COVID-19 Immunity Monitoring Using a Multiplexed Paper-Based Assay and Machine Learning

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Introduction. Monitoring the level of COVID-19 immunity within populations is crucial for understanding the disease trend and guiding public health policies, as SARS-CoV-2 remains a threat. Serological testing is essential in assessing immunity by detecting antibodies, IgG, and IgM, the immune system's response developed against infection or after vaccination. To rapidly and cost-effectively monitor COVID-19 immunity levels, we developed a paper-based multiplexed vertical flow immunoassay (xVFA) which detects the IgG and IgM levels in less than 20 mins, aiming to monitor the COVID-19 immunity levels of individuals longitudinally and categorize immune levels into three groups: protected, unprotected, and infected.

Materials & Methods. The multiplexed xVFA platform for monitoring COVID-19 immunity comprises a bottom case housing a nitrocellulose membrane serving as a paper-based assay panel, along with two top cases (Fig. 1a) designed for introducing the serum sample and gold conjugate solution, respectively. The assay panel consists of ten capture spots representing five structural proteins of SARS-CoV-2 (RBD-1, RBD-2, S1-protein, S2-protein, N-protein). We monitored colorimetric changes on these spots following an immunoreaction in the presence of the analyte (IgG and IgM) and gold conjugates (Fig. 1b). Using 40 µL of human serum sample per test, we conducted two xVFA tests. The images of the sensing panel were captured with a mobile phone-based reader (Fig. 1c) and analyzed using our serodiagnostic algorithm. We optimized a neural network architecture with 30 serum samples from the training/validation set to develop the serodiagnostic algorithm. Subsequently, the optimized model was blindly tested on a testing set comprising 31 serum samples from 8 individuals.

Results & Discussion. Following the optimization of both the serodiagnostic algorithm and the xVFA platform, specific test spots, including RBD-1, RBD-2, S1-protein, S2-protein, N-protein, and negative control were chosen for detecting IgG, while RBD-1, RBD-2, S1-protein, and N-protein spots were selected for IgM detection in human serum samples (Figures 1d and 1e). These optimized selections of IgG and IgM signals were utilized to automatically classify each patient sample into three predetermined categories of COVID-19 immunity (i.e., protected, unprotected, and infected), as shown in Fig. 1f. Furthermore, the temporal dynamics of immune responses were effectively monitored, and COVID-19 immunity levels were classified with 89.5% accuracy, correlating well with the ground truth IgG and IgM levels obtained from an FDA-cleared analyzer.

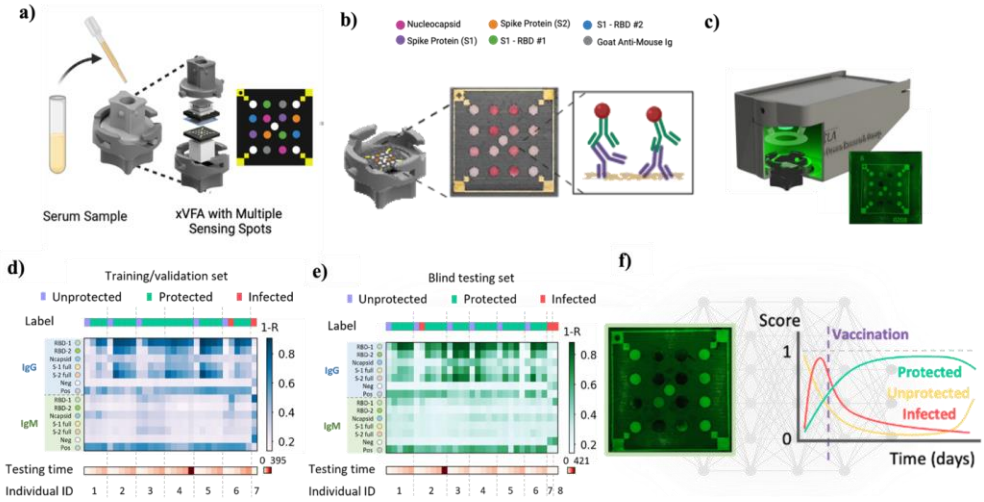

Figure 1. a) Design of the xVFA and details of the cases, (b) Immunoreaction on the capture spots and color change, (c) Mobile phone- based reader. Heatmap of xVFA signals from: (d) training/validation set and (e) blind testing set for the IgG and IgM.

Conclusions. Our xVFA platform for COVID-19 immunity monitoring using a neural-network-based serodiagnostic algorithm enabled robust classification of the immune levels of patients over time. With its simple operation, speed and cost-effectiveness, the presented xVFA provides an accessible POC (point-of-care) testing technology for immunity profiling of individuals.

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