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Medicine in Novel Technology and Devices 11 (2021) 100084

Contents lists available at ScienceDirect

Medicine in Novel Technology and Devices

journal homepage: www.journals.elsevier.com/medicine-in-novel-technology-and-devices/

Short Communication

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

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ARTICLE INFO

Keywords:
Inactivated SARS-CoV-2 vaccine
IgM-IgG antibody
Colloidal gold immunization

ABSTRACT

Vaccination interventions is consideredan 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-2infection 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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https://doi.org/10.1016/j.medntd.2021.100084
Received 19 May 2021; Received in revised form 17 June 2021; Accepted 21 June 2021

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-RR120, 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 vector pET30-RBD-His/pET30-NTD-His was constructed and transformed into BL21 competent state. Splitting buffer (pH=7.6) 30 mmol/L sodium dihydrogen phosphate, 1 mol/L sodium chloride, 20 mmol/L imidazole, 10% glycerol; Scrubbing and softening solution (pH=7.6) 30 mmol/L sodium dihydrogen phosphate, 1 mol/L sodium chloride, 250 mmol/L imidazole, 10% glycerol; Desalination Buffer (pH=7.6): 30 mmol/L sodium dihydrogen phosphate, 500 mmol/L sodium chloride, 10% glycerol. Single colonies were isolated into a 5 mL LB test tube (including 50 μg/mL Kanamicin) and cultured overnight at 37 °C at 220 r/min, and then transferred the tube to 160 mL LB flask (50 μg/mL kanamycin), 3 vials each, with an initial OD value of about 0, cultured at 30 °C at 200 r/min for 3～4 h Isopropy-β-D-thiogalactoside (IPTG) was induced at 3 mmol/L at 16 °C for 180 r/min for 20 h. The bacteria were centrifuged (8000 r/min, 4 °C, 10 minutes) and collected. After the supernatant was removed, 1/10 volume of lysate was added to resuspend the bacteria and centrifuged again. The supernatant was dumped and the precipitate was resuspended with 1/30 volume of lysate. The thalli were broken under high pressure and dithiothreitol (DTT) with a final concentration of 1 mmol/L was added, and then centrifuge at 12700 r/min, 4 °C, 20 minutes. The supernatant was transferred to a clean high-speed dedicated centrifuge tube and centrifuged at a high speed (20000 r/min, 4 °C, 10 minutes). The supernatant was transferred to a new 50 mL centrifuge tube, and samples were injected through pump tube. FPLC protein purification system (Histrap-5 mL affinity chromatography column) was used for linear gradient elution of 0–250 mmol/L imidazole. Samples were collected according to the peak conditions. The concentration was determined by microUV spectrophotometer, and the required proteins were determined by SDS-PAGE protein electrophoresis of 10 μL sample. The higher concentration of 2. The desalination process of 5 mL sample was performed on a GE PD-10 desalination column. BCA protein quantification kit was used to determine the protein concentration after desalination. Packed into multiple tubes with 50 μl, then added with 1 mmol/L final concentration DTT, and stored at −80 °C for later use.The detail of gold label protein preparation and purification methods are available in Supplementary Materials and Methods.

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 beater 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.The supernatant was discarded and redissolved to 50 mL with colloidal gold working solution. Pour 20 cm^2 of 1 mL solution on the glass fiber membrane evenly, and then put it in a drying room. The temperature is 21 °C–25 °C, the humidity is less than 30%, and the drying time is 2–5 h to make the marking pad.The detail of colloidal gold preparation , test strip selection and treatment are available in Supplementary Materials and Methods.

The SARS-CoV-2 protein S receptor binding domain (RBD) and N terminal domain were extracted by combining and matching different concentrations of markers and coated reagents. NTD was used to test the detection reagents, and the best combination was selected to determine the detection formula. After the preparation of the test strip, all samples were tested according to the test method and the test results were analyzed.

2.5. Detection method

Unseal the aluminum foil bag after the the blood diluent 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 diluent 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 was determined to be positive for the Novel Coronavirus protein IgG antibody, as shown in Figure 1D. Invalid results: no color on the Line Cindicates the test is invalid, no matter whether the Line G or Line M shows color, as shown in Figure 1E.

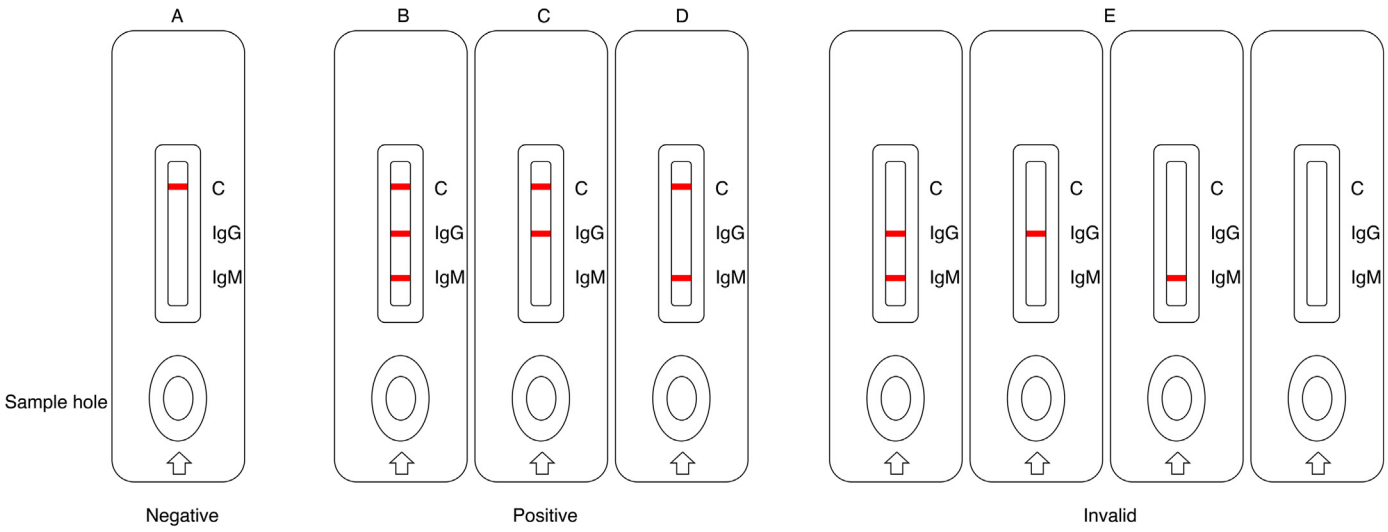

Fig. 1. Schematic diagram of interpretation of detection results. A: Negative; B: Positive of IgG and IgM; C: Positive of IgG antibody; D: Positive of IgM antibody; E: Invalid.

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 (%) = 100 × [true negative/(true negative + false positive)];

Sensitivity (%) = 100 × [true positive/(true positive + false negative)].

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

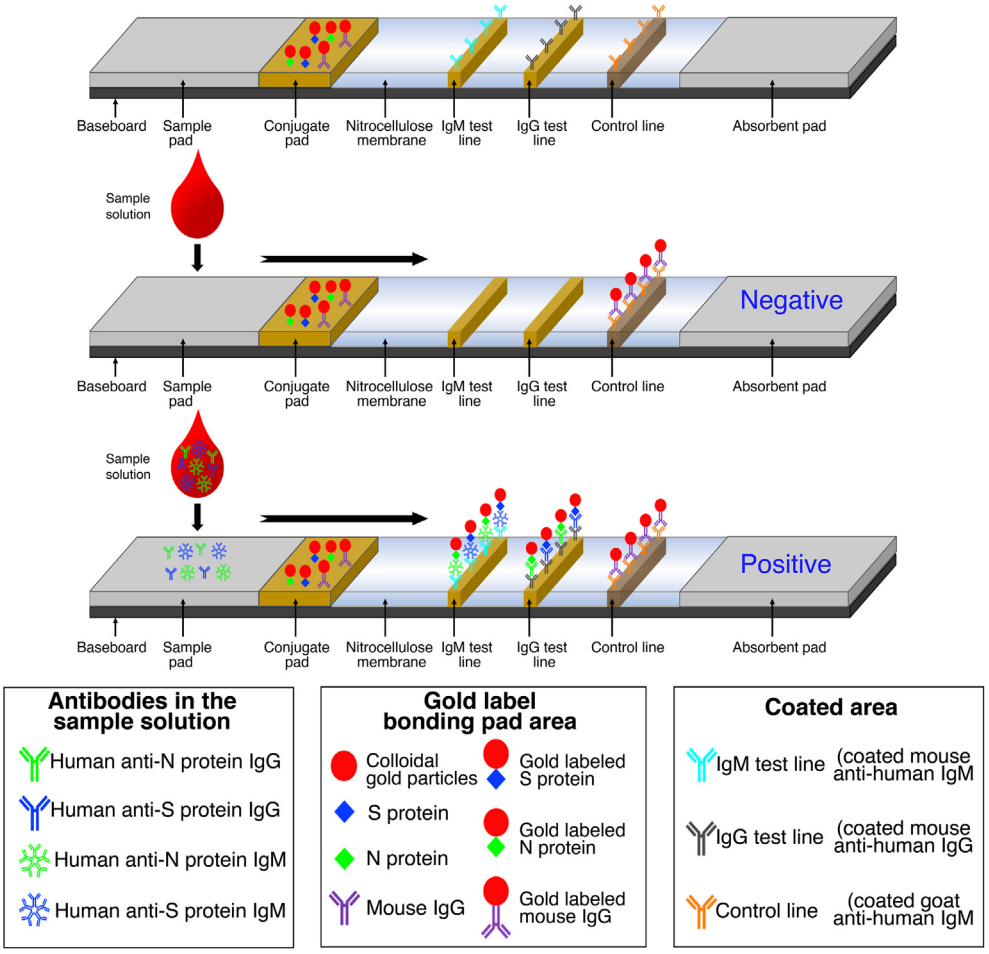

Fig. 2. Schematic diagram of the colloidal gold-based immunochromatographic strip.

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 colloid 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 8–10minutes. 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.

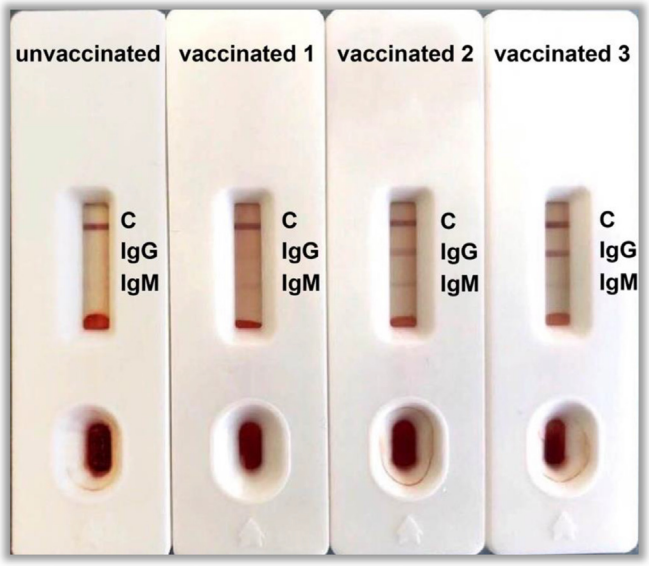

Fig. 3. Test results of serum samples.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81970186].

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. Zhuojun 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.

References

[1] Nomburg J, Meyerson M, Decaprio JA. Pervasive generation of non-canonical subgenomic RNAs by SARS-CoV-2. Genome Med 2020;12:108. https://doi.org/10.1186/s13073-020-00802-w.

[2] Flower TG, Buffalo CZ, Hooy RM, Allaire M, Ren XHurley JH. Structure of SARS-CoV-2 ORF8, a rapidly evolving immune evasion protein. Proc Natl Acad Sci USA 2021;118. https://doi.org/10.1073/pnas.2021785118.

[3] Bakhiet M, Taurin S. SARS-CoV-2: targeted managements and vaccine development. Cytokine Growth Factor Rev 2021;58:16–29. https://doi.org/10.1016/j.cytogfr.2020.11.001.

[4] Cervia C, Nilsson J, Zurbuchen Y, Valaperti A, Schreiner J, Wolfensberger A, et al. Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. J Allergy Clin Immunol 2021;147:545–57. https://doi.org/10.1016/j.jaci.2020.10.040. e9.

[5] Wang Z, Fu Y, Guo Z, Li J, Li J, Cheng H, et al. Transmission and prevention of SARS-CoV-2. Biochem Soc Trans 2020;48:2307–16. https://doi.org/10.1042/BST20200693.

[6] Ben-Shmuel A, Brosh-Nissimov T, Glinert I, Bar-David E, Sittner A, Poni R, et al. Detection and infectivity potential of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) environmental contamination in isolation units and quarantine facilities. Clin Microbiol Infect 2020;26:1658–62. https://doi.org/10.1016/j.cmi.2020.09.004.

[7] Wu Z, Mcgoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese center for disease control and prevention. J Am Med Assoc 2020;323:1239–42. https://doi.org/10.1001/jama.2020.2648.

[8] Dagotto G, Yu J, Barouch DH. Approaches and challenges in SARS-CoV-2 vaccine development. Cell Host Microbe 2020;28:364–70. https://doi.org/10.1016/j.chom.2020.08.002.

[9] Awadasseid A, Wu Y, Tanaka Y, Zhang W. Current advances in the development of SARS-CoV-2 vaccines. Int J Biol Sci 2021;17:8–19. https://doi.org/10.7150/ijbs.52569.

[10] Che Y, Liu X, Pu Y, Zhou M, Zhao Z, Jiang R, et al. Randomized, double-blinded and placebo-controlled phase II trial of an inactivated SARS-CoV-2 vaccine in healthy adults. Clin Infect Dis 2020. https://doi.org/10.1093/cid/ciaa1703.

[11] Li YD, Chi WY, Su JH, Ferrall L, Hung CF, Wu TC. Coronavirus vaccine development: from SARS and MERS to COVID-19. J Biomed Sci 2020;27:104. https://doi.org/10.1186/s12929-020-00695-2.

[12] Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020;369:77–81. https://doi.org/10.1126/science.abc1932.

[13] Xia S, Duan K, Zhang Y, Zhao D, Zhang H, Xie Z, et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: interim analysis of 2 randomized clinical trials. J Am Med Assoc 2020;324:951–60. https://doi.org/10.1001/jama.2020.15543.

[14] Xiong S, Wang YF, Zhang MY, Liu XJ, Zhang CH, Liu SS, et al. Immunogenicity of SARS inactivated vaccine in BALB/c mice. Immunol Lett 2004;95:139–43. https://doi.org/10.1016/j.imlet.2004.06.014.

[15] Zhou J, Wang W, Zhong Q, Hou W, Yang Z, Xiao SY, et al. Immunogenicity, safety, and protective efficacy of an inactivated SARS-associated coronavirus vaccine in rhesus monkeys. Vaccine 2005;23:3202–9. https://doi.org/10.1016/j.vaccine.2004.11.075.

[16] See RH, Petric M, Lawrence DJ, Mok CPY, Rowe T, Zitzow LA, et al. Severe acute respiratory syndrome vaccine efficacy in ferrets: whole killed virus and adenovirus-vectored vaccines. J Gen Virol 2008;89:2136–46. https://doi.org/10.1099/vir.0.2008/001891-0.

[17] Darnell ME, Plant EP, Watanabe H, Byrum R, St Claire M, Ward JM, et al. Severe acute respiratory syndrome coronavirus infection in vaccinated ferrets. J Infect Dis 2007;196:1329–38. https://doi.org/10.1086/522431.

[18] Deng Y, Lan J, Bao L, Huang B, Ye F, Chen Y, et al. Enhanced protection in mice induced by immunization with inactivated whole viruses compare to spike protein of middle east respiratory syndrome coronavirus. Emerg Microb Infect 2018;7:60. https://doi.org/10.1038/s41426-018-0056-7.

[19] Dong Y, Dai T, Wei Y, Zhang L, Zheng M, Zhou F. A systematic review of SARS-CoV-2 vaccine candidates. Signal Transduct Target Ther 2020;5:237. https://doi.org/10.1038/s41392-020-00352-y.

[20] Ye T, Zhong Z, Garcia-Sastre A, Schotsaert M, De Geest BG. Current status of COVID-19 (Pre)Clinical vaccine development. Angew Chem Int Ed Engl 2020;59:18885–97. https://doi.org/10.1002/anie.202008319.