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Lab Note

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Assessment and comparison of recombinant proteins from different sources for the detection of SARS-CoV-2 infection by using protein microarray

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COVID-19 (The COVID-19 is a type of disease and resulted from being infected by virus SARS-CoV-2) pandemic is threatening tens of millions of lives around the world [1]. To date, specific drugs or vaccines are still not available for fighting the SARS-CoV-2. Diagnosis and quarantine are still the effective strategies for preventing the virus from spreading. Up to now, various detection methods for SARS-CoV-2 have been developed [2], which play critical roles in controlling the pandemics. According to the targets to be detected, these methods can be divided into three classes: methods for the nucleic acid [3], antigens [4], and antibodies elicited by the virus in the host [5]. Among these methods, the ones for antigens and antibodies could produce results in 10–15 min [6], which are suitable for point-of-care diagnosis, especially the ones for antibodies [7]. Meanwhile, the asymptomatic cases [8] and the false-negative results of nucleic acid testing [9] make the detection methods based on antibodies the best alternatives. The proteins, used in methods for detection of antibodies elicited by SARS-CoV-2 in the host, are critical agents, which could affect the diagnosis results. Given the strong immunogenicity of the nucleocapsid (N protein), spike protein (S protein), and related domains [10] and the potential diagnosis values, we collected 9 S1 proteins, 3 N proteins, 5 S2-related proteins, and receptor-binding domains (RBDs) from ACROBiosystems (Beijing, China), ABclonal (Wuhan, China), Novoprotein (Shanghai, China), Sino Biological (Beijing, China), and KactusBiosystems (Shanghai, China) and fabricated a protein microarray (**Fig. 1A**) to assess their diagnosis performance. In addition, other proteins such as the angiotensin converting enzyme 2 (ACE2), envelop protein (E), 3C-like protease, and papain-like protease were also included (**Fig. 1B**).

According to the protocol described by Jiang *et al.* [10], the fabricated protein microarray is evaluated by a standard serum, the mixture [11] of the serum collected from the COVID-19 patients (**Fig. 1A**). Firstly, as expected, the secondary antibody DyLight^{TM} 549 affinipure goat anti-human IgG (Jackson ImmunoResearch, West Grove, USA) could interact with the human IgG, not human IgM. The other secondary antibody DyLight^{TM} 649 affinipure donkey anti-human IgM (Jackson ImmunoResearch) interacts with the human IgM, not human IgG. No interference between human IgG and IgM exists. These results demonstrated that both secondary antibodies can be used to measure the human IgG and IgM respectively. Secondly, each spot for investigation of IgG and IgM in the serum has good morphology and uniform fluorescence intensity (FI). Thirdly, many IgG and IgM against N protein, S protein, and related domains exist in the standard serum. No or few antibodies against ACE2, E protein, 3C-like proteinase, and papain-like proteinase exist. These results are the same as our earlier results [10]. In a word, the protein microarray with high quality was fabricated.

According to the protocol described previously [10], a set of 36 COVID-19 serum and 36 control serum are profiled by protein microarray. There is no significant difference between the two groups with regard to age and gender. One section of the protein microarray, with the protein concentration of 0.125 mg/ml, demonstrated the best performance, such as enough sensitivity, no interference between the two adjacent spots, and no smearing. As a result, the FI of this section for every serum is used to estimate the diagnosis value of each protein. Meanwhile, the proteins, including ACE2, E protein, 3C-like proteinases, and papain-like proteinase, were not analyzed further for low FI.

According to the FI of immobilized human IgG/IgM on the protein microarray, all the FI values of other proteins in protein microarray for investigating the IgG/IgM in the serum are normalized. For the SARS-CoV-2 specific IgG in serum, as shown in **Fig. 2A**, all the S1 domains, S2-related domains, RBDs, and N proteins (expressed in either mammalian cells or baculovirus-insect cells) told the COVID-19 patients from the healthy persons. Furthermore, analysis by receiver operating characteristic (ROC) curve for each protein was carried out. The area under curve (AUC) for each protein, except protein 3#, was 1.00, which demonstrated that these proteins could tell the COVID-19 patients from the healthy ones thoroughly.

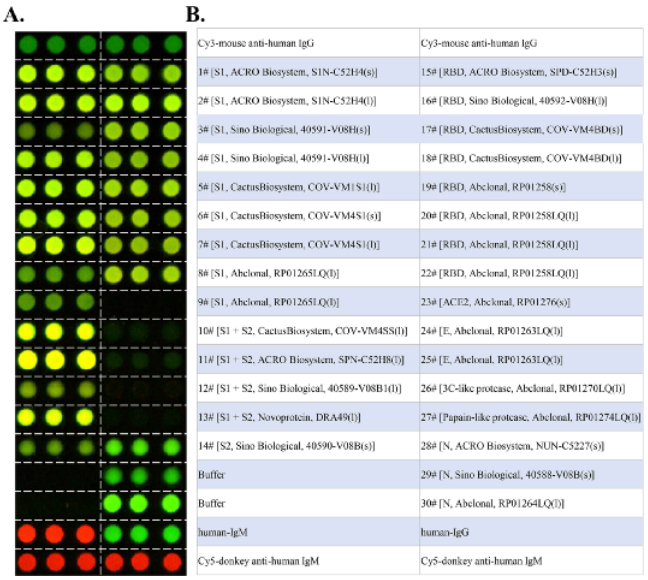

Figure 1. Protein microarray fabricated with high quality (A) The IgG/IgM in standard serum against S protein and related domains and N protein. (B) The pattern of protein microarray fabricated in this study and the information of proteins, such as source, catalog number, and format, of which 'l' in the bracket stands for the proteins provided in liquid format and 's' stands for the proteins provided in lyophilized format.

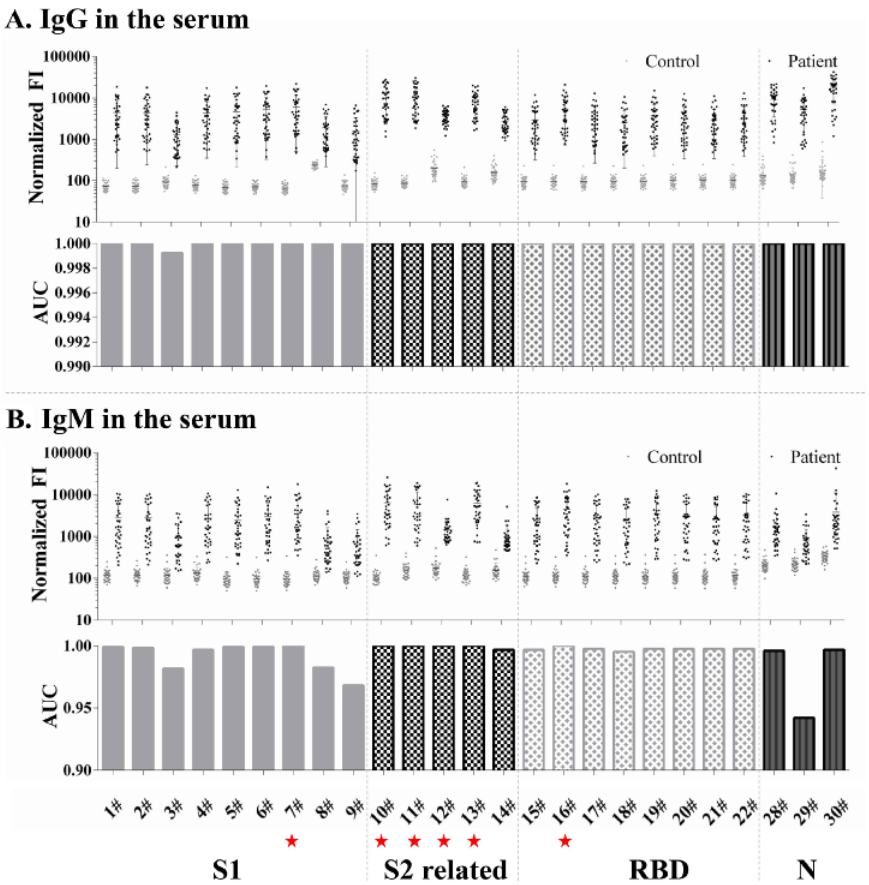

Figure 2. The performance of all proteins in distinguishing the COVID-19 patients from healthy ones All S1 domains, S2 related domains, RBDs , and N protein could tell the COVID-19 patient from the healthy ones by the SARS-CoV-2-specific IgG (A, up) and IgM (B, up) in the serum against corresponding proteins. The AUC values of each protein for SARS-CoV-2-specific IgG (A, bottom) and IgM (B, bottom) are all more than 0.94. The proteins, labelled with the red star, demonstrate 100% sensitivity and 100% specificity in detecting SARS-CoV-2-specific IgG or IgM for COVID-19 diagnosis.

For the SARS-CoV-2 specific IgM in serum, as shown in Fig. 2B, all S1 domains, S2-related domains, RBDs, and N proteins also told the COVID-19 patients from the healthy persons. Similarly, ROC analysis for each protein was also performed. The AUC of proteins 7#, 10#, 11#, 12#, 13#, or 16# was 1.00 (Fig. 2B, bottom). The AUC of protein 29# is 0.94206, which is the smallest one. However, its diagnosis performance is still good.

Compared with their performances in profiling SARS-CoV-2 specific IgM in serum, some proteins, such as 8#, 9#, 15# or 17# were better in profiling SARS-CoV-2 specific IgG in serum. This was resulted from the serum samples. The serum might be with low titer IgM for antigens of SARS-CoV-2, because they were collected from COVID-19 patients at the convalescent stage.

The last but not least, the normalized FI of some antigens demonstrated significant difference, even from the same supplier with different formulation, liquid or lyophilized. All these results suggest that differences, such as the glycan modification, exist in the same antigens.

Taken together, we estimated the diagnosis value of antigens from several suppliers by protein microarray. All the S1 domains, S2-related domains, RBDs, and N proteins, produced from the mammalian or insect cells, can be used to develop the diagnosis method for COVID-19, of which all the S proteins with full length, and a smaller substitute S1 from CactusBiosystem with liquid format, even the smallest substitute RBD from Sino Biological demonstrated 100% sensitivity and 100% specificity (labeled as the red star in the bottom of Fig. 2B). These might provide the guidelines for preparation and choice of antigens in developing diagnosis methods for COVID-19.

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Conflict of Interests

The authors declare there is no any confilct of interests.

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