

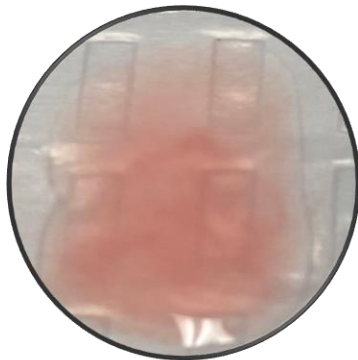
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**Supplemental information**

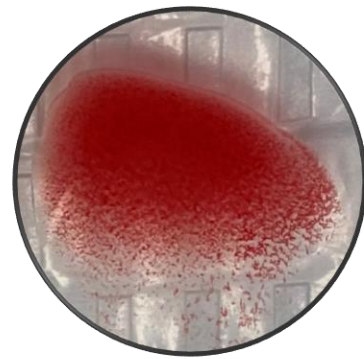
**Rapid and accurate agglutination-based  
testing for SARS-CoV-2 antibodies**

**Sally Esmail, Michael J. Knauer, Husam Abdoh, Courtney Voss, Benjamin Chin-Yee, Peter Stogios, Almagul Seitova, Ashley Hutchinson, Farhad Yusifov, Tatiana Skarina, Elena Evdokimova, Suzanne Ackloo, Lori Lowes, Benjamin D. Hedley, Vipin Bhayana, Ian Chin-Yee, and Shawn S.-C. Li**

**A**

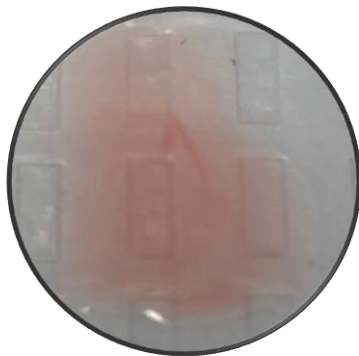


S-RBD-RBC  
SARS-CoV2<sup>-</sup> plasma

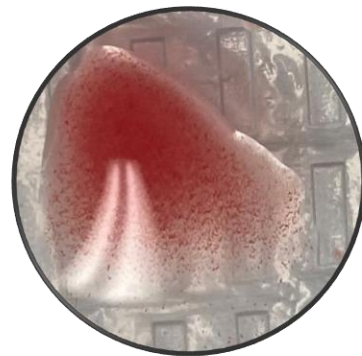


S-RBD-RBC  
SARS-CoV2<sup>+</sup> plasma

**B**



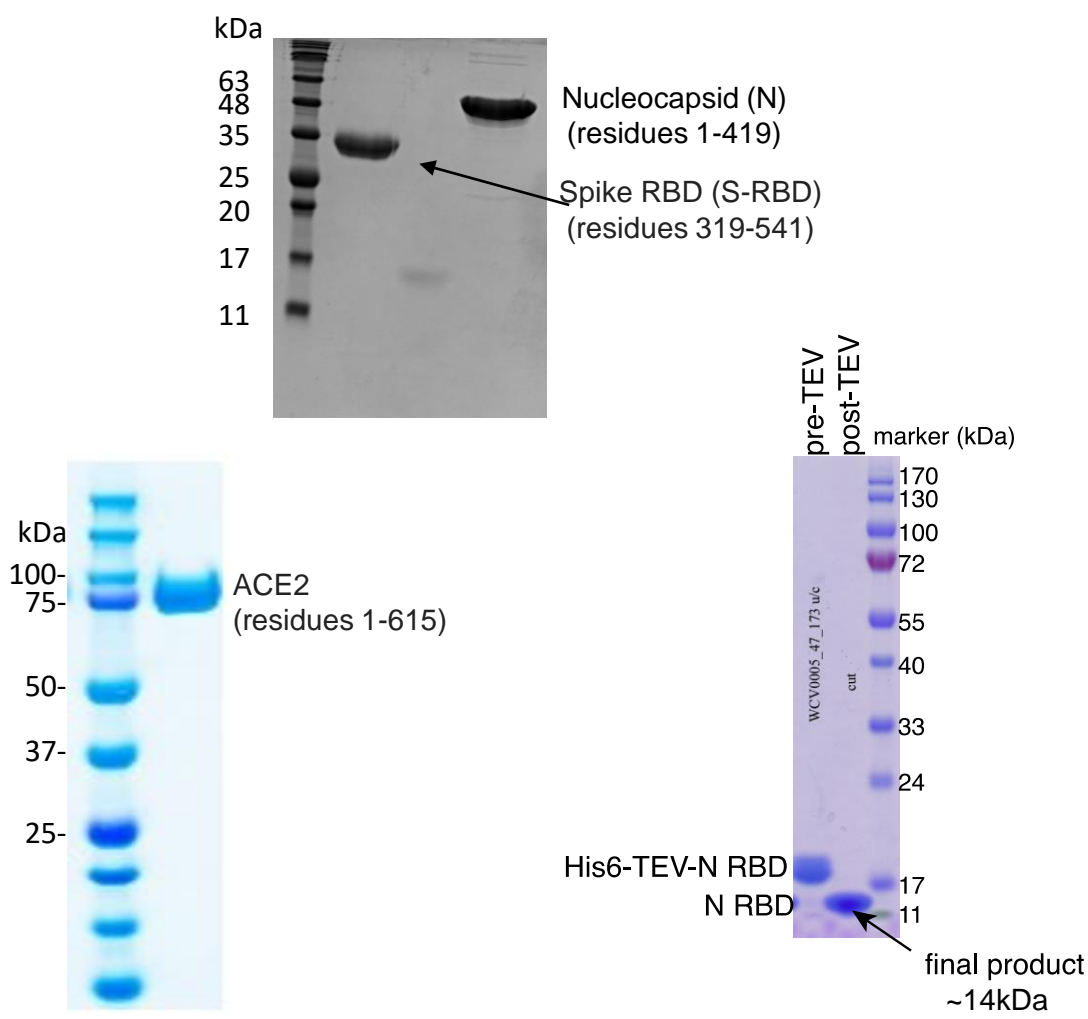
N-RBD-RBC  
SARS-CoV2<sup>-</sup> plasma



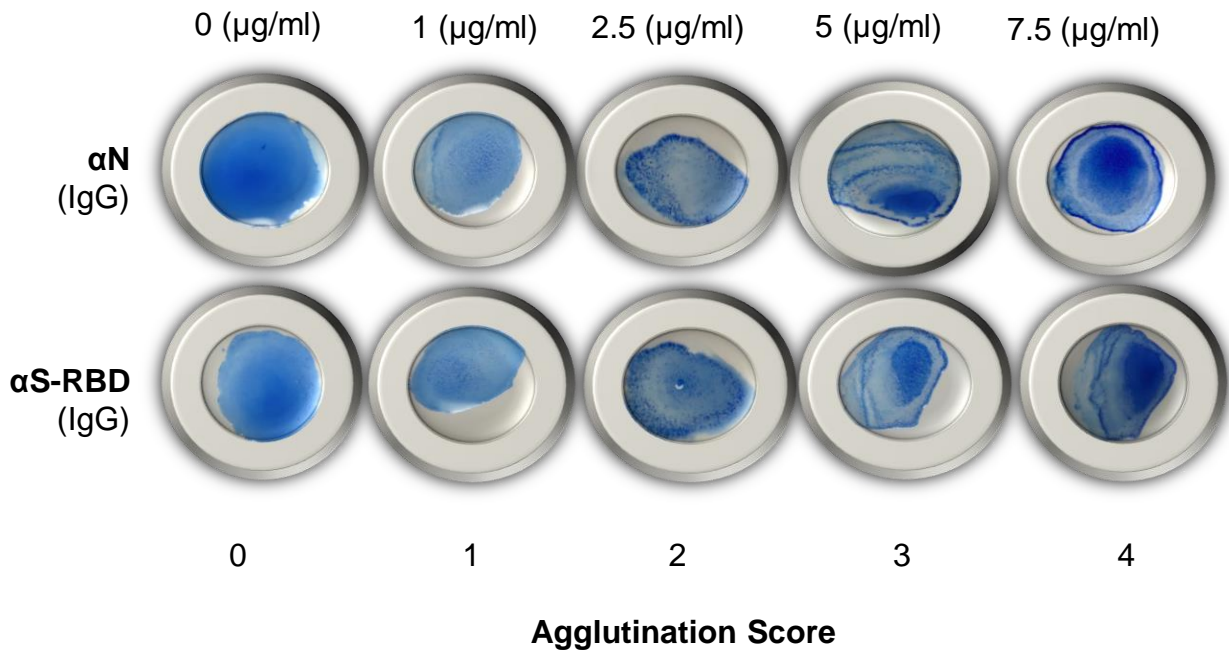
N-RBD-RBC  
SARS-CoV2<sup>+</sup> plasma

**Figure S1. SARS-CoV2 antibody testing based on RBC agglutination, related to Figure 1.**

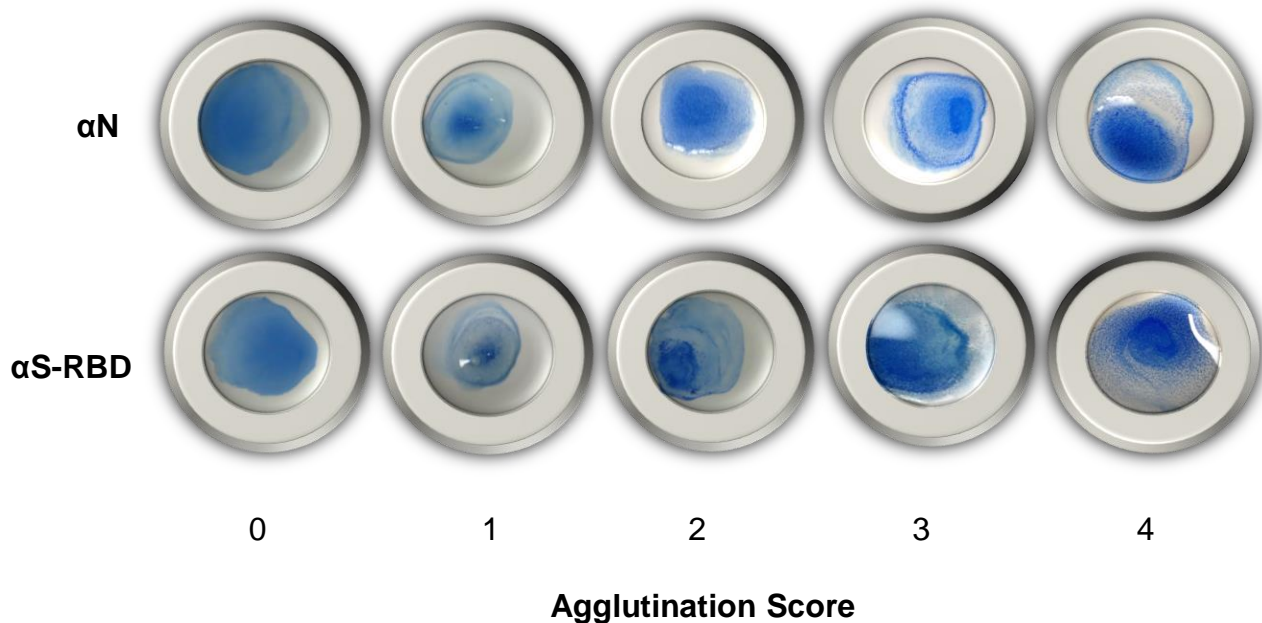
Red blood cells (RBC, group O; R2R2) carrying the D antigen were labelled with anti-D IgG conjugated to recombinant S-RBD or N-RBD through streptavidin-biotin (i.e., IgG-streptavidin conjugated to biotin-RBD). (A) S-RBD labeled RBCs were mixed with either SARS-CoV2<sup>-</sup> (NAAT) or SARS-CoV2<sup>+</sup> plasma (right). (B) N-RBD labeled RBCs were mixed with either SARS-CoV2<sup>-</sup> (left) or SARS-CoV2<sup>+</sup> plasma (right). Images shown were taken after 1 min incubation at room temperature.



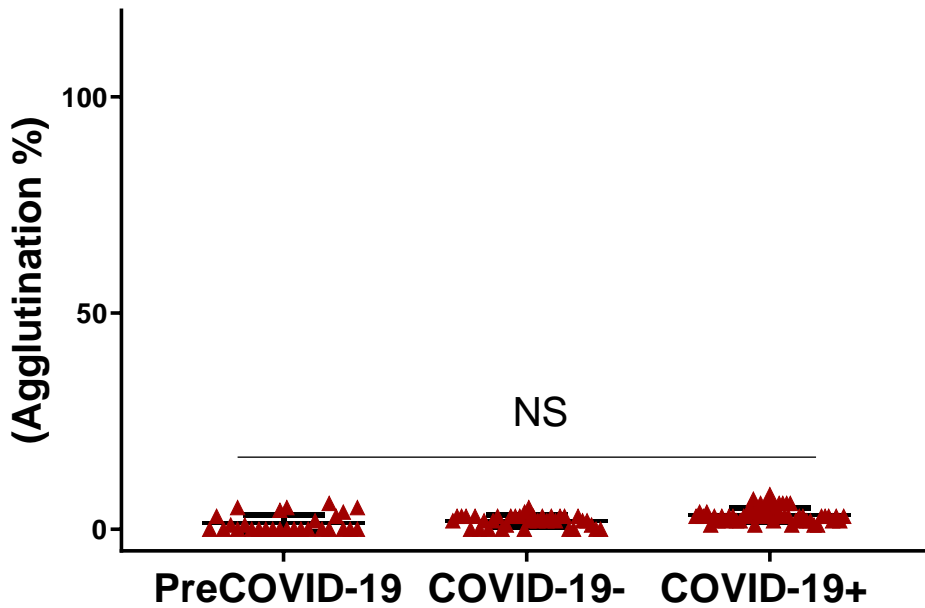
**Figure S2. SDS-PAGE images of recombinant SARS-CoV-2 proteins employed in the current study, related to Figure 1-4.**



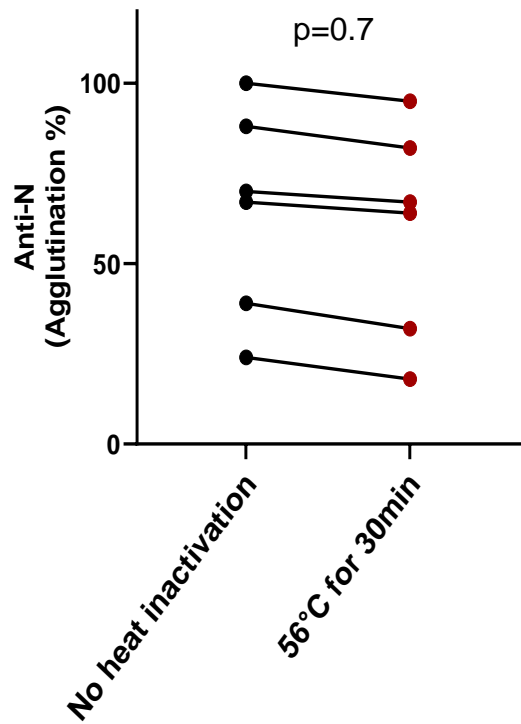
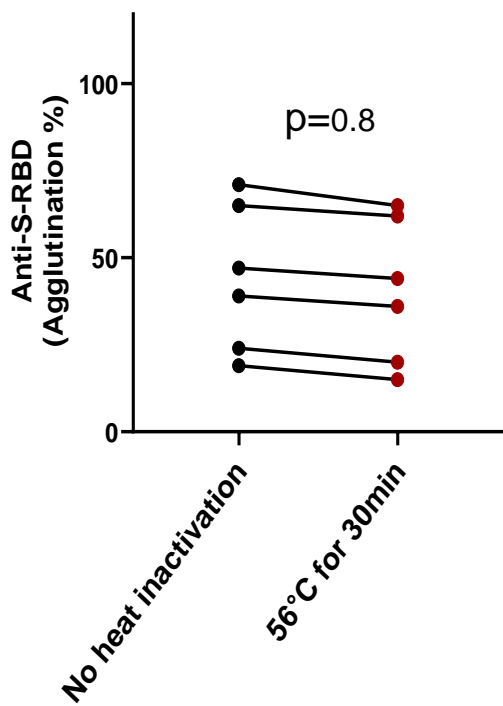
**Figure S3. Agglutination of blue latex particles in response to different concentrations of antibody (IgG) against the nucleocapsid ( $\alpha$ N) or S-RBD ( $\alpha$ S-RBD), related to Figure 3. Anti-S-RBD (monoclonal, NBP2-90980) was obtained from Novus Biologicals; Anti-Nucleocapsid (polyclonal, PA5-81794) was from ThermoFisher Scientific.**



**Figure S4. Representative images of agglutination induced by plasma with different agglutination scores, related to Figure 3.** Shown are five samples with distinct agglutination scores (0-4) in the nucleocapsid ( $\alpha N$ ) (upper row) or the S-RBD antibody ( $\alpha S-RBD$ ) test (lower row). The scores were assigned as 4 = 75-100% agglutination; 3 = 50-75% agglutination; 2 = 25-50% agglutination; 1 = 5-25% agglutination; 0 (or negative)  $\leq 5\%$  agglutination.



**Figure S5. Unconjugated latex beads did not show non-specific antibody induced agglutination, related to Figure 3.** Comparison of agglutination percentage between COVID-19<sup>+</sup> (n=169), COVID-19<sup>-</sup> (n=121) and Pre-COVID-19 (n=100) plasma samples using unconjugated latex particles (Latex particles without viral antigen conjugation). Statistical analyses were performed using One-Way ANOVA (NS, not significant).



**Figure S6. Heat inactivation of plasma did not affect antibody induced agglutination, related to Figures 3 and 4.** Shown are agglutination percentages between samples without or with heat-inactivation in the anti-S-RBD (left) or anti-N (right) agglutination assay.  $p$ -values calculated from paired two tailed t-test (no assumption of equal variance,  $n=6$ ).