Development of chemiluminescent lab-on-fiber immunosensors for rapid point-of-care testing of anti-SARS-CoV-2 antibodies and evaluation of longitudinal immune response kinetics following three-dose inactivation virus vaccination

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Abstract
Developing reliable, rapid, and quantitative point-of-care testing (POCT) technology of SARS-CoV-2-specific antibodies and understanding longitudinal vaccination response kinetics are highly required to restrain the ongoing coronavirus disease 2019 (COVID-19) pandemic. We demonstrate a novel portable, sensitive, and rapid chemiluminescent lab-on-fiber detection platform for detection of anti-SARS-CoV-2 antibodies: the chemiluminescent lab-on-fiber immunosensor (c-LOFI). Using SARS-CoV-2 Spike S1 RBD protein functionalized fiber bio-probe, the c-LOFI can detect anti-SARS-CoV-2 immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies with high sensitivity based on their respective horseradish peroxidase-labeled secondary antibodies. The limits of detection of anti-SARS-CoV-2 IgG and IgM antibodies were 0.6 and 0.3 ng/ml, respectively. The c-LOFI was successfully applied for direct detection of anti-SARS-CoV-2 antibodies in whole blood samples with simple dilution, which can serve as a finger prick test to rapidly detect antibodies. Furthermore, the longitudinal immune response (>12 months) kinetics following three-dose inactivated virus vaccines was evaluated based on anti-SARS-CoV-2 IgG detection results, which can provide important significance for understanding the immune mechanism against COVID-19 and identify individuals who may benefit from the vaccination and booster vaccination. The c-LOFI has great potential to become a sensitive, low-cost, rapid, high-frequency POCT tool for the detection of both SARS-CoV-2-specific antibodies and other biomarkers.

KEYWORDS
anti-SARS-CoV-2 antibody, chemiluminescent immunoassay, immune response kinetics, lab-on-fiber immunosensor, vaccination
INTRODUCTION

Since 2019, the world experienced unprecedented morbidity and mortality because of the coronavirus disease 2019 (COVID-19) pandemic that was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).\(^1\)\(^-\)\(^4\) To restrain the spread of COVID-19, many vaccines in the world have obtained emergency use certification.\(^5\) The objective of vaccination is to induce anti-SARS-CoV-2 antibodies to reduce infection and form a population immune barrier.\(^6\)\(^-\)\(^8\) Quantitative measurement of specific antibodies is essential for assessing the immune acquisition and long-term protection effect after vaccination and improving vaccination deployment based on the herd immunity level. Particularly, more protection effect after vaccination and improving vaccination globulin G (lgG) antibodies detection.\(^18\)\(^,\)\(^21\) However, most of these significance in SARS automation, and low detection risk, CLIA has important clinical application for quantitative detection of specific antibodies.\(^17\)\(^,\)\(^18\) For this, developing novel reliable quantitative POCT technology of anti-SARS-CoV-2 IgG and IgM antibody (IgG-S and IgM-S) detection according to the previous method with minor modifications (details in Supporting Information Section S1).

2.1 Reagents and chemicals

See Supporting Information Section S1 for reagents and chemicals used in this study.

2.2 Functionalization of bio-probe for anti-SARS-CoV-2 IgM/IgG antibody detection

The S-protein was immobilized on the fiber bio-probe for the detection of anti-SARS-CoV-2 IgG and IgM antibody (IgG-S and IgM-S) detection according to the previous method with minor modifications (details in Supporting Information Figure S1).

2.3 Design of the c-LOFI and its application for antibody detection

Scheme 1A shows the schematic of the c-LOFI, consisting of a microfluidic system, optical system, signal treatment and control system, and mini-computer. The photo of the c-LOFI is shown in Figure S2. Optical system, including fiber bio-probe, fiber patch cord, and photodetector, is the key section of the entire sensor because it determines the sensitivity, portability, and stability. In the c-LOFI, the S-protein functionalized fiber bio-probe is employed not only as a biorecognition element but also as a separated element and transducer. The chemiluminescence is collected by the bio-probe and detected by a PD-C1 detector after transmission of the fiber patch cord and filtering using a bandpass filter. In the microfluidic system, the peristaltic pump and six-way injection valve are used for sequentially introducing various fluids into the metal cylindrical microfluidic cell. The fiber bio-probe is embedded in the microfluidic cell (\(\phi 0.8 \times 30.0\) mm) with an effective volume of 15.0 \(\mu\)L. Signal treatment and control system is built based on a microcontroller unit, which is used for the treatment of the chemiluminescence signal and for controlling the pump and valve operation. The dynamic detection curve and final detection result are shown on the display screen.
Taking IgG-S for example, the quantitative detection of the anti-SARS-CoV-2 antibodies is achieved based on the sandwich chemiluminescent immunoassay principle (Scheme 1B). First, S-protein functionalized bio-probe is placed in the microfluidic cell, and 15.0 μl of the sample containing various concentrations of IgG-S is introduced into it for a certain time. In this phase, IgG-S specifically bind with S-protein on the surface of fiber bio-probe. Second, after phosphate-buffered saline (PBS) buffer is pumped into the cell to remove free IgG-S, a certain concentration of the HRP-labeled anti-IgG secondary antibodies are added into the cell, and part of them binds with the IgG-S. Third, after washing the free secondary antibodies using PBS buffer, 15.0 μl CL substrate solution is pumped into the surface of fiber bio-probe where the CL intensity is real-time detected from the HRP-catalyzed luminescence of substrates. The detectable CL intensity is related to the concentration of IgG-S, which is used for their quantitative detection. Finally, the bio-probe is

**SCHEME 1** Design of chemiluminescent lab-on-fiber immunosensor (c-LOFI) and detection mechanism of anti-SARS-CoV-2 antibodies. (A) Schematic of the c-LOFI consisting of a microfluidic system, optical system, signal treatment, and control system, and minicomputer. (B) Detection mechanism of IgG-S based on chemiluminescent sandwich immunoassay principle.
regenerated for 300 s with sodium dodecyl sulfate solution (0.5%, pH 1.9) and rinsed with PBS buffer to reuse. The whole process of detection is less than 30 min.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of S-protein functionalized bio-probe and c-LOFI

To evaluate the c-LOFI performance, the S-protein functionalized bio-probe was placed in a microfluidic cell for immunoassay of IgG/S-IgM-S. First, IgG-S was detected using c-LOFI, and Figure 1A shows the typical signal curve of IgG-S detection; 15.0 µl IgG-S of 5.0 µg/ml was introduced into the microfluidic cell for a 300 s incubation reaction, and part of them bound with the S-protein immobilized on the fiber surface. After washing with PBS, 15.0 µl HRP-labeled secondary antibodies of 0.5 µg/ml were then delivered to the microfluidic cell. Some of the secondary antibodies reacted with IgG-S for 400 s. After the excess secondary antibodies were washed away using PBS, 15.0 µl CL substrate solution was introduced to perform the CL detection. From Figure 1A, an abruptly increasing chemiluminescence signal was detected by the PD-C1 detector after addition of the CL substrate solution due to the HRP catalysis. The CL signal ($I_p$) for detection is calculated as the difference between the CL intensity at the peak ($I_p$) and the baseline ($I_b$).

$$I_p = I_p - I_b.$$  (1)

Second, IgM-S was also determined based on a similar immunoassay principle (Figure 1B). Fifteen microliters IgM-S of 100 ng/ml was primarily pumped over the S-protein functionalized bio-probe for incubation reaction. After washing, HRP-labeled anti-IgM secondary antibody was added to bind with IgM-S bound on the bio-probe surface. Then, CL substrate solution was added to perform the CL detection. The same as the IgG-S detection, the CL signal also increased after the addition of CL substrate solution (Figure 1B).

Third, to further verify the CL signal generated from the immunoassay reaction, several control experiments were performed (Figure 1C). (a) The CL substrate solution was directly delivered to the microfluidic cell, and no CL signal was detected. (b and c) The HRP-labeled anti-IgG secondary antibody or HRP-labeled anti-IgM secondary antibody was initially added for incubation, and the CL substrate solution was then delivered. A low CL signal was detected, which originated from the nonspecific adsorption of secondary antibodies. (d) The IgM-S, HRP-labeled anti-IgG secondary antibody, and the CL substrate solution was sequentially introduced. The CL signal could also be detected due to the nonspecific adsorption of HRP-labeled anti-IgG secondary antibody, but it was significantly lower than that using HRP-labeled anti-IgM secondary antibody. (e) The IgG-S, HRP-labeled anti-IgM secondary antibody, and the CL substrate solution was sequentially introduced. Similarly, a lower CL signal was obtained compared with that using HRP-labeled anti-IgG secondary antibody. For comparison, the functionalized fiber bio-probe was also characterized using the confocal fluorescence microscope (Nikon A1; 638 nm). From Figure 1Da, no fluorescence is observed on the surface of the S-protein modified bio-probe. A little fluorescence is demonstrated after Cy5.5-labeled secondary antibody is pumped over the bio-probe (Figure 1Db), which contributes to nonspecific adsorption. However, when IgG-S and Cy5.5-labeled secondary antibody was sequentially introduced, a clear fluorescence image was obtained (Figure 1Dc). The above results demonstrated that the S-protein was successfully immobilized on the bio-probe. The detected CL signal mainly originated from the specific binding of S-protein, IgG-S, and anti-IgG secondary antibody, or S-protein, IgM-S, and anti-IgM secondary antibody. Although both IgG-S and IgM-S could specifically bind with S-protein, they might bind with different sections of S-protein and did not affect each other. Therefore, using the S-protein modified fiber bio-probe, either IgG-S or IgM-S could be detected just using HRP-anti-IgG secondary antibody or HRP-anti-IgM secondary antibody, respectively.

To further verify the specificity of the S-protein functionalized fiber bio-probe, the other three antibodies (anti-MERS-CoV antibody, anti-HCoV-HKU1 antibody, and normal human IgG) were tested. From Figure 1E, the individual addition of three antibodies does not induce the increase of CL, which is similar to that of a blank sample. However, the mixture of anti-MERS-CoV, anti-HCoV-HKU1, normal human IgG, and IgM-S antibodies was introduced into the optofluidic cell, and a significant CL intensity was obtained, which is almost identical to that of IgM-S antibody. Therefore, the proposed method was highly specific for IgM-S and IgG-S detection, which depended on the high specificity of IgM-S and IgG-S to S-protein and the secondary antibodies to IgG-S or IgM-S.

Finally, the reusability of the S-protein functionalized fiber bio-probe was also investigated. From Figures 1 and S3, when the regeneration solution was pumped over the cell, the signal curve returned to the baseline. In this process, all the bound antibodies were dissociated from the surface of the optical fiber. After washing by PBS buffer, a similar CL signal was again detected when the IgG-S/IgM-S and HRP-anti-IgG/IgM-S secondary antibodies were sequentially added (Figure S3A,B). The experimental result shows that the functional bio-probe could be reused more than 15 times without obvious detection performance loss (Figure S3C,D). The reusability of bio-probe not only saves detection cost but also increases the accuracy of detection results. All these results show that the proposed c-LOFI can provide a good solution for the quantitative detection of IgG-S/IgM-S based on the sandwich immunoassay mechanism.

3.2 | Detection parameter optimization

Several parameters, including SARS-CoV-2 S-protein concentration, the incubation time of primary antibody, and the concentration and incubation time of HRP-labeled secondary antibody, were optimized. The concentration of S protein covalently attached to the fiber surface is a key factor to affect the signal output. S-protein of various
FIGURE 1  Characterization of S-protein functionalized bio-probe and chemiluminescent lab-on-fiber immunosensor (c-LOFI). (A) Typical detection process and signal curve for IgG-S antibody detection. 5.0 μg/ml IgG-S and 0.5 μg/ml horseradish peroxidase (HRP)-labeled anti-IgG secondary antibody. (B) Typical detection process and signal curve for IgM-S detection. 100 ng/ml IgM-S and 1.0 μg/ml HRP-labeled anti-IgM secondary antibody. (C) Verification of the CL signal came from the binding reaction between antibody and antigen. (a) CL substrate solution was directly added; (b) 0.5 μg/ml HRP-labeled anti-IgG secondary antibody and CL substrate solution was sequentially added; (c) 1.0 μg/ml HRP-labeled anti-IgM secondary antibody and CL substrate solution was sequentially added; (d) 100 ng/ml IgM-S, 0.5 μg/ml HRP-labeled anti-IgG secondary antibody, and CL substrate solution was sequentially added; (e) 5.0 μg/ml IgG-S, 1.0 μg/ml HRP-labeled anti-IgM secondary antibody, and CL substrate solution was sequentially added; (f) 5.0 μg/ml IgG-S, 0.5 μg/ml HRP-labeled anti-IgG secondary antibody, and CL substrate solution was sequentially added; (g) 100 ng/ml IgM-S, 1.0 μg/ml HRP-labeled anti-IgM secondary antibody, and CL substrate solution was sequentially added. (D) Images of confocal fluorescence microscope for S-protein functionalized bio-probe (a), only addition of Cy5.5-labeled anti-IgG secondary antibody (b), and sequential addition of IgG-S and Cy5.5-labeled anti-IgG secondary antibody (c). (E) Specificity of the S-protein functionalized fiber bio-probe, and the concentration of anti-MERS-CoV antibody, anti-HCoV-HKU1 antibody, and normal human IgG is 100 ng/ml, 100 ng/ml IgM-S, 1.0 μg/ml HRP-labeled anti-IgM secondary antibody. The error bars were the standard deviation of three repeated experiments (n = 3).
concentrations ranging from 0.05 to 0.5 mg/ml was applied for the functionalization of the bio-fiber probe (Supporting Information). Figure S4A,B shows that the CL intensity increases with increasing the concentration of S protein and reaches a plateau for the detection of both IgM-S and IgG-S. The optimal concentration of S-protein was 0.3 mg/ml considering the CL intensity and the reagent consumption.

To determine the optimal incubation time between IgG-S/IgM-S and S-protein immobilized onto the bio-probe surface, the incubation time ranging from 0 to 9 min was tested. Figure S4C,D show that the CL intensity increased rapidly over time when the incubation time of IgM-S and IgG-S was less than 5 and 3 min, respectively, and then reached a plateau, which was used as the optimal time. The secondary antibody reaction time was optimized. Figure S4E,F displayed that the CL signal increased obviously when the reaction time of anti-IgM and IgG secondary antibodies was less than 7 min and remained basically the same over time. Thus, the optimal time was set at 7 min.

The concentration of HRP-labeled secondary antibody is one of the key factors for the detection of IgG-S/IgM-S. To study the optimum secondary antibody concentration, a sensitivity index ($\varepsilon$) was defined as follows:

$$\varepsilon = \frac{l_0 - l_i}{l_i},$$

where $l_0$ and $l_i$ are the CL intensities of the sandwich chemiluminescent immunoassay without or with primary antibody (IgG-S/IgM-S), respectively. In practical use, the best secondary antibody concentration was determined considering several criteria. First, the stronger CL intensity should be obtained in the presence of IgG-S or IgM-S. Second, a higher $\varepsilon$ should be achieved (>0.60) to improve detection sensitivity. Figure S4G,H shows the CL intensities without or with primary antibody significantly increased with increasing secondary antibody concentration for both IgG-S and IgM-S, respectively. The maximum $\varepsilon$ for IgG-S and IgM-S were 0.62 and 0.67 when secondary antibodies were 0.5 and 1.0 $\mu$g/ml, respectively, which were used as the optimum condition for subsequent IgG-S and IgM-S measurements.

### 3.3 | Dose–response curve of anti-SARS-CoV-2 IgM/IgG antibody in PBS

Once optimizing detection parameters, quantification analysis of IgM-S/IgG-S was done based on the sandwich chemiluminescent immunoassay principle. The typical CL signal traces for IgM-S/IgG-S detection was shown in Figure 2A,B, respectively, which illustrated increasing concentration of both IgM-S and IgG-S led to proportional enhancement of their CL intensities. To obtain the dose–response curves, the normalization value of a certain IgM-S or IgG-S, $N_{\text{IgM}}$, and $N_{\text{IgG}}$, is calculated as follows:

$$N_{\text{IgM}} = \frac{l_{\text{s,IgM}} - l_{\text{b,IgM}}}{l_{\text{s,IgM,\max}} - l_{\text{b,IgM}}},$$

$$N_{\text{IgG}} = \frac{l_{\text{s,IgG}} - l_{\text{b,IgG}}}{l_{\text{s,IgG,\max}} - l_{\text{b,IgG}}}$$

where $l_{\text{s,IgM,\max}}$ and $l_{\text{s,IgG,\max}}$ are the CL intensities at the maximum IgM-S or IgG-S concentration, respectively; $l_{\text{s,IgM}}$ and $l_{\text{s,IgG}}$ are the CL intensities of the blank samples without IgM-S or IgG-S, respectively; $l_{\text{b,IgM}}$ and $l_{\text{b,IgG}}$ are the CL intensities of the samples with different antibody concentrations.

The dose–response curves for IgM-S and IgG-S were plotted against the concentration logarithm using a four-parameter logistic equation, respectively (Figure 2C,D). The error bars shown in Figure 2C,D are less than 10%, indicating the excellent stability of the proposed methods. The limit of detection (LODs) of IgM-S and IgG-S is 0.3 and 0.4 ng/ml using three times the standard deviation of the mean blank values, respectively. These LODs are lower than those of ELISA (4.2 and 0.6 ng/ml for IgM-S and IgG-S, respectively, shown in Figure S5), Considering between 20% and 80% of inhibition, the linear response of IgM-S and IgG-S ranged from 0.5 to 19.1 ng/ml and from 2.1 to 1084.5 ng/ml, respectively. Table S1 presents the biosensing features of our platform and other previous works, including the targets and LODs.

### 3.4 | Detection of anti-SARS-CoV-2 IgG/IgM antibody in blood

Direct on-site detection of IgM-S and IgG-S antibodies in whole blood greatly benefits for rapid diagnosis and appropriate treatment of COVID-19 patients. Unfortunately, the complexities of whole blood, containing many organic components including proteins, amino acids, or lipids, are challenging for their immunoassay due to the matrix effect on immunoassay. Herein, we explored the matrix effect of whole blood on the detection of IgG-S or IgM-S. The IgG-S or IgM-S was spiked into PBS, primary whole blood, 5, 10, 20, and 30 times diluted blood, respectively, with a final concentration of 50.0 ng/ml. These samples were detected by the proposed method. Figure S6 demonstrates that the CL intensity of primary whole blood is quite lower than that of PBS, which resulted from the inhibition of the blood matrix on the antibody-antigen interaction. With increasing the dilution times, the detectable CL intensity increased and was similar to that in PBS when the whole blood was diluted more than 20 times, suggesting that the dilution blood could be directly used for IgG-S and IgM-S detection. The common methods to eliminate blood matrix effects, such as sample extraction and cleanup procedures, are generally time-consuming, laborious, and not suitable for POCT. Dilution of the blood sample is a simple and useful method to achieve
on-site POCT detection. Therefore, blood samples diluted 20 times were used in subsequent experiments.

After confirming the capability of c-LOFI to detect antibodies in whole blood, standard curves were established to measure IgG-S and IgM-S using healthy donors’ blood without vaccination. After spiking with various amounts of IgG-S and IgM-S, the whole blood samples were detected using c-LOFI. Similar to those in PBS, increasing the concentration of both IgG-S and IgM-S also resulted in proportional enhancement of their CL intensities. The dose–response curves for IgM-S and IgG-S were plotted against the logarithm of their concentrations (Figure 3A,B). The LODs of IgM-S and IgG-S are 0.3 and 0.6 ng/ml, respectively, which are similar to those in PBS. Considering between 20% and 80% of inhibition, the linear response of IgM-S and IgG-S ranged from 0.6 to 23.7 ng/ml and from 1.8 to 984.9 ng/ml, respectively (Figure 3C,D).

To further prove the feasibility of our device in the real scenario, we applied the c-LOFI to quantify IgG in negative finger blood samples and positive finger blood samples with the vaccine. In five negative finger blood samples, no IgG-S was detected because they did not either vaccine or infect the SARS-CoV-2 virus. Then, these samples were spiked with various concentrations of IgG-S and IgM-S detected using the c-LOFI. Tables S2 and S3 show that the recovery rates of the spiked samples are in the range of 77%–136% with relative standard derivations of less than 10%. Furthermore, the IgG-S originating from five vaccination volunteers’ finger blood samples were also detected using the c-LOFI. The concentrations of IgG-S were from 17.1 to 182.1 ng/ml, which were consistent with those detected by ELISA (Table S4). These results demonstrated that the c-LOFI system can be regarded as a reliable method of quantitative analysis of IgG-S and IgM-S. More importantly, the
c-LOFI can directly be applied for the rapid detection of the whole blood sample, which is especially useful for on-site patient diagnosis.

Compared with various reported POCT devices, c-LOFI has outstanding advantages in high sensitivity and quantitative analysis. First, the c-LOFI has a low background signal and a high signal-to-noise ratio, which allows a high sensitivity. Second, the specific recognition sites of IgM-S/IgG-S can be increased by modifying the number of the biorecognition molecules on the surface of the bio-probe, which can decrease the amount of IgM-S/IgG-S to obtain the same CL intensity. Third, the S-protein immobilized on the surface by chemical modification keeps high activity toward IgM-S/IgG-S to reduce the nonspecific adsorption. In addition, the c-LOFI has other superiority, such as easy-to-use, portability, cost-effectiveness, and rapidity.

3.5 | Immune kinetics with three-dose inactivation virus vaccination

Anti-SARS-CoV-2 humoral response kinetics benefits for COVID-19 diagnosis and vaccine development. Lacking protective immunity may be induced by decreasing antibody levels. Many vaccines have been officially approved for emergency use including inactivated virus vaccines, RNA/DNA vaccines, and subunit vaccines. Among them, inactivated virus vaccines are one of the most promising choices because they retain all of the antigenic components of the corresponding virus and have several significant features, such as potentially high efficacy, low cost, high feasibility, and high safety. The IgG is the most robust and durable antibody against the SARS-CoV-2 virus after vaccine and infection. Therefore, evaluating anti-SARS-CoV-2 IgG antibodies level is pivotal to monitoring the immune response. Studies have reported that IgG could persist for

![FIGURE 3](image-url)

**FIGURE 3** Typical signal curves of (A) IgM-S detection and (B) IgG-S detection using chemiluminescent lab-on-fiber immunosensor (c-LOFI) system in blood; (C) dose–response curve of IgM-S detection in blood (1.0 μg/ml secondary antibody); (D) dose–response curve of IgG-S detection in blood (0.5 μg/ml secondary antibody) (n = 3).
3-8 months in the human body after SARS-CoV-2 infection symptoms. However, IgG antibodies with longer-term kinetics especially for inactivated virus vaccines, remain to be investigated.

In line with this, we analyzed immune dynamics for up to 12 months using finger blood samples from four healthy young people immunized with the following three-dose inactivation virus vaccination (Coronavac). Figure 4 shows the response dynamic curves of IgG-S detected by c-LOFI4, in which 4 negative samples and 48 positive samples, obtained before and after vaccination, respectively, were tested. Before vaccination, no anti-SARS-CoV-2 antibodies were detected in all finger blood samples. After a week of one-dose vaccination, the IgG-S was detected in all vaccination donors, indicating that the vaccine induced the humoral immune reaction. The higher IgG antibody concentration was obtained after 2 weeks and then decreased, which was similar with previous reports. On the 21st day, the second-dose vaccine of all donors was injected. Similarly, the IgG-S concentration of all donors rapidly increased and by far higher than that of the first vaccine. The highest IgG-S concentration was 73 μg/ml. However, different from previous studies, the high concentration of IgG-S did not last a long time, but rapidly decreased and then reached a platform. Even though 200 days later, the concentration of IgG-S kept a stable value. After the third-dose vaccine, a higher IgG-S concentration was obtained in all donors, which was almost three times higher than those of the first-dose vaccine. Surprisingly, the IgG-S concentration also rapidly decreased and then reached a platform similar to that of the second vaccine. This decline in seropositivity over time might explain why an increased infection breakthrough was observed in cases among vaccinated individuals, even for the third booster vaccine. Even though our data provided humoral immune response for the observation of vaccination cohorts, a deeper investigation carried out by c-LOFI will be needed because of the limitation of the small sample size.

4 CONCLUSION

A portable and rapid detection platform (c-LOFI) was proposed for detection of anti-SARS-CoV-2 antibodies, which can meet the growing demand for extensive antibody testing facing the ongoing pandemic and the population-wide vaccine deployment. The c-LOFI can quantitatively detect IgG-S or IgM-S in whole blood with simple dilution using functional fiber bio-probe and their respective HRP-labeled secondary antibodies. Using the c-LOFI, the IgG-S and IgM-S gave excellent linearity over the range of 1.8–984.9 ng/ml and from 0.6–23.7 ng/ml, and displayed a LOD of 0.6 and 0.3 ng/ml for IgG and IgM antibody, respectively. The real-time simultaneous detection of IgG-S and IgM-S is helpful for infection progression tracking. Furthermore, the longitudinal immune response kinetics following vaccination was analyzed based on the proposed method, which provided valuable information for the diagnosis, treatment, vaccine research, and epidemiological research for COVID-19.

AUTHOR CONTRIBUTIONS

Wenjuan Xu: Conceptualization, methodology, data curation, writing original draft, writing—review and editing. Dang Song: Methodology, investigation, writing—review and editing. Jiayao Liu: Methodology, investigation. Xiangzi Han: Methodology. Jiaxin Xu: Methodology. Anna Zhu: Conceptualization, supervision, writing—original draft, writing—review and editing. Feng Long: Conceptualization, methodology, funding acquisition, project administration, supervision, writing—original draft, writing—review and editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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