Machine Learning-enabled Classification and Monitoring of COVID-19 Immunity Levels Using a Paper-based Multiplexed Sensor

Introduction

Serological testing refers to the detection of antibodies, e.g., IgM and IgG, and these tests play a critical role in defining the immune levels of individuals after vaccine administration or an infection. Recognizing the importance of COVID-19 immunity across populations, it is essential to employ rapid tests for identifying disease patterns and establishing public health strategies in the face of the persistent threat of SARS-CoV-2. To track COVID-19 immunity efficiently in point-of-care (POC) settings, we present a paper-based multiplexed vertical flow immunoassay (xVFA) along with a custom-designed serodiagnostic algorithm. During the development and testing of our algorithm, we utilized serum samples from individuals who had received mRNA-COVID-19 vaccines, tracking their antibody levels before and after each vaccine dose. By categorizing these samples based on their IgM and IgG levels into three categories (i.e., protected, unprotected, and infected), we trained and blindly evaluated a neural network-based algorithm for its inference accuracy. Leveraging this serodiagnostic algorithm, our cost-effective, paper-based xVFA platform swiftly measured the IgG and IgM levels from serum samples, facilitating the accurate monitoring of COVID-19 immunity levels. With its simple operation, scalability, and cost-effectiveness, our xVFA technology offers accessible COVID-19 serology testing to classify patients' immunity status rapidly.

Materials & Methods

The multiplexed xVFA platform consists of a bottom case and two top cases. These components have a nitrocellulose membrane and several paper layers to facilitate the introduction of gold conjugates and the human serum sample. The paper-based assay panel was designed using five proteins of SARS-CoV-2: nucleocapsid (N-protein), spike protein subunit-2 (S2-protein), receptor binding domains (RBD-1, RBD-2), and spike protein subunit-1 (S1-protein), and they were dispended on the testing spots in two replicates (Figure 1a). After employing two separate xVFAs to simultaneously detect the IgM and IgG using 40 µL of human serum sample, a color change was monitored on the testing spots in the presence of the antibody and its gold conjugate (Figure 1b). Once the assays were completed, the paper-based panel images were captured using a mobile phone-based custom-designed reader (Figure 1c) and analyzed by a neural network-based algorithm. For the training/validation of the neural network, 30 serum samples were used by conducting 120 xVFA tests, and we blindly tested our serodiagnostic algorithm by running 124 xVFAs with an additional 31 serum samples, which were not included in the training/validation set. In the neural network model, the output layer comprised three units, each representing an immunity level with a sigmoid activation function. The output unit with the highest predicted normalized score (0-1) determined the final classification of the COVID-19 immunity level (protected / unprotected / infected).

Results, Discussion and Conclusion

In our optimized xVFA platform, we used the negative control spots, N-protein, S1-protein, S2-protein, RBD-1, and RBD-2 spots in our paper-based assay panel for testing the IgG levels in human serum samples. In contrast, positive control spots, N-protein, S1-protein, RBD-1, and RBD-2 spots were selected for IgM detection with our xVFA platform (see Figure 1d). After validating our SARS-CoV-2 protein selection for the paper-based assay panel and optimizing the neural network-based algorithm, our serodiagnostic algorithm defined prediction scores (PS)

as "PS-Pro," "PS-Unp," and "PS-Inf" for protected, unprotected, and infected, respectively, which yielded an accuracy of 89.5% in classification of COVID-19 immunity levels (Figure 1e).

In addition, we successfully tracked the temporal dynamics of the immune responses of six individuals at various post-vaccination time points. For instance, one individual reached the maximum PS-Pro against SARS-CoV-2 60 days after vaccination and maintained this immunized level for 114 days (Figure 1f). Moreover, the same individual exhibited a relatively higher PS-Inf score on day 19 post-vaccination, potentially indicating high titer virus exposure or other immune-related complications. These PS-Pro scores at the corresponding time points were also consistent across blindly tested serum samples from all six individuals and correlated with ground-truth IgG and IgM levels (Figure 1f). In summary, our machine learning-based COVID-19 serology assay boasts numerous advantages: it requires only 40 µL serum sample for testing; results are obtained using a compact and cost-effective smartphone-based optical reader; the algorithm swiftly and reliably classifies the patient's immunity status, including identifying infected samples and tracking declining immunity over time.

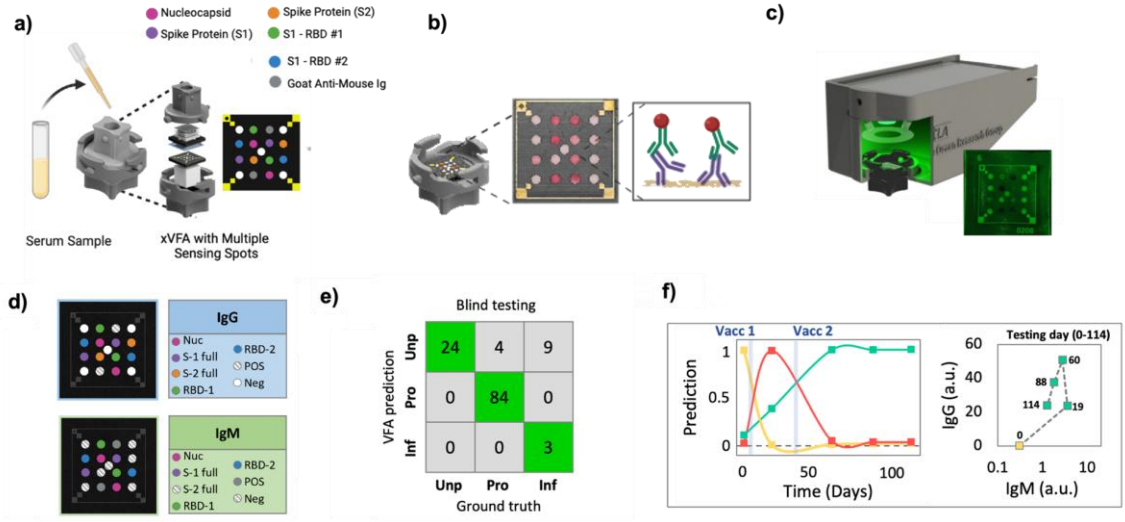

Figure 1. a) Design of the xVFA and details of the components of the platform; (b) Color change after the immunoreaction on the capture spots; (c) Mobile phone-based reader and the image of the paper-based assay panel; (d) Optimal subset of antigens from the IgG panel (top), Optimal subset of antigens from the IgM panel (bottom) (e) Confusion matrix for all the tested serum samples from the blind testing set; f) Blind testing result for one individual that was tracked over time: neural network predictions of immunity level over time (left) and ground truth IgG/IgM levels (right).