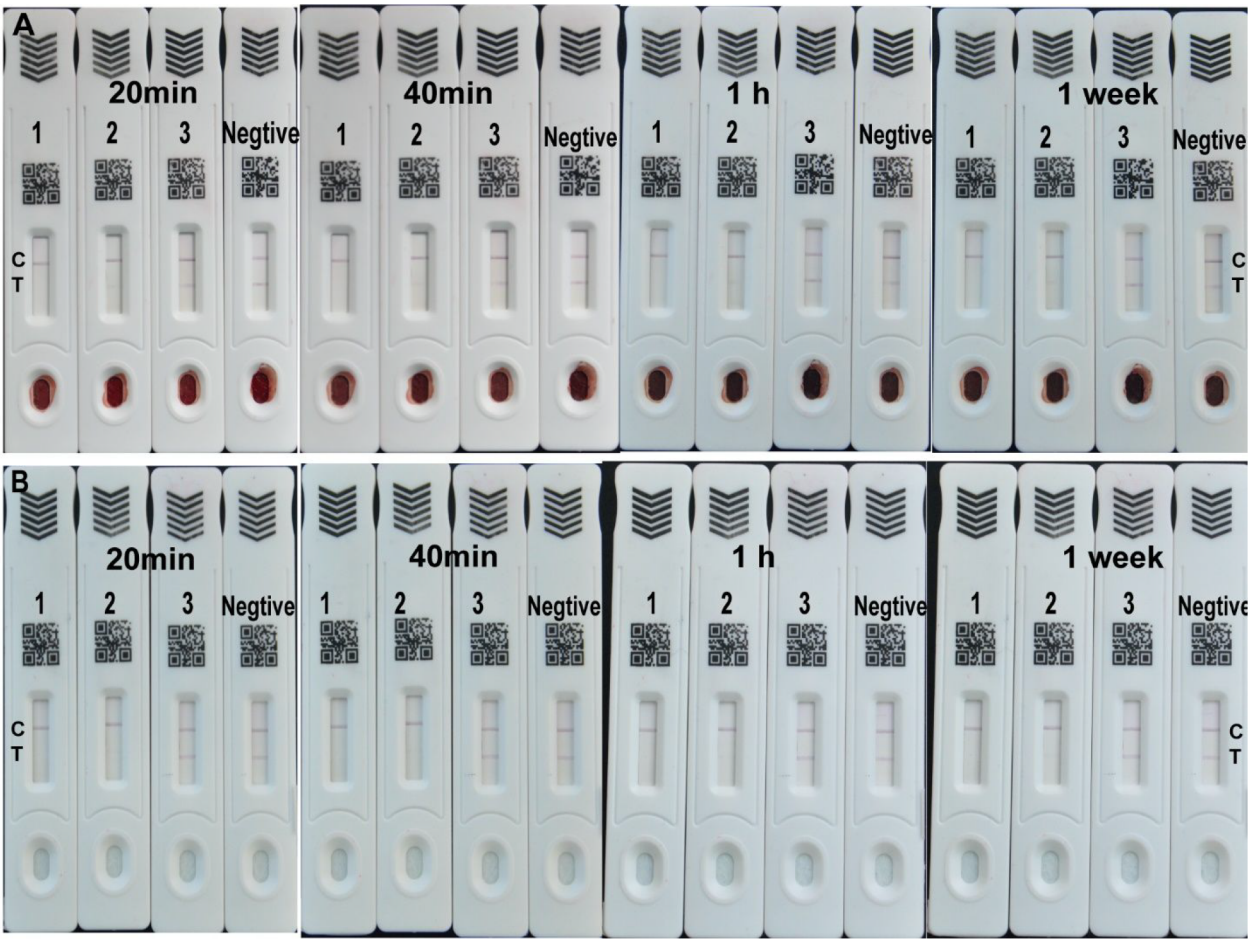

Figure 4. Stability of GICA test strips results in serum (A) and whole blood (B).

Table 1. Comparisons of the Detection Rates of GICA, PN, and ELISA Tests for Vaccinated People in Different Phases

Groups	Number of serum	ELISA-positive cases	GICA-positive cases	PN-positive cases
4 weeks	53	50(94.34%)	49(92.45%)	51(96.25%)
12 weeks	52	12(23.08%)	10(19.23%)	11(21.15%)
24 weeks	60	9(15.00%)	4(6.67%)	6(10.00%)
In total	165	71(43.03%)	63(38.18%)	68(41.21%)

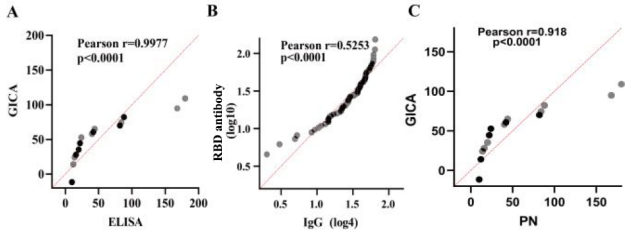

Figure 5. (A) Correlation analysis of GICA and ELISA methods for detecting neutralizing antibody titer results (n = 67). (B) Correlation analysis of specific IgG antibodies and anti-RBD neutralizing antibodies (n = 67). (C) Correlation analysis of GICA and PN assays for detecting neutralizing antibody titer results (n = 67).

1.95 in W4, which was significantly lower ($p < 0.05$) than anti-RBD neutralizing antibody titers in serum (Figure 6D). Interestingly, in this study, 23 cases of specific IgM (+) and IgG (+) named "W4+" and 18 cases of IgM (−) and IgG (+) named "W4−" were found in the serum of W4. There was no significant difference ($p > 0.05$) in the results of specific IgG antibodies and neutralizing antibodies between W4+, W4−, and W4 (Figure 6B,C), indicating that IgM (+) and IgG (+) individuals after vaccination cannot produce higher antibody titers.

3.2. Changes in Neutralizing Antibodies and Specific IgG Antibodies in Serum after Inoculation with the Homologous Booster Inactivated Vaccine and Recombinant Vaccine. The specific IgG antibody titers in serum before (W0) and after (W4, W12, W24) inoculation with homologous booster inactivated vaccine were 0.09, 10.80, 8.89, and 5.53 (S/CO), and W4 was 122.72, 1.21, and 1.95 times that of W0, W12, and W24, increased to a plateau at 4 weeks and then began to decrease gradually (Figure 7A). The anti-RBD antibody titers in serum were 0.00, 28.33, 15.00, 5.00, and in W4 were 28.33, 1.89, and 5.67 times those of W0, W12, and W24, respectively, and reached a plateau within 4 weeks and began to decrease rapidly (Figure 7B). The anti-RBD omicron variant (B.1.1.529) antibody titers were 0.00, 11.67, 5.00, and 3.33, and in W4 were 11.67, 2.33, and 3.48 times that of W0, W12, and W24, reached a plateau within 4 weeks and began to decrease rapidly (Figure 7C).

After inoculation with the recombinant vaccine, the specific IgG antibody titers in the serum of W4, W12, and W24 were 10.68, 9.18, and 7.21, respectively, and those of W4 were 1.16 and 1.48 times those of W12 and W24, respectively (Figure

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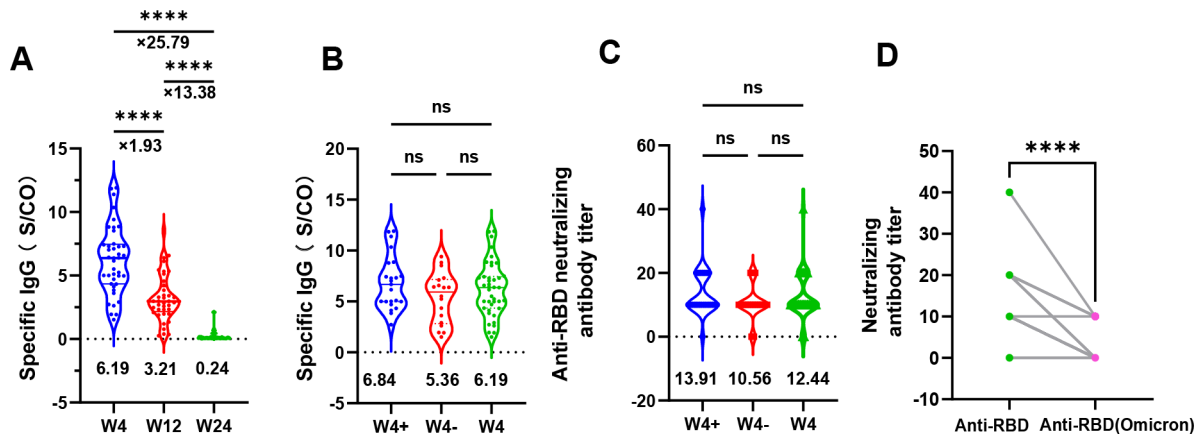

Figure 6. Specific IgG antibody, anti-RBD and anti-RBD omicron variant (B.1.1.529) neutralizing antibody titers in serum from individuals who had received two doses of inactivated vaccines. Specific IgG antibody assay using chemiluminescent method, anti-RBD and anti-RBD Omicron variant (B.1.1.529) neutralizing antibody titer assay using ELISA method. (A) Specific IgG antibody titer at 4 weeks (n = 41), 12 weeks (n = 40), and 24 weeks (n = 48). (B) Specific IgG antibody between W4+, W4−, and W4. (C) Anti-RBD neutralizing antibody titer between W4+, W4−, and W4. (D) Anti-RBD and anti-RBD omicron variant (B.1.1.529) neutralizing antibody titers.

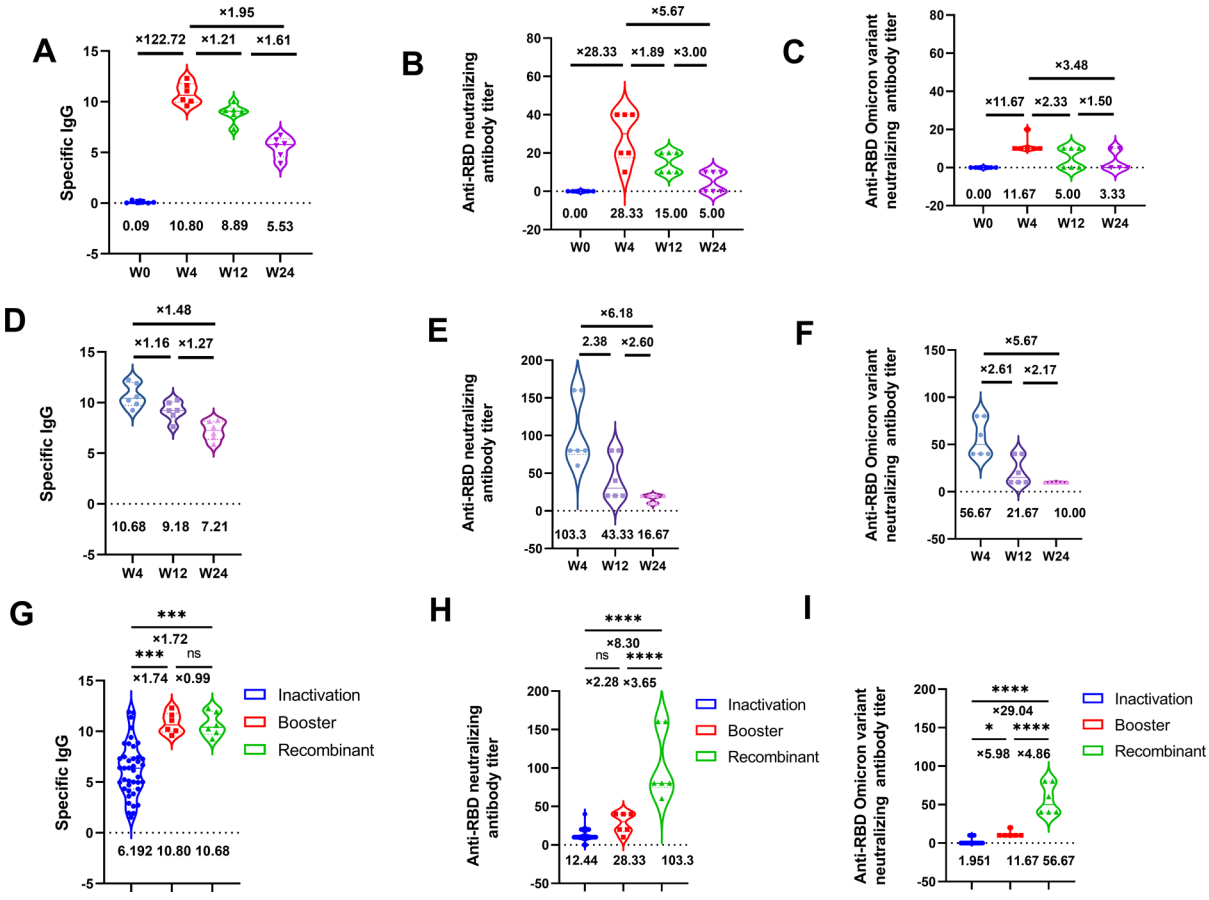

Figure 7. Specific IgG antibody (A), anti-RBD (B), and anti-RBD omicron variant (B.1.1.529) (C) neutralizing antibody titers in serum from individuals who had received homologous booster inactivated vaccines. Specific IgG antibody (D), anti-RBD (E), and anti-RBD omicron variant (B.1.1.529) (F) neutralizing antibody titers in serum from individuals who had received recombinant vaccination. Comparison between specific IgG antibody (G), anti-RBD (H), and anti-RBD Omicron variant (B.1.1.529) (I) neutralizing antibody titers in postvaccination serum at 4 weeks. Specific IgG antibody assay using chemiluminescent method, anti-RBD and anti-RBD omicron variant (B.1.1.529) neutralizing antibody titer assay using ELISA method.

7D). The serum anti-RBD antibody titers were 103.30, 43.33, and 16.67, and those of W4 were 2.38 and 6.18 times those of W12 and W24, respectively. To compare the changes in antibodies in the serum after vaccination at W12 and W24, the levels began to decrease rapidly after 4 weeks (Figure 7E). The serum anti-RBD omicron variant (B.1.1.529) antibody titers were 56.67, 21.67, and 10.00, and W4 was 2.61 and 5.67 times

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higher than W12 and W24 and decreased rapidly after 4 weeks (Figure 7F).

The study found that antibodies generated by vaccination gradually began to decrease after 4 weeks. The differences in the results of specific IgG antibodies of the initial two doses of inactivated vaccine, the booster inactivated vaccine, and the recombinant vaccine were statistically significant ($p < 0.05$), and the booster inactivated vaccine and recombinant vaccine were 1.74 and 1.72 times higher than the initial two doses of inactivated vaccine, indicating that the booster vaccination and recombinant vaccine induced a more robust humoral response. The difference in results between the booster inactivated vaccine and recombinant vaccine was not statistically significant ($p > 0.05$), indicating that immunization with the booster vaccine and recombinant vaccine induced equivalent humoral responses (Figure 7G). Comparing the differences in serum results for anti-RBD antibody was statistically significant ($p < 0.05$), with the recombinant vaccine being 8.30 and 3.65 times higher than the initial two doses of inactivated vaccine and the booster inactivated vaccine (Figure 7H). Comparing the differences in serum results for anti-RBD omicron variant (B.1.1.529) antibody was statistically significant ($p < 0.05$), with the recombinant vaccine being 29.04 and 4.86 times higher than the initial two doses of inactivated vaccine and the booster inactivated vaccine (Figure 7I). Comparing the results of the above studies, it is concluded that recombinant vaccines produce higher titers of neutralizing antibodies and higher immunogenicity than other types of vaccines.

4. DISCUSSION

In the context of universal vaccination for COVID-19, many patients ask doctors every day whether they can test for neutralizing antibodies to understand the effect of vaccination. Therefore, in this study, we selected the spike RBD protein and SARS-CoV-2 antigen expressed in HEK293 cells and developed an anti-SARS-CoV-2 neutralizing antibody (RBD) kit based on colloidal gold immunochromatography. The sensitivity, specificity, and stability (Figures 3 and 4) of the kits were evaluated and met the clinical standard, which can not only meet the needs of laboratory testing but also allow everyone to test neutralizing antibodies at home. There was a good correlation between specific IgG antibodies and anti-RBD antibodies in postvaccination serum (Figure 5B). It has been shown that the GICA method can be applied for periodic self-assessment of the protective effect of neutralizing antibodies in primary screening of individuals recovering from COVID-19 and in postvaccination populations.[17] Additionally, there was a good correlation in recovered COVID-19 patients.[18] Because specific IgG antibodies are present in vivo for longer than neutralizing antibodies, they can be used to evaluate neutralizing antibody titers in the serum of vaccinated and recovered COVID-19 patients.

In the present study, the specific IgG antibody and neutralizing antibody titers produced by two doses of inactivated vaccine were significantly reduced after 4 weeks (Figure 6A), so our government recommends a third dose of vaccine. Some studies have reported rapidly generating large amounts of neutralizing antibodies after booster vaccination with homologous or heterologous inactivated vaccines, which will further enhance protection against mutant strains[19,20] without specifically addressing changes in neutralizing antibodies. Our study showed that after 4 weeks of inoculation with the homologous booster inactivated vaccine, a large amount of specific IgG and a certain amount of neutralizing antibodies against RBD of SARS-CoV-2 and omicron variant (B.1.1.529) were rapidly produced in the serum, which was 1.74, 2.28, and 5.98 times that of the two doses of inactivated vaccine (Figure 7G,H,I).

Although research showed that vaccination with heterologous recombinant or mRNA vaccine could produce larger amounts of neutralizing antibodies against the prototype strain and variant strain than homologous inactivated vaccines at early stages, it did not specify how long neutralizing antibodies would be present in the body.[19] We selected the group of individuals who received the recombinant vaccine for further study. Four weeks after vaccination with the recombinant vaccine, a large amount of specific IgG and a certain amount of neutralizing antibodies against RBD of SARS-CoV-2 and omicron variant (B.1.1.529) were rapidly produced in the serum, which was 1.72, 8.3, and 29.04 times higher than those of the two doses of vaccine and 0.99, 3.65, and 4.86 times higher than those of the booster vaccine (Figure 7G,H,I), significantly raising the concentration of antibodies in the serum. Experiments have confirmed that the recombinant vaccine will produce many neutralizing antibodies and highly immunogenic specific IgG increases. The recombinant vaccine produces neutralizing antibodies and immunogenicity significantly better than the two-dose and homologous booster vaccines. It can be deduced that the heterologous vaccination booster can produce higher neutralizing antibodies. Vaccine-induced immunity typically wanes over time, and emerging SARS-CoV-2 variants, especially omicron, may evade immunity initiated by vaccines against an older variant.[21] Therefore, whether it is a natural first-time infection or a postimmunization infection, understanding the changes in the amount and kinetics of SARS-CoV-2-specific antibodies in vivo can help evaluate the duration of protection.

The *Technical Guidelines for New Coronavirus Vaccination (First Edition)* states that COVID-19 nucleic acid and antibody testing is not required before vaccination, and routine antibody testing is not recommended as a basis for judging the success of immunization.[22] SARS-CoV-2 neutralizing antibody reagent is currently only carried out in vaccine research and development and has not been popularized in medical institutions. Thus, there is no neutralizing antibody reagent that has obtained a medical device registration certificate. As shown in this study, kits can be used as a laboratory test for successful 2019-nCoV vaccination. This test will be a tool of great significance in the fight against pandemics, as it can be used to monitor the immunity of the population and guide interventions when immunity declines. At the same time, acclimation can reduce the incidence of serious diseases and mortality.

5. CONCLUSION

The newly identified SARS-CoV-2 poses a continual threat to human health due to its rapid transmission worldwide. Unprecedented vaccination campaigns have now begun with multiple candidates. Therefore, it is helpful to develop the Anti-SARS-CoV-2 Neutralizing Antibody Titer Assay Kit to test the protective neutralizing antibody levels in postvaccination serum or convalescent serum.

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■ ASSOCIATED CONTENT

Supporting Information

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Eighteen clinical serum samples with different concentrations, comparison between GICA and ELISA, comparison between GICA and PN, comparison between PN and ELISA, the colloidal gold SARS-CoV-2 neutralizing antibody rapid test kit test results and standard colorimetric cards (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Xuefeng Guan − *Liaoning University of Traditional Chinese Medicine, Shenyang 110847 Liaoning, China*; Email: lnzygxf@163.com

Authors

Heshan Yu − *Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang 110032 Liaoning, China*

Huan Liu − *Liaoning University of Traditional Chinese Medicine, Shenyang 110847 Liaoning, China*

Yongju Yang − *Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang 110032 Liaoning, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03677

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Notes

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■ ABBREVIATIONS

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; GICA, gold immunochromatography assay; COVID-19, coronavirus disease 2019; RBD, receptor-binding domain; ELISA, enzyme-linked immunosorbent assay

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