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Article

Development and Application of Human Coronavirus Protein Microarray for Specificity Analysis

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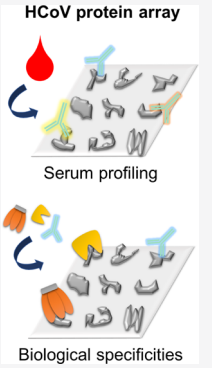
ABSTRACT: Coronavirus is an enveloped RNA virus that causes mild to severe respiratory diseases in humans, including HKU1-CoV, 229E-CoV, NL63-CoV, OC43-CoV, SARS-CoV, MERS-CoV, and SARS-CoV-2. Due to the outbreak of SARS-CoV-2, it is important to identify the patients and investigate their immune responses. Protein microarray is one of the best platforms to profile the antibodies in the blood because of its fast, multiplexed, and sensitive nature. To fully understand the immune responses and biological specificities, this study developed a human coronavirus (HCoV) protein microarray and included all seven human coronaviruses and three influenza viruses. Each protein was printed in triplicate and formed 14 identical blocks per array. The HCoV protein microarray showed high reproducibility and sensitivity to the monoclonal antibodies against spike and nucleocapsid protein with detection limits of 10−200 pg. The HCoV proteins that were immobilized on the array were properly folded and functional by showing interactions with a known human receptor, e.g., ACE2. By profiling the serum IgG and IgA from 32 COVID-19 patients and 36 healthy patients, the HCoV protein microarray demonstrated 97% sensitivity and 97% specificity with two biomarkers. The results also showed the cross-reactivity of IgG and IgA in COVID-19 patients to spike proteins from various coronaviruses, including that from SARS-CoV, HKU1-CoV, and OC43-CoV. Finally, an innate immune protein named surfactant protein D showed broad affinities to spike proteins in all human coronaviruses. Overall, the HCoV protein microarray is multiplexed, sensitive, and specific, which is useful in diagnosis, immune assessment, biological development, and drug screening.



INTRODUCTION

COVID-19 is an infectious disease caused by SARS-CoV-2, which leads to mild to severe respiratory syndromes with an average case fatality rate of 2%. Comparing with other types of coronaviruses, SARS-CoV-2 shares 76−95% protein similarity with SARS-CoV and 29−46% protein similarity with MERS-CoV. Despite the striking similarities between SARS-CoV-2 and SARS-CoV, the latter has a 10% case fatality rate.[1] Due to the global COVID-19 pandemic, it is important to develop an accurate, fast, and flexible platform for its diagnosis and biological development.

The most widely used method for COVID-19 diagnosis is based on a reverse transcription−polymerase chain reaction (RT-PCR). This method detects SARS-CoV-2 RNA in the nasal swab.[2] However, the nasal swab is very uncomfortable to the subjects and increases the risk of transmission due to sneezing or coughing. In contrast, serological testing provides an easier solution for both the subjects and medical practitioners. Moreover, it provides valuable information about the host's immunity. The serological antibodies against nucleocapsid (N) and spike (S) proteins can be detected around 7 days and remain detectable for more than 30 days postinfection.[3] To date, there are over a hundred serological rapid tests based on antibodies against N and S proteins.[4−7] However, the readout only based on the immunogenicity of the N or S proteins is quite risky because the humoral responses vary from person to person and may cross-react due to other human coronaviruses.[8] Therefore, serological tests showed a wide range of sensitivities from 45% (Epitope Diagnostics) to 95% (Creative Diagnostics). To overcome the host antibody variations and cross-reactivity, it is necessary to include the SARS-CoV-2 antigens and other coronavirus antigens including HKU1-CoV, 229E-CoV, NL63-CoV, OC43-CoV, SARS-CoV, and MERS-CoV.

Protein microarray is one of the best platforms to profile the antibodies in the blood due to its fast, multiplexed, and sensitive nature. Protein microarrays have been widely used to profile molecular interactions and identify disease-relevant antibodies in the blood, including autoimmune diseases, cancers, and infectious diseases.[9,10] One of the recent developments is the Zika/Dengue protein microarray to differentiate viral infections[11] and SARS-CoV-2 proteome microarray for total IgG and specific IgG, IgM, and IgA detection.[12−14] Since MERS-CoV, SARS-CoV, and SARS-CoV-2 share a great similarity, it is important to know the

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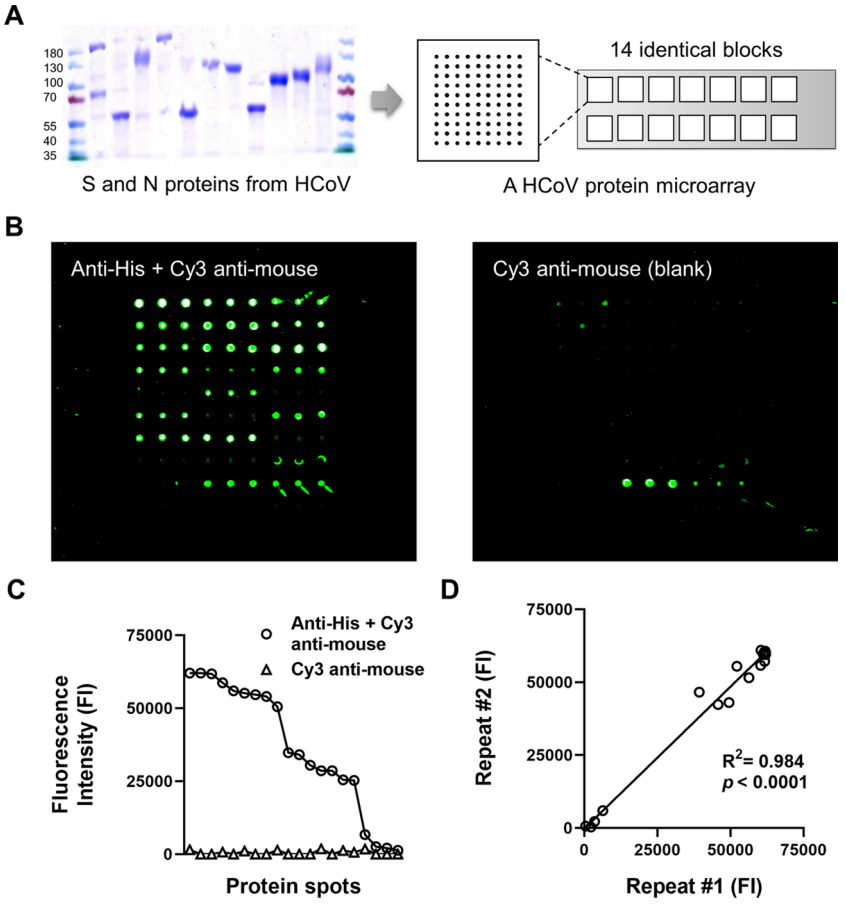

Figure 1. Fabrication and quality control of HCoV protein microarray. (A) Seventeen His-tagged spike and nucleocapsid proteins from several coronavirus species, 3 His-tagged hemagglutinin proteins from influenza viruses, and 10 controls were used in the HCoV protein microarray fabrication. The protein quality was checked by the SDS-PAGE followed by coomassie staining. Once the proteins passed the quality control, they were transferred along with the control samples into 384 well for contact printing. Each sample was printed three times in each block (with the order listed in Table 1) and 14 identical blocks per slide. (B, C) Immobilization of the His-tagged proteins was checked by anti-His staining with Cy3-labeled anti-mouse. Certain blocks were incubated only with Cy3-labeled anti-mouse acts as blank control to show the background of fluorescence staining. (D) As for the reproducibility, the anti-His signal in different blocks was plotted against each other and showed high correlations (R^2 = 0.984).

contribution of these similarities in the immune responses, receptor binding affinities, antibody specificities, and drug specificities. The common cold coronaviruses that are widely spread, e.g., HKU1-CoV, 229E-CoV, NL63-CoV, and OC43-CoV, were suspected to contribute some nonspecific immune responses.[13,15] In recent months, there are several studies utilizing the SARS-CoV-2 and other coronaviruses to fabricate an antigen microarray for serum profiling, including antibody and antigen combinations.[13,14,16,17] This strategy has been used to differentiate SARS-CoV-2 antibodies from that of other pathogens.[18] However, the sensitivities in these studies are limited perhaps due to the lack of quality assessment for the antigen microarray, due to the decision making based on a single type of immunoglobin, or due to the cross-reactivity to form other common coronaviruses. Thus, this study developed a human coronavirus (HCoV) protein microarray, which includes all of the human coronaviruses to significantly reduce the false-positive results that is quality controlled by immunostaining and receptor bindings. The validated HCoV protein array will be used to profile serum IgG/IgA and biological specificities. The HCoV protein microarray developed in this study demonstrates broad serological recognition toward various coronavirus species in COVID-19 patients. The findings will be useful in the diagnosis, disease surveillance, epidemiologic research, antibody drugs, and vaccine assessment, and may help to develop personalized therapies based on humoral immunity.

EXPERIMENTAL SECTION

Collection of HCoV Proteins. Twenty viral proteins, which consist of 10 spike proteins (both full and s1 domains); 7 nucleocapsid proteins from coronavirus that causes human diseases including MERS-CoV, SARS-CoV, SARS-CoV-2, HKU1-CoV, 229E-CoV, NL63-CoV, and OC43-CoV; and 3 HA proteins from popular influenza strains, e.g., H1N1, H3N2, and FluB, were purchased from Sino Biological. Those 6x His-tagged proteins were expressed in insect cells. The proteins were dissolved in 30% glycerol at a 25 μg/μL concentration and kept at −80 °C. The quality of the proteins was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and showed in a single band with a correct molecular weight (Figure 1).

Fabrication of HCoV Protein Microarray. Twenty viral proteins and 10 control samples were transferred to the 384 well as source plate for printing. The slides were coated with aldehyde and stored at 4 °C. Each sample was printed three

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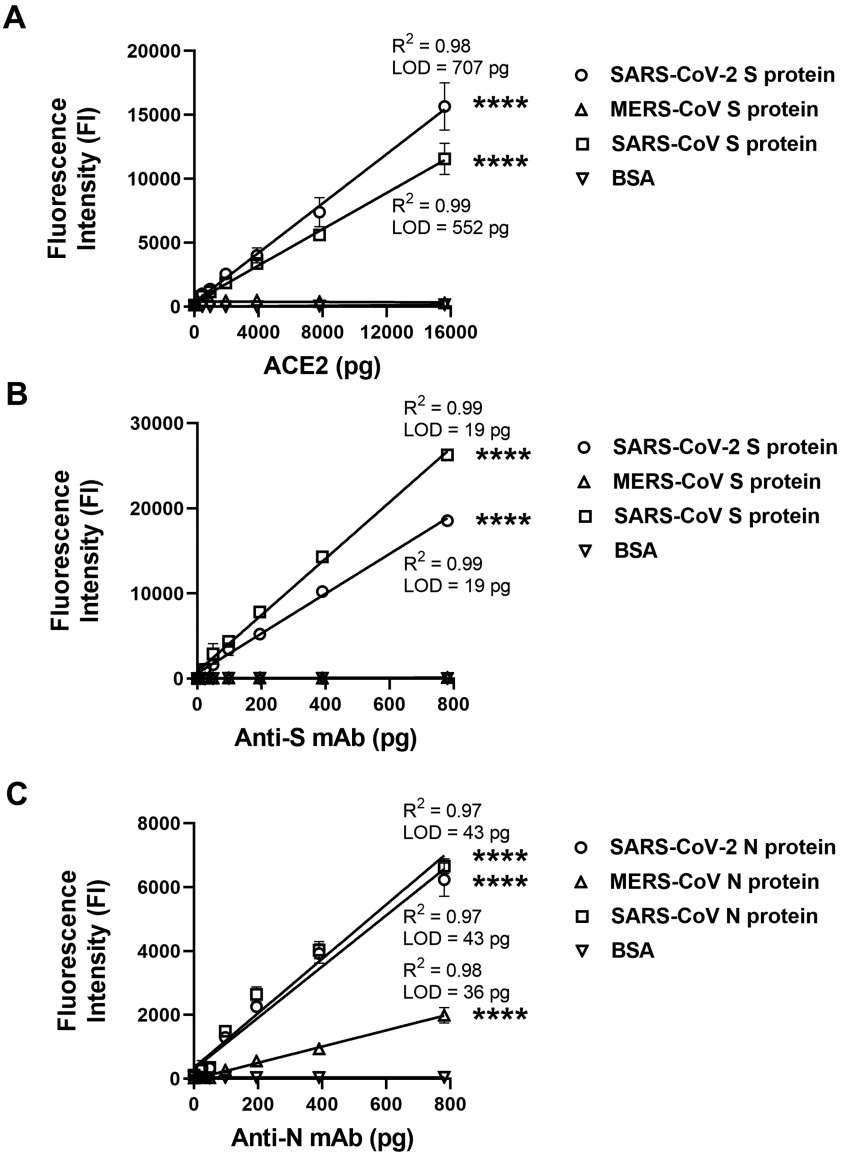

Figure 2. Specificity profiling of antibody and ACE2 using the HCoV protein microarray. The HCoV protein microarray was incubated with (A) a serial dilution of ACE2 and detected with Cy3-labeled anti-human IgG, (B) a serial dilution of anti-spike and detected with Cy5-labeled anti-rabbit, and (C) a serial dilution of anti-nucleocapsid and detected with Cy5-labeled anti-rabbit. The calibration line and R^2 value were calculated by linear regression. The absolute limit of detection (LOD) was calculated by 3-fold SD of the Y-intercept/slope of the calibration line. Data were analyzed by two-way ANOVA with multiple comparisons. ****$p < 0.0001$, compared with BSA.

times in each block (9 × 10 format) and 14 identical blocks per slide using a contact printer (CapitalBio SmartArrayer 136, China). After printing, the HCoV protein microarrays were immobilized overnight, vacuum-sealed, and stored at 4 °C for a short term (<6 months) or at −80 °C for a long term (6 months to a few years). The quality control of the array was performed using anti-6x His staining (1000x dilution, Abbkine, #A02050) with Cy3-labeled anti-mouse (2000x dilution, Jackson Immuno Research, #109-165-003) and showed strong immobilizations of proteins.

Antibody and Receptor Specificity Profiling. Monoclonal antibodies against SARS-CoV-2 S1 domain (Sino Biological, 40150-R007) and SARS-CoV-2 nucleocapsid protein were purchased (Sino Biological, #40143-R019). The purchased antibodies were used to test their specificity and the detection limits of the array. The HCoV protein microarray was incubated with a serial dilution of the antibody for 1 h, washed, incubated with Cy5-labeled anti-rabbit (1000x dilution, Jackson Immuno Research, #111-605-003) for 30 min, washed, dried, and then scanned for fluorescence image (SpinScan, Caduceus Biotechnology). ACE2 (Sino Biological, #10108-H02H) was used to examine the function and the specificity against various viral proteins. The HCoV protein microarray was incubated with a serial dilution of ACE2 for 1 h, washed, incubated with Cy3-labeled anti-human IgG (1000x dilution, Jackson Immuno Research, #109-165-008) for 1 h, washed, dried, and then scanned for fluorescence image.

Serum Profiling. Thirty-two COVID-19 and 36 control serum samples were tested from NHRI Biobank. The study protocol was approved by the institutional review board of National Cheng Kung University Hospital, which is organized and operated according to the laws and regulations of ICH-GCP (IRB No. A-ER-109-225). To speed up the testing time, a novel nonprotein blocking for the protein microarray,

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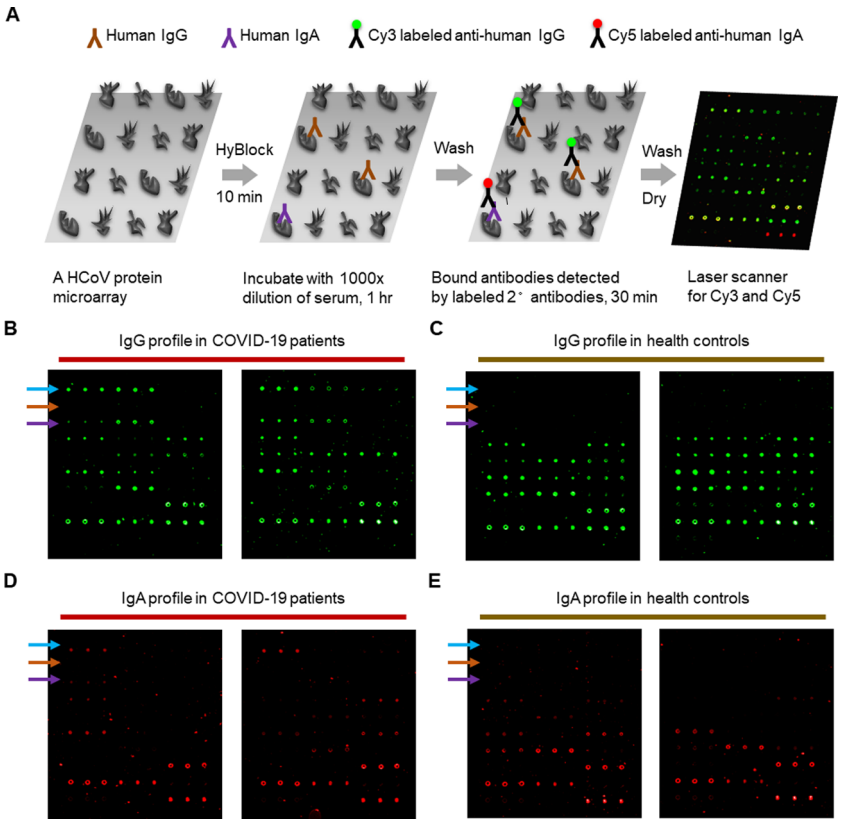

Figure 3. Serum IgG and IgA profiling in COVID-19 and control subjects. (A) HCoV protein microarray was blocked by HyBlock for 10 min and applied with 100 μL of 500-fold diluted serum in TBST supplement with 1% BSA (0.2 μL of original serum). After 1 h incubation, the array was washed and incubated with labeled anti-IgG/IgA antibodies for 30 min. After a final wash, the array was dried and scanned for fluorescence signals. The total procedure took about 150 min. (B, C) Representative images of the IgG in two COVID-19 patients and two healthy controls. (D, E) Representative images of IgA in two COVID-19 patients and two healthy controls. The blue arrow indicates spike protein, nucleocapsid protein, and spike S1 domain from SARS-CoV-2 (from left to right). The orange and purple arrows indicate the same order but from MERS-CoV and SARS-CoV, respectively.

HyBlock (Hycell International, #W-3400), was used. After 10 min of blocking, the array was applied with a 16-well adhesive and incubated with 100 μL of 500-fold diluted serum in TBST supplement with 1% BSA (0.2 μL of original serum) for an hour. After washing, the array was incubated with Cy3-labeled anti-human IgA (1000x dilution, Jackson Immuno Research, #109-165-011) and Cy5-labeled anti-human IgG (1000x dilution, Jackson Immuno Research, #109-605-008) for 30 min, washed, dried, and scanned for fluorescent images.

Specificity Profiling of Recombinant Fragment of Human Surfactant Protein D. The recombinant fragment of human SP-D (rfhSP-D) was purified according to a recent publication.[19] The microarray was placed in a 16-well chamber, washed by TBST + 5mM $CaCl_2$ for 5 min, blocked by SuperBlock (Thermo Fisher Scientific, # VG299792) for 20 min, and incubated with purified surfactant protein D (prepared in 1% BSA in TBST + 5mM $CaCl_2$). After 1 h incubation, the microarray was washed with TBST + 5mM $CaCl_2$ for 10 min, incubated with a mouse antibody against surfactant protein D (R&D Systems, # MAB1920), and followed by Cy5-labeled anti-mouse antibody. After a 10 min wash, the microarray was dried and scanned for analysis.

Data Analysis. The images were analyzed by GenePix Pro software and output as signal minus background or signal-to-noise ratio. The statistics were analyzed by GraphPad Prism software. Linear regression was used to plot the dose-response curve and R^2 value. The absolute limit of detection (LOD) was calculated by 3-fold SD of the *Y*-intercept/slope of the calibration line. For serum profiling, the multiple blocks were normalized based on BSA and buffer to minimize the background from different serum samples. Data were analyzed using unpaired *t*-test for two groups and analysis of variance (ANOVA) with Dunnett's post-test for multiple comparisons. All data were presented as mean ± SD, where n is the number of subjects. Significant differences were defined as $p < 0.05$.

RESULTS AND DISCUSSION

Several proteins are characterized, sequenced, and identified, including the four main structural proteins, specifically the E, S, N, and M proteins of many coronaviruses.[20,21] High levels of structural protein abundance are known as the major targets used to diagnose coronaviruses. S and N proteins are the key antigens used to diagnose COVID-19 as well as other coronavirus infections.[22,23] Also, the cross-reactivity of SARS-CoV-2 to other coronaviruses such as the most common cold-causing ones, 229E, HKU1, NL63, and OC43, which are widely distributed in the general population,[15] can cause false-positive results. To overcome this gap, in the present study, we fabricated a multiplexed HCoV protein microarray utilizing the S and N proteins of several coronaviruses and some influenza viruses that was used to systematically investigate the specificity of the immune response in COVID-19 patients.

Fabrication of the HCoV Protein Microarray. Among 17 SARS-CoV-2 proteins (10 S proteins and 7 N proteins),

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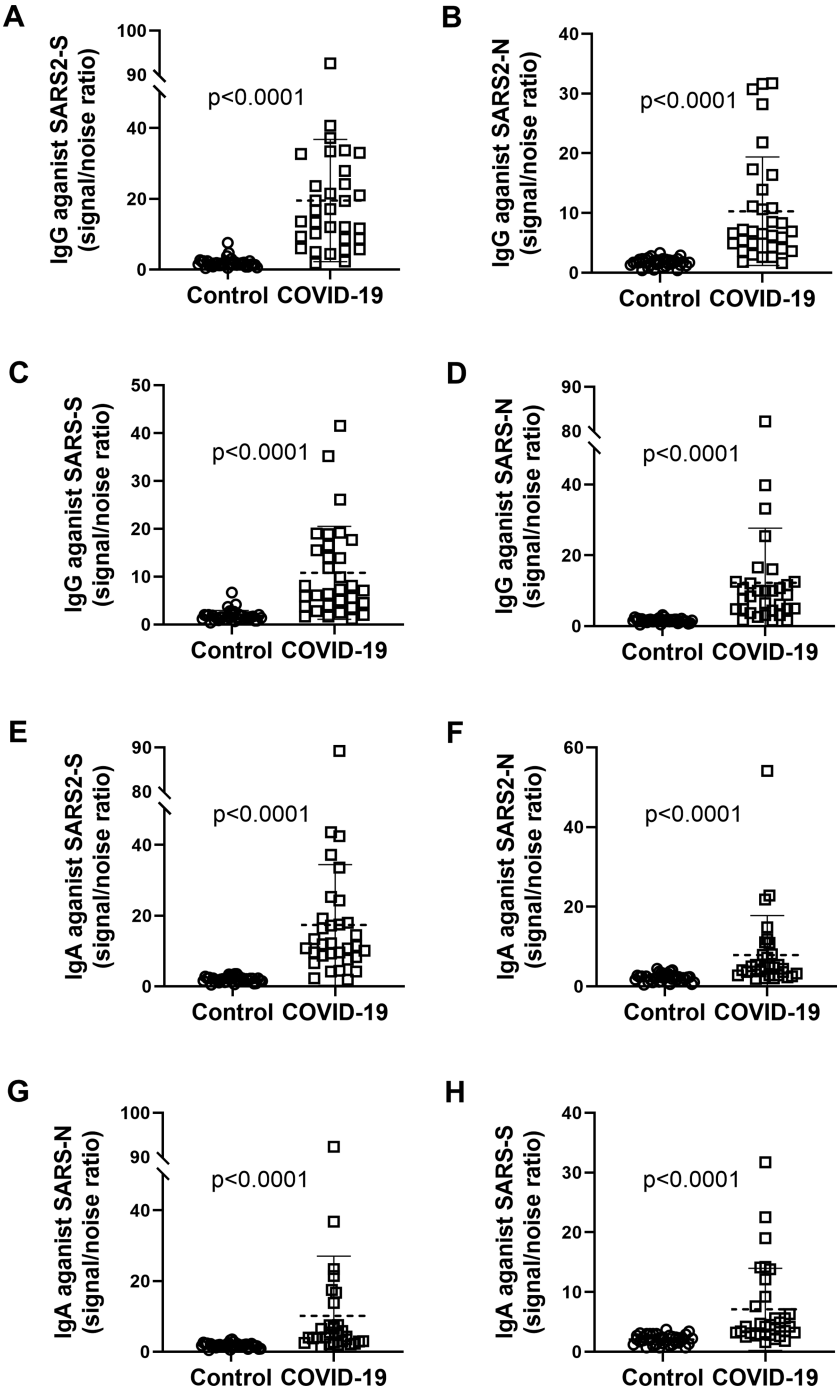

Figure 4. Serum IgG and IgA responses against the spike and nucleocapsid proteins. (A, B, E, F) The serum IgG and IgA reactivity to the spike and nucleocapsid proteins from SARS-CoV-2. (C, D, G, H) The serum IgG and IgA reactivity to the spike and nucleocapsid proteins from SARS-CoV. Data were analyzed by a *t*-test.

only spike and nucleocapsid proteins showed strong immune responses in the patients.[12] Therefore, this study mainly focused on the spike and nucleocapsid proteins and included all of the species of coronaviruses that cause human diseases, e.g., MERS-CoV, SARS-CoV, SARS-CoV-2, HKU1-CoV, 229E-CoV, NL63-CoV, and OC43-CoV. Due to the high occurrence of the influenza virus infection, hemagglutinin proteins were also included from the three most common strains. The full list of the proteins and control samples that were used in this study is presented in Table S1. The proteins and control samples were then spotted on the homemade aldehyde slides in triplicate in 14 identical blocks and formed a high content of HCoV protein microarray (Figure 1).

Quality Control of the HCoV Protein Microarray. To ensure that the proteins were efficiently immobilized, slides were stained with anti-His and Cy3-labeled anti-mouse (Figure 1B). The results showed significant anti-His signals on the array compared to the secondary antibody only (Figure 1C). The anti-His signal can be reproduced in different blocks with $R^2 = 0.984$ (Figure 1D). All of these indicated the high quality of the HCoV protein microarray.

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Receptor Profiling and Antibody Specificities Using HCoV Protein Microarray. To demonstrate that the HCoV protein microarray is functional and able to profile receptor specificity, we select ACE2, a known human receptor for the SARS-CoV and SARS-CoV-2 infection.[24] The results showed that the HCoV protein microarray can detect low concentrations of the ACE2 and showed significant binding to the spike proteins in SARS-CoV-2 (the absolute LOD = 707 pg) and SARS-CoV (the absolute LOD = 552 pg) but not to MERS-CoV (Figures 2A and S1A). Indeed, the MERS-CoV infected human cells with DPP4 other than ACE2.[25]

Currently, the antibody is a powerful tool to block SARS-CoV-2 infection and can be used to detect SARS-CoV-2 viral particles. Here, we selected a monoclonal antibody against the SARS-CoV-2 S1 domain and a monoclonal antibody against SARS-CoV-2 nucleocapsid protein to demonstrate the use of the HCoV protein microarray. The anti-spike antibody showed the highest signal in SARS-CoV-2 and SARS-CoV but not in MERS-CoV (Figures 2B and S1B). Similarly, the anti-nucleocapsid antibody showed the highest signal in SARS-CoV-2 and SARS-CoV, followed by MERS-CoV (Figures 2C and S1C). The absolute LODs were 19 pg for anti-spike and 43 pg for anti-nucleocapsid in SARS-CoV-2, which are 2000- to 5000-fold more sensitive than rapid tests with a 100 ng detection limit.[26]

Serum IgG Profiling Using HCoV Protein Microarray. To accelerate the readout for diagnosis, we tried BSA blocking and nonprotein blocking (HyBlock). HyBlock showed a cleaner background with superior blocking time compared to the traditional BSA blocking (Figure S2). Therefore, the 10 min HyBlock was used in the serum profiling. The serum IgG played an important role in humoral immunity, while IgA played a major role in mucosal immunity. Detection of serum IgA is a novel way to diagnose SARS-CoV-2 infection. The experimental procedures of profiling serum IgG and IgA are described in Figure 3A. Since the detection limit was low in the HCoV protein microarray, only 0.2 μL of serum was needed in the IgG/IgA profiling. The duration of the assay is about 150 min, which was far less than the 20 h procedures for serum profiling.[12]

In our pilot study, serum IgG was profiled in six COVID-19 patients and six healthy controls. They have large differences in IgG reactivity to the proteins from SARS-CoV and SARS-CoV-2. Using signal-to-noise ratio > 5 can perfectly group to COVID-19 patients and healthy controls. Based on the pilot study, we analyzed a larger cohort containing 32 COVID-19 sera and 36 control sera. The full list of IgG profiling results is presented in Table S2, and the representative IgG images from four COVID-19 patients and four healthy controls are shown in Figure 3B,C. There was a significant difference between COVID-19 and control subjects regarding the IgG reactivity to the spike and nucleocapsid proteins from SARS-CoV and SARS-CoV-2 (Figure 4A−D). Therefore, the results demonstrated the usefulness of the HCoV protein microarray in profiling serum IgG and applied it to separate COVID-19 patients from healthy controls.

Serum IgA Profiling Using HCoV Protein Microarray. It is established that N and S proteins are the main antigens of SARS-CoV-2 that induce IgM and IgG antibodies, and the antibody response against N protein is generally stronger.[12] The results in the present study are in agreement with previously published literature wherein they found that the antibody response against N protein was higher compared to S protein.[8,27,28] Besides the IgG and IgM antibody responses, it is important to profile IgA antibodies since it plays a role in mucosal immunity against SARS-CoV-2 infection.[29,30] Secreted IgA can neutralize SARS-CoV-2 before binding and reaching the epithelial cells.[29] In serum, the recent study showed a strong IgA signal in COVID-19 patients, which indicates that IgA could be a valuable diagnostic target.[31,32] However, it is not clear how the specificity of serum IgA in COVID-19 patients. Therefore, we took advantage of the HCoV protein microarray to profile the serum IgA in COVID-19 patients and controls. The full list of IgA profiling results is presented in Table S3, and the representative IgA images from two COVID-19 patients and two healthy controls are shown in Figure 3D,E. Although the IgA concentration was much lower than the IgG in blood, here, IgA has significant reactivity against HCoV proteins. There was a significant difference between COVID-19 and control subjects pertaining to the IgA reactivity to the spike proteins from SARS-CoV-2 and SARS-CoV (Figure 4E−H). Here, we demonstrated the usefulness of the HCoV protein microarray in profiling novel antibody responses and applied it to separate COVID-19 patients from healthy controls.

Biomarker Discovery and Other Relationship That Is Useful in Diagnosis and Epidemiology. Since we were able to separate COVID-19 from controls using the HCoV protein microarray, we further evaluated the sensitivity and specificity of each biomarker or two biomarkers in the combine (Table 1). The most effective single biomarker was IgG against spike protein from SARS-CoV-2 (sensitivity, 90.6%; specificity, 97.2%), followed by IgA against spike protein from SARS-CoV-2 (sensitivity, 84.4%; specificity, 100%). It is noteworthy that the S1 domain of spike protein was not quite immune active and did not generate significant IgG or IgA in most patients. If two biomarkers were combined, (e.g., IgG against spike and nucleocapsid proteins from SARS-CoV-2), the sensitivity and specificity can reach 97%.

Table 1. Sensitivity and Specificity of Single and Dual Biomarkers

single/dual biomarkers	sensitivity	specificity
SARS2-S IgG + SARS2-N IgG	31/32 = 96.9%	35/36 = 97.2%
SARS2-S IgG + SARS2-S IgA	30/32 = 93.8%	35/36 = 97.2%
SARS2-S IgG + SARS−N IgG	30/32 = 93.8%	35/36 = 97.2%
SARS2-S IgG + SARS−S IgG	29/32 = 90.6%	35/36 = 97.2%
SARS2-S IgG	29/32 = 90.6%	35/36 = 97.2%
SARS2-S IgA	27/32 = 84.4%	36/36 = 100%
SARS2-N IgG	21/32 = 65.6%	36/36 = 100%
SARS−N IgG	21/32 = 65.6%	36/36 = 100%
SARS−S IgG	21/32 = 65.6%	35/36 = 97.2%

The HCoV protein microarray can be used to show the cross-reactivity between different species in COVID-19 patients (Figure S3). The results demonstrated that the IgG or IgA against SARS-CoV-2 spike protein had showed cross-reactivity with SARS-CoV spike protein, HKU1-CoV spike protein, and OC43-CoV spike protein. In Figure S4, the amino acid similarity among those proteins compared with the SARS-CoV-2 spike was SARS-CoV spike (74.9% identity, 90.5% similar), HKU1-CoV spike (29.3% identity, 57.9% similar), and OC43-CoV spike (30.3% identity, 58.8% similar). Based on the low similarity of the amino acid sequences, we believe that the IgG and the IgA produced in COVID-19 patients

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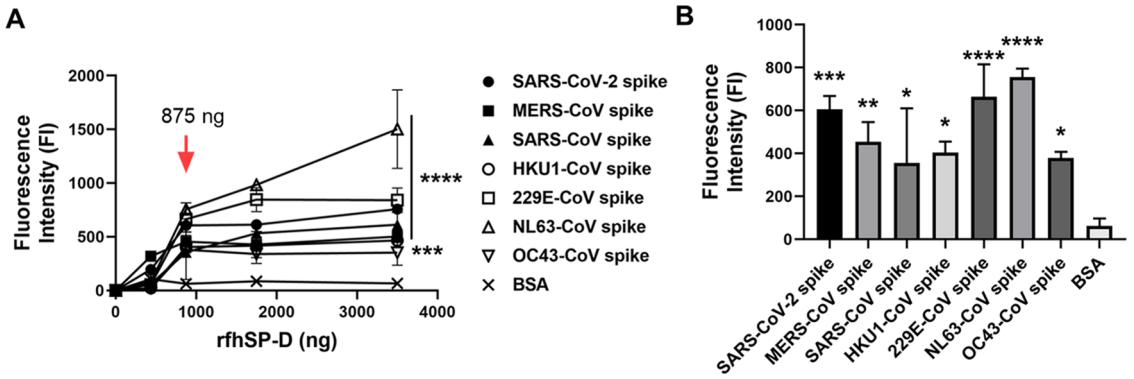

Figure 5. Specificity profiling of rfhSP-D using the HCoV protein microarray. The rfhSP-D was purified and incubated with the HCoV protein microarray with calcium supplementation. (A) The spike proteins from SARS-CoV-2, MERS-CoV, SARS-CoV, HKU1-CoV, 229E-CoV, NL63-CoV, and OC43-CoV showed significant binding signals compared with BSA. Data were analyzed by two-way ANOVA with multiple comparisons. ****$p < 0.0001$, ***$p < 0.001$, compared with BSA. (B) The 875 ng of rfhSP-D showed significant bindings to the spike proteins from all human coronaviruses. Data were analyzed by One-Way ANOVA with multiple comparisons. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, compared with BSA.

reacted with numerous epitopes on the spike proteins and show somewhat broad but low specificity. This indicates the possible tolerance for humoral immunity against the evolving mutations of the SARS-CoV-2 spike protein. Also, the broad immune reactivity to several coronaviruses may indicate the possible protection if found to have prior exposure to the common cold coronaviruses. Interestingly, our findings matched with the recent epidemiological study, which showed that the symptoms of COVID-19 patients were less severe if found to have prior exposure to the common cold coronaviruses.[33]

On the contrary, the IgG or IgA against the SARS-CoV-2 nucleocapsid protein only cross-reacts with the SARS-CoV nucleocapsid protein. The similarity of nucleocapsid protein between SARS-CoV-2 and SARS-CoV was high, 90.5% identity and 97.2% similar. Therefore, the IgG and IgA produced in COVID-19 patients reacted more specifically to the nucleocapsid protein.

Binding and Specificity of rfhSP-D. Surfactant protein D was recently found to recognize spike protein in SARS-CoV-2.[19] However, the binding and specificity of rfhSP-D to other coronaviruses are currently unknown. Strikingly, rfhSP-D showed broad binding activity to the spike proteins not only from SARS-CoV-2 but also from all of the human coronaviruses (Figure 5). The broad binding spectrum of rfhSP-D could allow maintaining the binding ability against various ongoing mutations.

CONCLUSIONS

Altogether, this study developed highly sensitive and reproducible HCoV protein microarrays and applied them in specificity tests and biological and serological profiling. The HCoV protein microarray and our findings will not only shed some light on the diagnosis and immunological research in COVID-19 patients but also accelerate the biological advancements, vaccine assessments, and drug developments. Due to limited time and resources, this study only assessed COVID-19 patients and did not include influenza serum samples. The future directions would be developing competition assays against ACE2 to evaluate the serum neutralizing antibodies in patients or individuals after vaccination. Also, SARS-CoV-2 variants will be included in the HCoV protein microarray for a wider range of detection and analysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c00614.

Content of a HCoV protein array, list of IgG/IgA responses in COVID-19 patients and healthy controls, blocking reagents for serum profiling, and the similarity of spike proteins from several coronavirus species (PDF)

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Author Contributions

¶P.-X.D. and Y.-Y.C. are first authors.

Author Contributions

P.-X.D., Y.-Y.C., H.M.S., G.-D.S., and M.-H.H. conceived and designed the experiments. Data analysis was performed by P.-X.D. and G.-D.S. P.-X.D., H.M.S., B.B.K., M.-H.H., T.-S.H., J.-Y.W., Y.-L.L., and G.-D.S. wrote the paper.

Notes

The authors declare no competing financial interest.

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