Implementation and testing of enzyme linked immunosorbent assay for the detection of SARS-CoV-2 receptor binding domain antibodies

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I. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has caused over 4 million deaths worldwide[1] and is a major ongoing public health concern. COVID-19 is caused by the novel, highly contagious severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Importantly, the quantitative and qualitative host-immune response to SARS CoV-2 remains only partially characterized. To evaluate longitudinal effects of SARS-CoV-2 exposure and vaccination, we designed an epidemiological study to collect and analyze biospecimens (including blood, serum, stool samples etc.,) from a cohort of MD Anderson employees. This comprehensive evaluation is expected to highlight novel biomarkers associated with immunity. The receptor binding domain (RBD) of the SARS CoV-2 spike protein contains the critical neutralizing domain (CND) which is the target of highly potent host neutralizing antibodies[2]. Therefore, the detection of anti-RBD antibodies in serum (i.e., seroconversion) is considered predictive of immune protection from COVID19. To this end, we implemented an enzyme linked immunosorbent assay (ELISA)[3] to detect circulating antibodies against the RBD of the virus' Spike (S) protein as a screening assay to triage samples into subsequent anti-Spike antibody titration, in vitro neutralization and immune monitoring assays. Testing and implementation of this RBD-based ELISA to detect serum antibodies in volunteers is the first step in recognizing seropositivity trends and subsequently, understanding host immune responses correlative with microbiome diversity, dietary patterns, and T-cell responses.

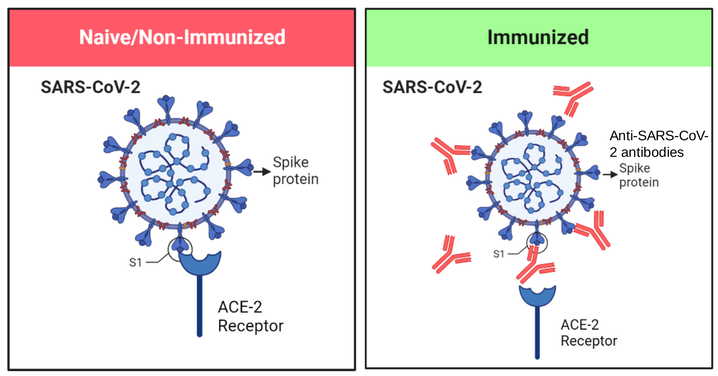

Figure 1. SARS CoV-2 seroconversion is defined as the development of neutralizing antibodies as a result of natural infection or vaccine mediated exposure

II. Immunity Surveillance Study

Involvement of MD Anderson Employees in COVID-19 Immunity Surveillance

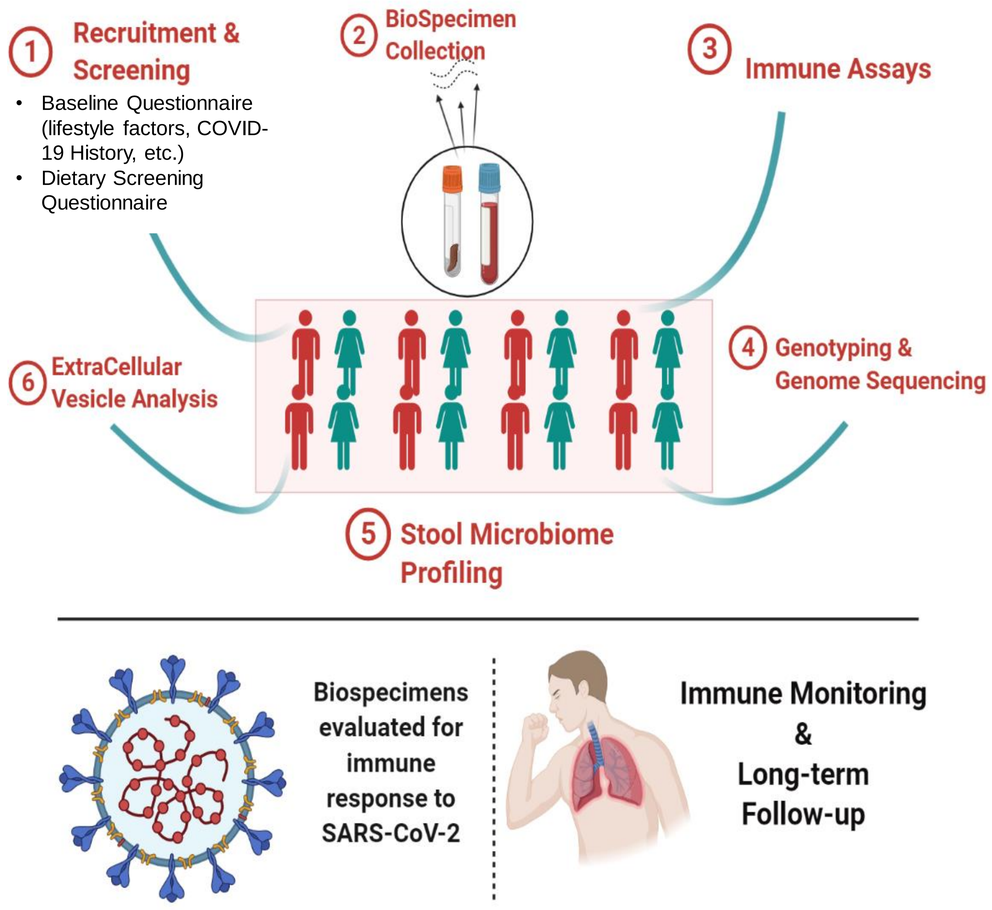

Figure 2. Graphical workflow of COVID-19 immunity surveillance study for MD Anderson employees regardless of vaccination history.

III. Serology Assay Validation

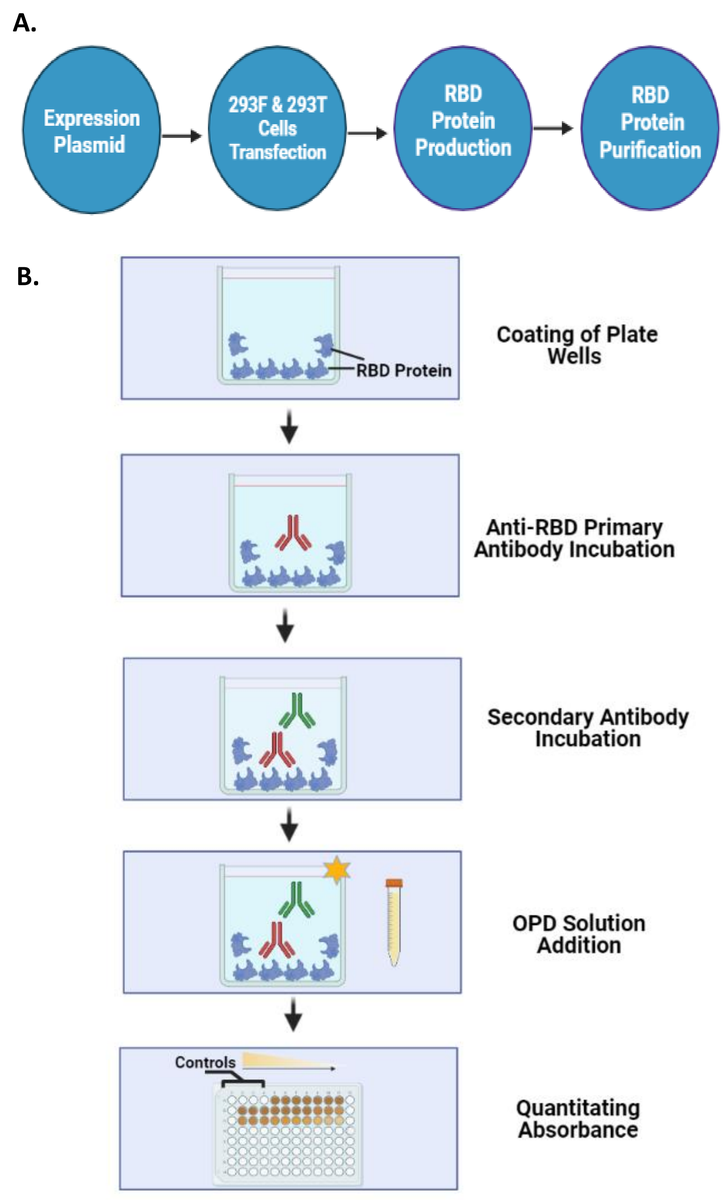

Figure 3. A) HEK 293F & 293T cell transfection and RBD protein purification protocol developed by Stadlbauer et al. for generating of SARS-CoV-2 RBD. **B)** Graphical workflow of indirect ELISA used to test for RBD reactivity.

IV. Results

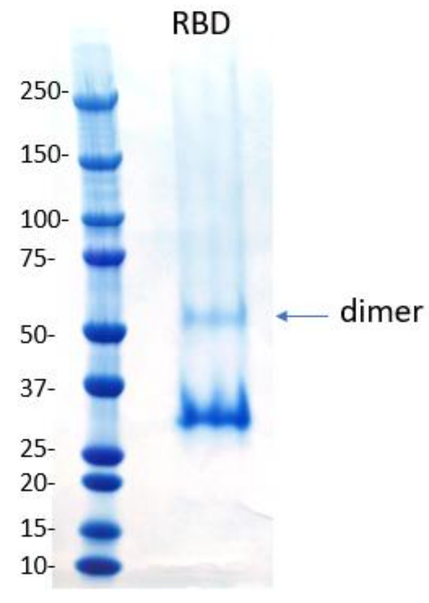

Figure 4. SDS-PAGE gel stained with Coomassie blue showing validating size and purity of RBD isolated from transfected cells.

IV. Results (Continued)

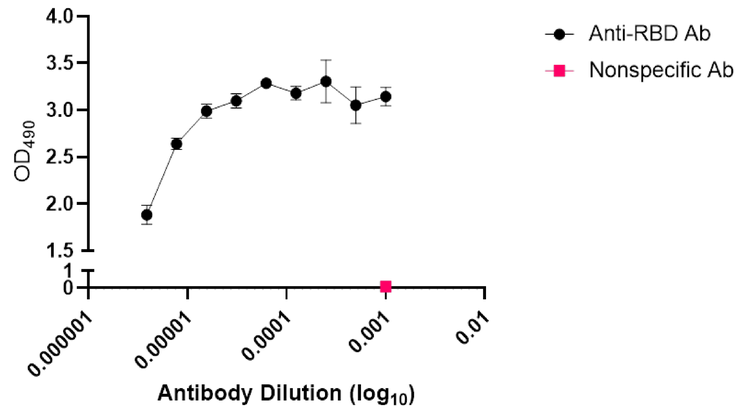

Figure 5. Cloned RBD is detectable in indirect ELISA using anti-human RBD antibody

V. Conclusion

The transfection of HEK293 cells with the RBD encoding expression plasmid resulted in the production of size matched RBD. The purified RBD was used in subsequent ELISAs where serial dilutions of an anti-human RBD antibody demonstrated a dilution-dependent decrease in OD_{490} absorbance suggesting successful recognition and antigenicity of the cloned RBD protein. Nonspecific negative control antibodies when tested at the highest concentration failed to impart any color to corresponding experimental wells highlighting the accurate diagnostic value of this workflow thanks to low non-specific reactivity. Taken together, the implementation of the RBD ELISA presents a specific and accurate assay to test for presence of anti-RBD antibodies in collected volunteer specimen sera.

VI. Future Directions

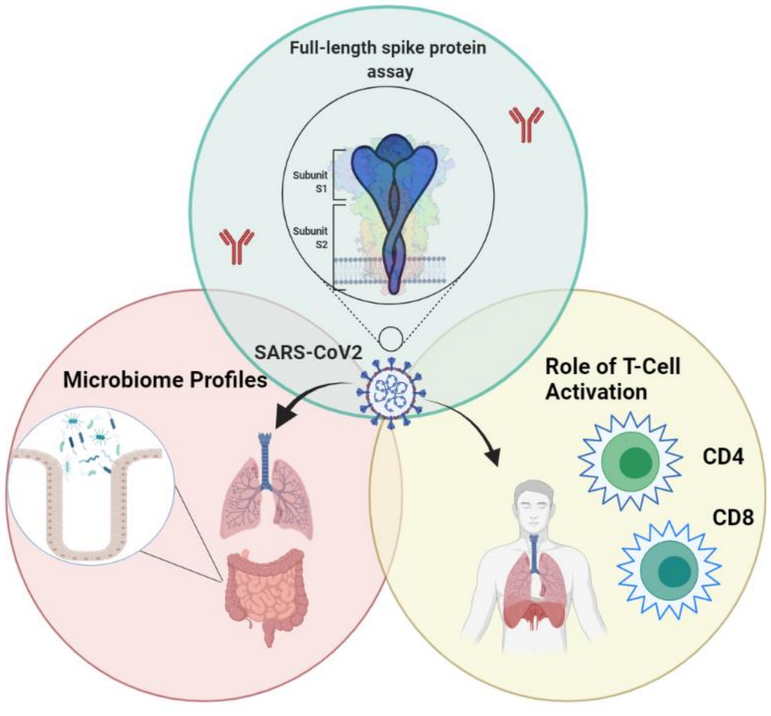

Figure 6. Proposed exploration of novel immune and gut microbial biomarkers associated with seroconversion.

References

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2. Stadlbauer, D., Amanat, F., Chromikova, V., Jiang, K., Strohmeier, S., Arunkumar, G. A., Tan, J., Bhavsar, D., Capuano, C., Kirkpatrick, E., Meade, P., Brito, R. N., Teo, C., McMahon, M., Simon, V., & Krammer, F. (2020). SARS-CoV-2 seroconversion in humans: A detailed protocol for a serological assay, antigen production, and test setup. *Current Protocols in Microbiology*, 57, e100. doi: 10.1002/cpmc.100
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