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Short Communication

A colloidal gold-based immunochromatographic strip for rapid detection of SARS-CoV-2 antibodies after vaccination

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ABSTRACT
Vaccination interventions is considered an important preventive measure to block the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and protect the organism from pathogen infection effectively. However, a quick and accurate technique to evaluate the immune efficacy of the SARS-CoV-2 inactivated vaccine remains scarce. In this paper, an IgM-IgG antibody combined detection colloidal gold immunochromatography assay kit was optimized and developed, which can assess the efficacy of the inactivated SARS-CoV-2 vaccine. We collected fingertip blood samples from 3 vaccinees and 1 unvaccinated sample. The results showed that the proportion of antibody was high after the second shots immunization. The colloidal gold-based immunochromatographic strip is rapid, convenient and easy to operate. It can be used as an auxiliary method for preliminary evaluation of the antibody effect of vaccine recipients, and provide a reference index for the potential clinical application value of the vaccine.

1. Introduction

With the continuous development of COVID-19, the research and prevention of COVID-19 are progressing and improving. SARS-CoV-2 coronavirus, the seventh coronavirus has been found to infect humans, is a single-stranded plus-stranded RNA virus and the seventh coronavirus has been found to infect humans [1]. The fatality rate of COVID-19 was lower than that of SARS (9.6%) and MERS (34.4%). However, the infectivity of COVID-19 is stronger than SARS and MERS [2,3]. The certain number of asymptomatic and mild patients make the prevention and control of COVID-19 difficult [4,5]. For this newly emerging infectious disease, isolation has been proved to be the most effective short-term prevention and control measure [6]. From a long-term perspective, safe and effective vaccines are of great significance for controlling the spread and preventing further outbreaks of the SARS-CoV-2 [7–9]. The inactivated vaccine is the most classical form of the vaccine, which is easy to prepare and can effectively induce the humoral immune response [10,11]. At the present stage, the SARS-CoV-2 inactivated vaccine developed in China are safety after inoculation [12, 13]. After inoculation with two shots of the vaccine, all of the vaccinators in the vaccine group produced antibodies with a high titer, and the positive conversion rate of neutralizing antibodies was 99.52% (Beijing Institute of Biological Products Co., LTD.). The protective efficiency of the vaccine against diseases caused by SARS-CoV-2 infection was more than 50%. The Chinese medicine vaccine was 79.34%, and the protective efficacy of CoronaVac in phase III clinical studies in different countries was up to 91.25% (Sinovac R&D Co., LTD.).

At present, China is constantly expanding the population vaccinated with COVID-19 inactivated vaccine. The methods for detecting the positive conversion rate of antibodies and to assess the duration of the antibodies in vaccinators are still lacking. This study has optimized a colloidal gold immunochromatography kit to quick assess the immune efficacy of the recipient of Novel Coronavirus inactivated vaccine within 10 min, with high specificity and sensitivity. This study provides a reference for further study on the antibody IgM-IgG changes in the recipients of COVID-19 inactivated vaccine lays a foundation for improving the vaccination efficiency.

2. Material and methods

2.1. Samples

Fingertip blood samples were collected from three vaccinees who received two shots of vaccine (Produced by the Sinovac R&D Co., LTD.)

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at Doudian Central Health Center, Fangshan District, Beijing from January to April 2021. At the same time, fingertip blood sample from one unvaccinated person who were negative for nucleic acid test were randomly selected as the negative control. This study was conducted with the permission of the donors. Fingertip blood samples should be detected immediately to avoid hemolysis. If the sample contains large amounts of lipids, hemolysis, or turbidity, it should not be used for testing.

2.2. Main membrane and instruments

Chloroauric acid, bovine serum albumin (BSA), hydrolyzed casein, goat anti-human IgG and IgM antibodies, mouse secondary IgG and goat anti-mouse IgG antibodies were purchased from Sartorius (Sigma, USA). Nitrocellulose (NC) membrane (CN140) were purchased from Sartorius, Germany. Other commonly used chemical reagents were analytical pure, purchased from Sangong, Shanghai. Instruments including pipet gun (Eppendorf, USA), gold spraying membrane ripping apparatus (Biodot-R120, USA), secondary biosafety cabinet (Eesco, Singapore), pH meter (Mettler Tolly, Switzerland), etc.

2.3. Expression and purification of antigenic proteins

SARS-CoV-2 S antigen protein expression plasmid pET30-RBD-His/pET30-NTD-His was constructed and transformed into BL21 competent state. Splitting buffer (pH=7.6) 30 mmol/L sodium dicyhrogen phosphate, 1 mol/L sodium chloride, 20 mmol/L imidazole, 10% glycerol; Scrubbing and softening solution (pH=7.6) 30 mmol/L sodium dicyhrogen phosphate, 1 mol/L sodium chloride, 250 mmol/L imidazole, 10% glycerol; Desalination Buffer (pH=7.6): 30 mmol/L sodium dicyhrogen phosphate, 500 mmol/L sodium chloride, 10% glycerol. Single colonies were isolated into a 5 mL LB test tube (including 50 μg/mL Kanamycin) and cultured overnight at 37 °C at 200 r/min for 5 h. Preparation process of binding pad: colloidal gold solution with particle size of 20 nm was prepared by trisodium citrate reduction method. After preparation, 100 mL colloidal gold solution was placed in a beaker and 0.1 mol/L K2CO3(pH 8.0) added. Recombinant antigens were stirred at room temperature for 2 h, then BSA with final concentration of 10% was added, sealed for 20 minutes, and then centrifuged at 12000 r/min for 20 minutes.

2.4. Preparation and condition optimization of colloidal gold reagent strip

Polyethylene fiberboard was selected as the supporting layer of colloidal gold test strip, sample pad and release pad were prepared with glass fiber dimension, NC membrane was selected to prepare the test layer, and absorbent paper was selected to prepare the absorption layer. The binding pad contains colloidal gold labeled SARS-CoV-2 recombinant protein S antigen, two test lines (G line, M line) and a quality control line (C line) were fixed on the NC membrane. The G line was coated with anti-human virus protein S IgG antibody, the M line was fixed with anti-human disease virus protein S IgM antibody, and the quality control line (C line) was coated with quality control antibody (goat anti-mouse IgG antibody). Coating process of NC membrane antibody: anti-human protein S IgG was dissolved in Phosphate buffers (PBS) to 0.4 to 1.5 mg/mL, goat anti-mouse IgG was dissolved in PBS to 0.6 – 1.5 mg/mL solution, using the gold spray membrane ripping instrument in the upper and lower parts of the NC membrane. The parameters of 1 μl/cm were crossed and coated with C, G and M lines. After the lines were drawn, the NC membrane were dried at 21 °C–25 °C, a humidity of less than 30%, and a drying time of 2–5 h. Preparation process of binding pad: colloidal gold solution with particle size of 20 nm was prepared by trisodium citrate reduction method. After preparation, 100 mL colloidal gold solution was placed in a beaker and 0.1 mol/L K2CO3(pH 8.0) added. Recombinant antigens were stirred at room temperature for 2 h, then BSA with final concentration of 10% was added, sealed for 20 minutes, and then centrifuged at 12000 r/min for 20 minutes.

2.5. Detection method

Unseal the aluminum foil bag after the the blood diluted balanced to room temperature. The test card was taken out from the bag and placed on the horizontal table top. 10 μl of whole blood was taken and added vertically into the sample well of the test card, then added 2 drops (about 60 μl) of sample diluted immediately. The determination results within 10 minutes.

2.6. Test result interpretation

Negative results: only the quality control line (Line C) appears, the detection line (Line G) and (Line M) showed no color, indicating that no Novel Coronavirus protein S antibody was detected, and the result was determined to be negative, as shown in Figure 1A. Positive results: the Line C, Line G and Line M all showed color, indicating that novel coronavirus protein IgM and IgG antibodies were detected, and the result determined that Novel Coronavirus protein IgM and IgG antibodies were positive, as shown in Figure 1B. Both the Line C and the Line M showed color, indicating that the Novel Coronavirus protein IgM antibody was detected, and the result determined that the Novel Coronavirus protein IgM antibody was positive, as shown in Figure 1C. Both the Line C and the Line G showed color, indicating that the Novel Coronavirus protein IgG antibody was detected, and the result determined to be positive for the Novel Coronavirus protein IgG antibody, as shown in Figure 1D. Invalid results: no color on the Line C indicates the test is invalid, no matter whether the Line G or Line M shows color, as shown in Figure 1E.
2.7. Statistics

After antibody detection of serum and whole blood samples from vaccine recipients by colloidal gold method, the specificity and sensitivity were calculated according to the following formula:

Specificity (%) = \( \frac{100}{C_2} \) \( \frac{\text{true negative}}{\text{true negative} + \text{false positive}} \);

Sensitivity (%) = \( \frac{100}{C_2} \) \( \frac{\text{true positive}}{\text{true positive} + \text{false negative}} \).

The comparison of counting results is expressed in the form of as a percentage (%).

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**Fig. 1.** Schematic diagram of interpretation of detection results. A: Negative; B: Positive of IgG and IgM; C: Positive of IgG antibody; D: Positive of IgM antibody; E: Invalid.

**Fig. 2.** Schematic diagram of the colloidal gold-based immunochromatographic strip.
3. Results

3.1. Design and preparation of colloidal gold-based immunochromatographic strip

We aimed to detect anti-IgM/IgG antibodies against spike (S) and nucleocapsid (N) protein of SARS-CoV-2. A colloidal gold-based immunochromatographic strip was designed and the detection principle was shown in Figure 2. Then, prepared and optimized the strips. The optimal concentration antigen labeling was 0.5mg/100 mL colloidal gold solution. The best working solution of colloidal gold was the phosphate buffer solution at 20 mmol/L (2% BSA, 10% sucrose, 5% trehalose, 0.01 mol/L Tris and pH 8.0). The optimal concentration of coated antigen was 2.0 mg/mL, and the optimal determination time was 8–10 minutes. The strip can be used for qualitative screening of IgM-IgG antibody in the recipients of COVID-19 vaccine.

3.2. Test results of whole blood samples

Next, the application of fingertip blood from donors was confirmed the sensitivity and specificity. 10μL blood sample were collected from vaccinees (n=3) and unvaccinated sample (n=1). The Line C, Line G and Line M all showed color, indicating that SARS-CoV-2 protein IgM and IgG antibodies were detected, and the result determined that SARS-CoV-2 protein IgM and IgG antibodies were positive, as shown in Figure 3. The positive detection rate of IgG in the samples was 100%, and that in the samples with both IgM and IgG positive was also high (Figure 3). Whole blood samples from randomly selected uninoculated individuals were tested for antibodies and finally found to be negative. The sensitivity and specificity of the kit in this study were high.

4. Discussion

SARS and MERS inactivated vaccines can induce the production of high-titer neutralizing antibodies in experimental animals [14–16], and SARS inactivated vaccines have completed the phase I clinical trials, which proved that they are safe in humans and can induce the generation of neutralizing antibodies [17]. However, T cell immune response caused by inactivated vaccines is generally weak [18]. Previous studies have proved that inactivated vaccines against SARS and MERS cannot effectively stimulate the body to produce cellular immune response, and the protective efficacy is not satisfactory even if high titer serum neutralizing antibodies are produced [17,19]. Nevertheless, there are still no effective antibody detection method to verify the positive antibody conversion rate and the protective efficacy of SARS-CoV-2 inactivated vaccine [20]. It is of great importance to develop a rapid detection method for antibodies produced by vaccines and provide accurate and effective reference data for further research on vaccine optimization. The results of this study indicated that the developed colloidal gold-based immunochromatographic strip has a high sensitivity to the antibodies generated by the NCOV, and can be used to detect the antibody duration of the NCOV in the recipients than 30 days after inoculation.

The detection of COVID-19 inactivated vaccine antibody by colloidal gold-based immunochromatographic strip is convenient. However, it may have certain constraints when applied on the vaccination cycle of the vaccinated patients in clinical detection. At the initial stage of full vaccination, IgM and IgG detection of antibodies are the most preferred detection methods. One of the limitation of this study is that the plasma or serum of the donors were not selected for antibody detection. In the subsequent studies, the plasma and serum of the donors will be tested with colloidal gold strip. Besides, the number of the whole blood samples are not large enough. Subsequent studies will conduct antibody detection on the whole blood of more donors at different times to dynamically describe the changes in antibody levels produced by the Novel Coronavirus. It can provide a theoretical foundation for evaluating the efficacy and duration time when testing the immunity efficacy of the Novel Coronavirus vaccine.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

Ethical approval and informed consent (if applicable)

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

The author should also clearly indicate in the Material and methods section of the manuscript that applicable guidelines, regulations and laws have been followed and required ethical approval has been obtained.

Author contribution to study

Jian Yu: Conceptualization, Methodology, Supervision. Jia Liu: Validation, Platform building, Writing-Original draft preparation. Weiqi Yan: Data curation: Writing- Reviewing and Editing. Zhaojun Liu: Validation, Supervision. Yizhao Han: Operation. Leqiang Zhang: Operation.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.medntd.2021.100084.

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