Rapid Detection of Anti-SARS-CoV-2 IgM and IgG Using a Selenium Nanoparticle-based Lateral Flow Immunoassay

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Abstract

**Background:** Coronavirus disease 2019 is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is highly transmissible. Early and rapid testing is necessary to effectively prevent and control the outbreak. Detection of SARS-CoV-2 antibodies with lateral flow immunoassay can achieve this goal. Antibody detection is especially effective for the detection of asymptomatic infection.

**Methods:** In this study, SARS-CoV-2 nucleoprotein was expressed by E. coli and purified by affinity chromatography. We used the highly stable and sensitive selenium nanoparticle as the labeling probe coupled with the SARS-CoV-2 nucleoprotein to prepare a new SARS-CoV-2 antibody (IgM and IgG) detection kit. The sensitivity and specificity of the kit were verified by plasma of COVID-19 patients and health persons. Separate detection of IgM and IgG, such as in this assay, was performed in order to reduce mutual interference and improve the accuracy of the test results.

**Results:** The SARS-CoV-2 nucleoprotein was purified on a nickel column, and the final purity was greater than 90%. The sensitivity of the kit was 94.74% and the specificity was 95.12% by 41 negative plasma samples and 19 positive plasma samples detection.

**Conclusions:** The assay kit does not require any special device for reading the results and the readout is a simple color change that can be evaluated with the naked eye. This kit is suitable for rapid and real-time detection of the SARS-CoV-2 antibody.

**Background**

Beginning in December 2019, several unexplained pneumonia cases occurred in Wuhan City, Hubei Province. Chinese researchers identified the cause of the disease as a new type of coronavirus, namely severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The virus belongs to the β-genus of the coronavirus family, which also includes the SARS coronavirus responsible for the outbreak in 2003 and the MERS coronavirus responsible for the outbreak in 2012. The virus is listed by the World Health Organization as the seventh coronavirus known to infect humans [2]. SARS-CoV-2 contains spike protein, envelope protein, membrane protein, and nucleoprotein. Of these proteins, nucleoprotein is the most highly conserved [3]. The incubation period after infection is 0–24 days, and all symptomatic infections involve pneumonia. Common symptoms are fever, cough, and myalgia or fatigue [4], and less common symptoms include expectoration, headache, hemoptysis, diarrhea, and dyspnea. The symptoms are similar to the common cold; however, if the patient is not diagnosed and treated in time, the disease can be fatal [1, 2]. SARS-CoV-2 is transmitted through droplets in the air, and current data do not exclude the possibility of fecal-oral transmission [5]. There is no age preference for viral infection [6]. Infections can be in family clusters, and there are also asymptomatic infections [7]. At present, more than 210 countries and regions have reported confirmed cases of the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2. SARS-CoV-2 has a long incubation period, a wide range of transmission channels, and screening
is required for a large population, all of which increases the difficulty in creating an optimal detection assay. On March 3, 2020, the General Office of the National Health Commission and the Office of the State Administration of Traditional Chinese Medicine jointly issued the *Diagnosis and Treatment Program for COVID-19* (trial version 7) [8] Gene sequencing and antibody (IgM/IgG) test results are recommended to be used as the basis for the diagnosis. When a pandemic outbreak occurs, portable, fast, and real-time screening methods are needed to help prevent and control the pandemic; a lateral flow immunoassay (LFIA) can meet these requirements.

The LFIA, described in Figure 1, is a fast, simple, accurate, stable, and portable diagnostic tool [9, 10]. LFIA utilize various types of labeling probes that either provide quantitative detection or qualitative detection [11]. Quantitative detection labeling probes include fluorescent microspheres [12], upconversion luminescence [13], quantum dots [14, 15], and magnetic particles [16]; qualitative detection labeling probes include colloidal gold [17], selenium nanoparticle [18], and nanocarbon [11].

Quantitative detection is the trend for these types of assays, but the drawback is that these methods do not provide a readout that is visible with the naked eye, and additional equipment is required to interpret the results. This practical limitation prevents the use of most quantitative lateral chromatography-based assays in SARS-CoV-2 screening. Currently, home self-tests are still mostly qualitative tests. Colloidal gold is the most widely used qualitative labeling probe [17]. Colloidal gold can easily label proteins, is relatively simple to prepare and widely used, but has low sensitivity. Selenium nanoparticles are another labeling probe used in lateral chromatography experiments. The nanoparticles have a surface plasmon effect, a small-size effect, and are used to label proteins or nucleic acids. Positive test results appear as orange lines visible to the naked eye because selenium nanoparticles are orange colored [18]. Selenium nanoparticles are prepared at room temperature, easily bind to proteins, and are not sensitive to electrolytes. They also have higher levels of sensitivity, and are more economical to prepare than other probe types [19]. The current study is based on our reported article on how to prepare selenium nanoparticles [18]. Here, we apply this technique to the development of a rapid detection kit for anti-SARS-CoV-2 IgM and IgG based on selenium nanoparticle probes. In the test, we separately detect IgM and IgG in order to reduce mutual detection interference and improve the accuracy of test results.

**Methods**

**Expression and purification of SARS-CoV-2 nucleoprotein**

The pET28a(+)–nCoV-N-his plasmid was constructed, and the SARS-CoV-2-N-His recombinant protein was produced using *E. coli* BL21. The plasmid was transformed into BL21 *E. coli*, and a single clone was inoculated into 5 mL of 2YT medium and cultured at 37°C and 220 rpm for 14 h. Subsequently, 2 mL of the bacterial solution was added into 1 L of 2YT medium and cultured at 37°C and 150 rpm for 2.5 h. The bacterial broth was incubated with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 30°C and 150 rpm for 6 h. The bacterial solution was centrifuged, the culture medium discarded, and the bacteria fully resuspended in 50 mL of phosphate-buffered saline (PBS). This solution was sonicated with a cell
ultrasonic disruptor, according to the following protocol: 250 W ultrasound for 10 s, pause for 10 s, for 30 cycles, at which time the bacterial solution became transparent. After sonication, the bacterial solution was centrifuged at 15,000 rpm for 20 min, and the supernatant was filtered through a 0.22 μm filter membrane, followed by affinity purification using a His Trap FF crude 5 mL nickel column. The purity of nucleoprotein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and quantified using ImageJ. The concentration of nucleoprotein was measured by BCA protein assay kit.

Preparation of selenium nanoparticles and nucleoprotein complex

The selenium nanoparticle solution (SeNps) was prepared using ascorbic acid to reduce selenite. The selenium nanoparticle preparation was used to label nucleoproteins at different pH levels, and the pH of SeNps was adjusted with K$_2$CO$_3$. Then, nucleoprotein was added in drops to 100 mL SeNps with gentle stirring at room temperature. The mixture was then softly agitated for 30 min to allow conjugation of nucleoproteins to selenium nanoparticle surfaces by physical adsorption. Subsequently, 1 g bovine serum albumin (BSA) was added to the mixture for 30 min to block the non-coated selenium nanoparticle surfaces, and then the mixture was centrifuged at 10,000 rpm for 10 min. Selenium nanoparticle-conjugated nucleoprotein in a soft-pellet form was collected. Finally, the labeled proteins were resuspended in a working solution (pH 7.4 10 mM PBS containing 0.05% Tween 20, 1% BSA, 5% sucrose, and 5% trehalose) and then stored at 4°C before use. The optimal pH was selected according to color development. At the selected optimal pH, 1.5 mg/mL, 2 mg/mL, and 4 mg/mL were selected as antibody coating concentration at the test line. 5 μg/mL, 10 μg/mL, and 20 μg/mL were the alternative conjugating concentrations of the nucleoproteins. 1 mg/mL was used as the coating concentration of the anti-His antibody at the control line to prepare the kit. The criteria for determining the optimal conditions were the number of false positive results and the degree of color development.

The internal structure of the kit is shown in Fig. 1a and includes a backing, a sample pad, a conjugate pad, a reaction pad, and an absorbent pad. The liquid matrix added to the sample pad is chromatographed to the conjugate pad to re-dissolve the selenium-labeled protein complex fixed to the conjugate pad, and, due to the capillary action, the selenium-labeled nucleoprotein is chromatographed with the liquid matrix toward the absorbent pad in the nitrocellulose membrane. The process for IgM detection is provided as an example in Fig. 1b. When the sample contains anti-SARS-CoV-2 IgM, the IgM conjugates to the selenium nanoparticle-labeled nucleoprotein. When this protein complex conjugate is chromatographed to the position of the test line, the IgM will bind to the anti-human IgM antibody, resulting in the accumulation of color at the position of the test line. When the excess complex conjugate is chromatographed to the control line area, the anti-His antibody coated in this region captures the selenium nanoparticle-labeled His-tag nucleoprotein, resulting in color accumulation. Therefore, when both the control line and the test lines develop color, it is determined that the sample contains anti-nucleoprotein IgM, and the test result is considered positive. When a negative sample is applied to the
test, there is no immune binding reaction in the detection area. Thus, no color will be accumulated at the test line, but the control line will develop color normally. Therefore, when the control line develops color and the test line does not develop color, it is determined that the sample does not contain anti-nucleoprotein IgM, and the test result is considered negative. If the control line does not develop color, the condition of color development at the test line is an invalid result. Interpretation of IgG test results works in the same way as for IgM. For the anti-SARS-CoV-2 IgG test, the anti-human IgG antibody was coated at the position of the test strip, and other the conditions remained unchanged.

**Preparation of the SARS-CoV-2 antibody detection kit**

The anti-human IgM antibody or IgG antibody was coated on the test line, and the anti-His antibody was coated on the control line. After the coating was completed, the reaction pad was dried at 37°C for 4 h. The sample pad was immersed in the sample pad treatment solution (10 mM PBS, pH 7.4, containing 0.05% Tween 20, and 5% serum) for 30 min and dried at 37°C for 4 h. The conjugate pad contained immobilized selenium nanoparticle-SARS-CoV-2 nucleoprotein complex. The test strips were prepared prior to performing the assay, assembled, cut, and ready for use.

**Clinical sample validation**

Fetal bovine serum (FBS) was chosen as the negative sample for the selection of kit conditions in the early stage. The serum of two healthy persons was used to verify the above conditions. SARS-CoV-2 positive specimen and negative specimen were used to validate the test strips. Among them, 19 cases were diagnosed as positive in Henan Provincial People's Hospital, the COVID-19 designated hospital in Henan Province; 41 cases were confirmed negatives by clinical detection from the First Affiliated Hospital of Henan University. For each kit, 16 μL of plasma and 64 μL of sample processing solution were added. The sample processing solution was 10 mM PBS containing 0.05% Tween-20 and 1% BSA, pH 7.4, and the results were observed after 5 min. The observation results are shown in Figure 1. When both the test line and the control line develop color, the result is positive; when only the control line develops the color, the result is negative; when the control line does not develop a color, the result is invalid. We confirm that the detection results of COVID-19 patients in this study have not been reported in any other submission by us or anyone else.

The sensitivity, specificity, positive predictive value, and negative predictive value of the kit were calculated according to the following formulas [20]:

\[
\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}} \times 100\%
\]

\[
\text{Specificity} = \frac{\text{True negative}}{\text{True Negative} + \text{False Positive}} \times 100\%
\]

\[
\text{Positive predictive value} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}} \times 100\%
\]
Results

Expression and purification of SARS-CoV-2 nucleoprotein

As shown in Fig. 2, after IPTG induction of protein production in *E. coli*, nucleoproteins were expressed and detected at approximately 50 kD. The target protein was present in the supernatant and the precipitate after sonication of the bacterial solution. The target protein was purified by affinity chromatography on a nickel column, and the final purity was greater than 90% tested by ImageJ. As determined by the BCA kit, the protein concentration was 1.22 mg/mL, which was sufficient yield for the preparation of the kit.

Preparation and validation of selenium nanoparticle anti-SARS-CoV-2 detection kit

Nucleoprotein was labeled with selenium nanoparticles as follows. The normal conjugating concentration was 10 μg/mL, and the coating concentration was 2 mg/mL. The selected test pH levels were 6.5, 7.0, 7.5, 8.0, 9.0, and 10.0. The protein was centrifuged and resuspended after labeling. At pH 6.5, 7.0, and 7.5, the protein stuck to the test tube bottom and was difficult to resuspend, especially at pH 6.5 and 7.0 (Fig. 3a). Therefore, pH 8.0, 9.0, and 10.0 were selected for subsequent experiments. During serum testing, no false positives were identified at pH 8.0, 9.0, or 10.0, irrespective of whether the IgG detection kit or IgM detection kit was used (Fig. 3b). However, the color rendering effect of pH 8.0 was slightly worse than at pH 9.0 or 10.0. Based on these results, as well as the predicted isoelectric point of nucleoprotein, pH 10.0 was finally selected as the conjugating pH for selenium nanoparticle-labeled nucleoprotein.

Using pH 10.0 as the conjugating condition, various conjugating concentrations and coating concentrations were tested. When the conjugating concentrations were 5 μg/mL, 10 μg/mL, and 20 μg/mL and coating concentrations were 1 mg/mL, 2 mg/mL, and 4 mg/mL, no false positive results occurred (Fig. 4a).

The SARS-CoV-2 antibody screening kit requires high sensitivity. A clinical sample validation study was therefore performed, using a conjugating concentration of 20 μg/mL and a coating concentration of 2 and 4 mg/mL to screen for false positive results. Two negative samples were selected for testing. It was found that when the coating concentration was 4 mg/mL, both the IgG and IgM tests showed false positives (Fig. 4b). When the coating concentration was 2 mg/mL, the IgG test showed no false positive results, but a weak false positive result occurred in the IgM test. When the coating concentration was 1.5 mg/mL, there was no false positive result in the IgM test. Therefore, 1.5 mg/mL and 2 mg/mL coating concentrations were selected for anti-IgM and IgG antibodies, respectively. A final conjugating
concentration of 20 \( \mu g/mL \) nucleoprotein was selected for use in the final product, as well as 1.5 mg/mL as the anti-IgM antibody coating concentration, 2 mg/mL as the anti-IgG antibody coating concentration, and 1 mg/mL as the anti-His antibody coating concentration.

Clinical specimen validation

A total of 41 samples were collected from the hospital and clinically diagnosed as negative for SARS-CoV-2. Thirty-nine patients were found to be IgG or IgM negative by testing and two were positive, including one IgG-positive sample and one IgM-positive sample. In addition, 18 cases were found to be positive using our kit (Fig. 5) in the 19 samples that tested positive by nucleic acid testing. Of the positive results, eleven (61.1%) were positive for either IgM or IgG, and four cases (22.2%) were double positive. From these data, the sensitivity of the kit is determined to be 94.74%, the specificity is considered 95.12%, the positive predictive value is 90%, and the negative predictive value is 97.5%.

Discussion

Selection of protein and conjugating conditions

The structural proteins of SARS-CoV-2 include spike protein, envelope protein, membrane protein, and nucleoprotein. Spike protein infects host cells by binding to the human ACE2 receptor. This spike protein shows great variability, but the receptor is suitable for drug development; however, the sample detection accuracy is likely to decrease as the SARS-CoV-2 mutates. Nucleoprotein is the most highly conserved of these structural proteins, so the sample detection rate can be improved through use of nucleoprotein.

When the effect of coating concentration was tested, FBS was selected to ensure the stability of the detection matrix. The serum component of FBS is less than that in human serum component resulting in less interfering factors. The coating concentration of the test strip 2 mg/mL anti-human IgG antibodies and 4 mg/mL anti-human IgM antibodies did not have false positives. However, when human serum matrix was tested, it was found that the anti-human IgG antibodies coated with 4 mg/mL showed false positives. In addition, coating with 2 mg/mL and 4 mg/mL anti-human IgM antibodies both showed false positive results. Therefore, 1.5 mg/mL anti-human IgM antibody was used for coating in test line.

Preparation of the kit

The detectable antibodies of SARS-CoV-2 include IgG and IgM, which can be completed using two kits separately or using one kit. The detection speed and method of interpreting the kit results are the same. In the two-kit strategy, there is no mutual interference between the two kits and the accuracy is higher compared with the combined-kit strategy. A combined test can reduce the sample volume but the process requirements are higher than those of using two separate tests. In order to quickly develop a SARS-CoV-2
antibody detection kit, we here selected the separate test method, and we planned to develop a test kit for combined detection.

SARS-CoV-2 is highly infectious, so the positive samples were under strict control and were difficult to obtain. However, the effectiveness of SARS-CoV-2 antibody detection kits requires clinical sample verification as there is no standard detection antibody for SARS-CoV-2. Thus, we were not able to quickly determine the optimal preparation conditions during research and development. Based on the test results of 19 positive samples, some samples showed weak test lines. This detection assay was improved by increasing the test line coating concentration and the conjugating concentration of selenium and nucleoprotein.

Clinical specimen validation

In this study, only preliminary testing of clinical samples was performed. A total of 19 positive samples were tested, and 18 cases were consistent with nucleic acid results. Of the 19 patients, 9 were male and 11 were female. They were all severe and critical condition patients. The patient who had the negative result had chronic bronchitis. Whether chronic bronchitis affects the detection of SARS-CoV-2 requires further research. To determine the true effectiveness of the kit, it is still necessary to expand the test sample size to validate the overall sensitivity and specificity of the kit. Moreover, patients having different stages of COVID-19 and a wide range of ages need to be tested to determine whether the detection results of the kit are affected by those factors.

Conclusion

We have developed a new assay for the rapid detection of SARS-CoV-2 antibody (IgM + IgG) using a selenium nanoparticle based method. The sensitivity of the kit is 94.74% and the specificity is 95.12%. The assay is portable and fast. The results can be obtained within 10 min. The assay kit does not require any special device for reading the results and the readout is a simple color change that can be evaluated with the naked eye. This kit is suitable for rapid and real-time detection of the SARS-CoV-2 antibody.

Abbreviations

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Immunoglobulin M: IgM

Immunoglobulin G: IgG

COVID-19: Corona virus disease 2019

LFIA: Lateral flow immunoassay
IPTG: Isopropyl β-D-1-thiogalactopyranoside
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis
PBS: Phosphate-buffered saline
SeNps: Selenium nanoparticle solution
BSA: Bovine serum albumin
FBS: Fetal bovine serum

**Declarations**

**Ethics approval and consent to participate**

This study is approved by the Biomedical Research Ethics Committee of Henan University (HUSOM2020-187) and the patient is consent to participate.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The data set generated and/or analyzed during the current study are not publicly available due to non-disclosure agreements with data providers, but are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**
Conceived and designed the study: ZZ. W, YH. W, YF. M. Implemented the study: Z. Z, XC. W, PM. Z, FC. C, QW. Z, HZ. H, XQ. L. Collected clinical samples: HL. Z, YX. W, G. L, X. L, J. Z. All authors contributed to review and revision and have seen and approved the final version of manuscript.

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Figures
Figure 1

Diagram and components of the SARS-CoV-2 antibody immunoassay test strip (a) and visual assessment guidelines for interpreting the test strip results (b). Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; His: Histidine tag; NP: Nucleoprotein of SARS-CoV-2; Se: Selenium nanoparticle; IgM: Human IgM; IgG: Human IgG; Ab: Antibody; C: Control line; T: Test line
Figure 2

SARS-CoV-2 nucleoprotein purification SDS-PAGE results. Abbreviation: M, marker lane. 1) Bacterial culture before induction. 2) Bacterial culture after induction. 3) Precipitate from centrifuged bacterial culture after induction. 4) Supernatant from centrifuged bacterial culture after induction. 5) Flow-through of nickel column supernatant. 6) Eluate of the target protein—nucleoprotein conjugate. 7) The reference protein. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Figure 3

pH value determination for selenium nanoparticle-conjugated nucleoprotein. Photographs of centrifuge, resuspending (a) the test strip (b) results are shown.
Figure 4

Selection of conjugating and coating conditions and test of conjugating conditions for anti-SARS-CoV-2 detection. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Figure 5

Detection results of anti-SARS-CoV-2 IgG and IgM. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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