Supplementary materials for

High-throughput quantitation of SARS-CoV-2 antibodies in a single-dilution homogeneous assay

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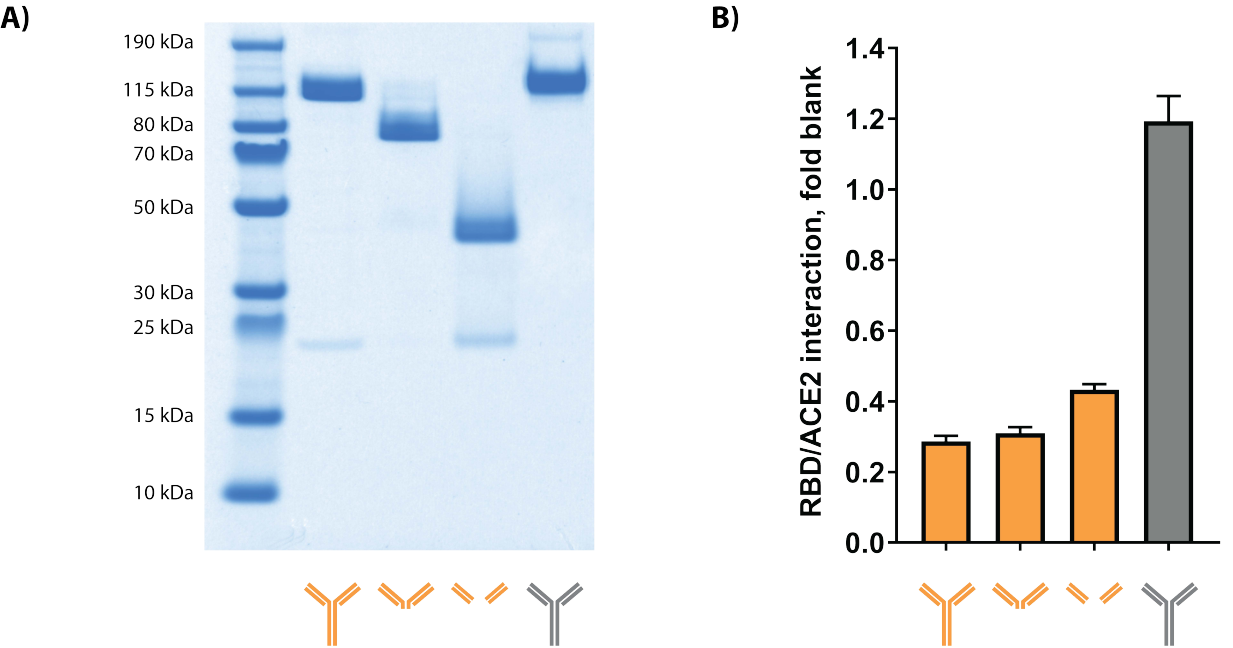
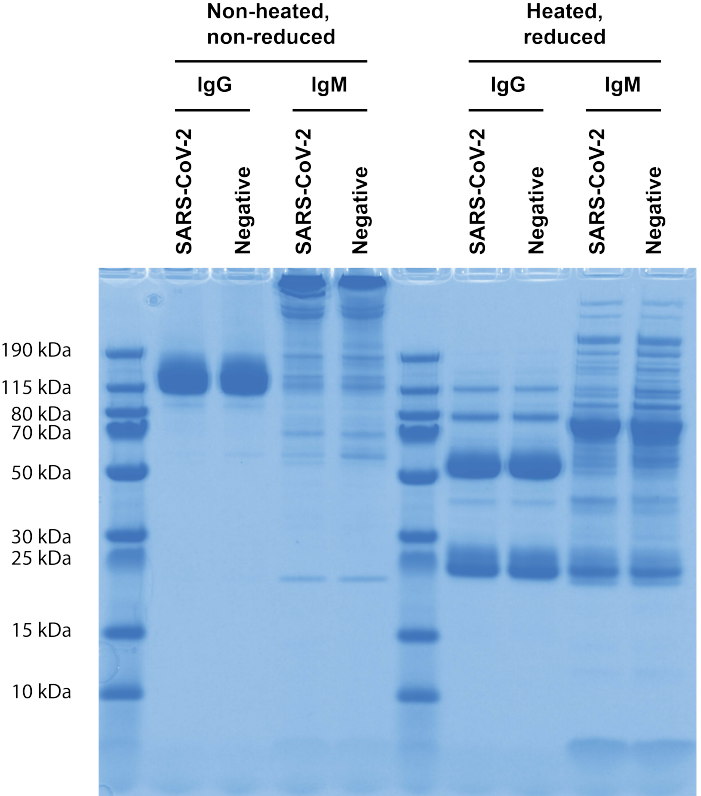
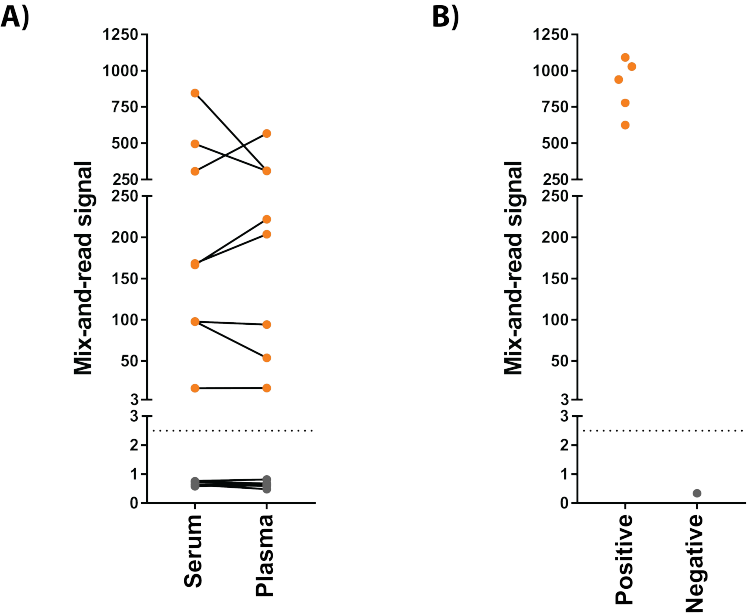


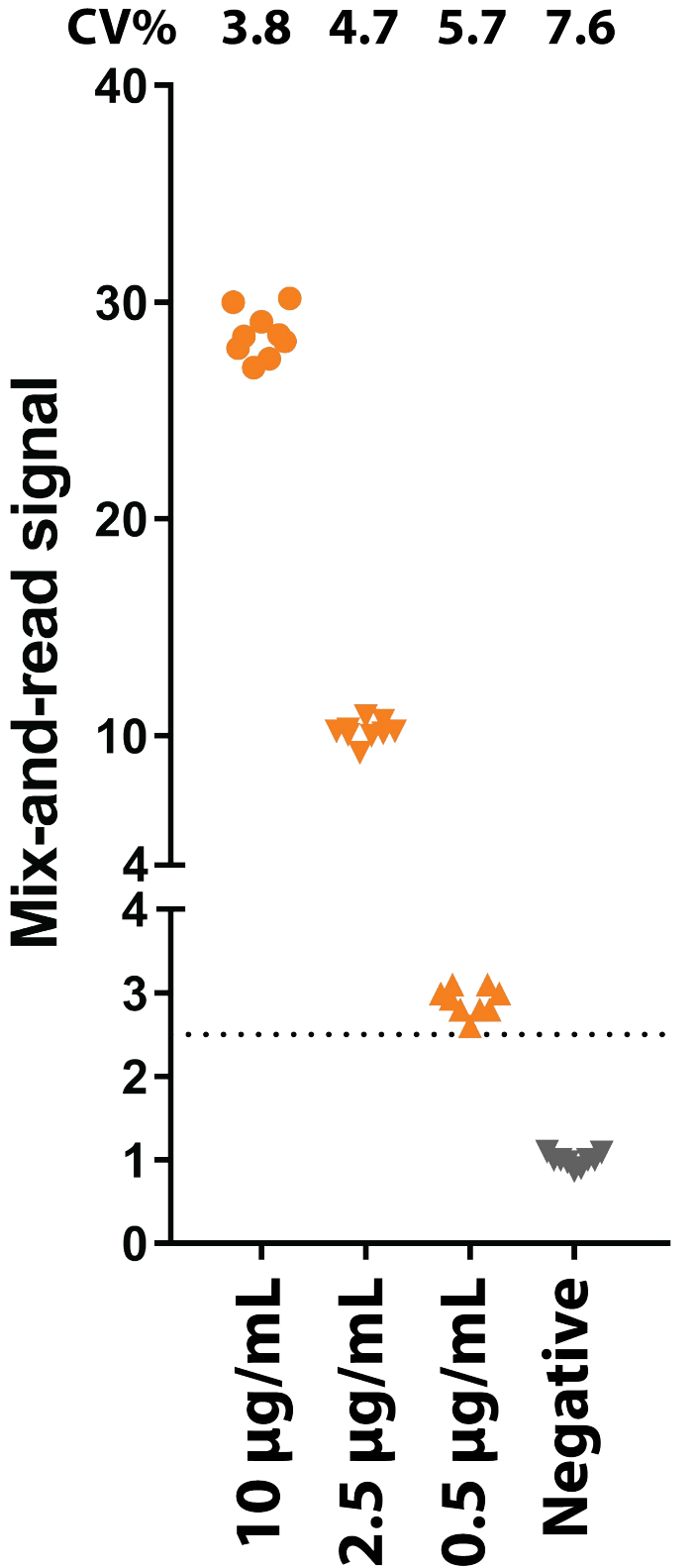
Supplementary Fig. 1: mAbs, F(ab')2 and Fab fragments analyzed by SDS-PAGE; RBD/ACE2 inhibition assay.
A) SARS-CoV-2 RBD-specific mAb 3A2 (orange) was analyzed with non-reducing, non-heated SDS-PAGE, both intact and after cleavege to F(ab')2 and Fab fragments as described in Materials and Methods. A non-targeting isotype control mAb (gray) was analyzed in parallel.
B) The ability of 3A2 mAb, 3A2 F(ab')2, 3A2 Fab, and isotype control mAb to inhibit the interaction between SARS-CoV-2 RBD and its receptor ACE2 was quantified using the assay described in Materials and Methods.



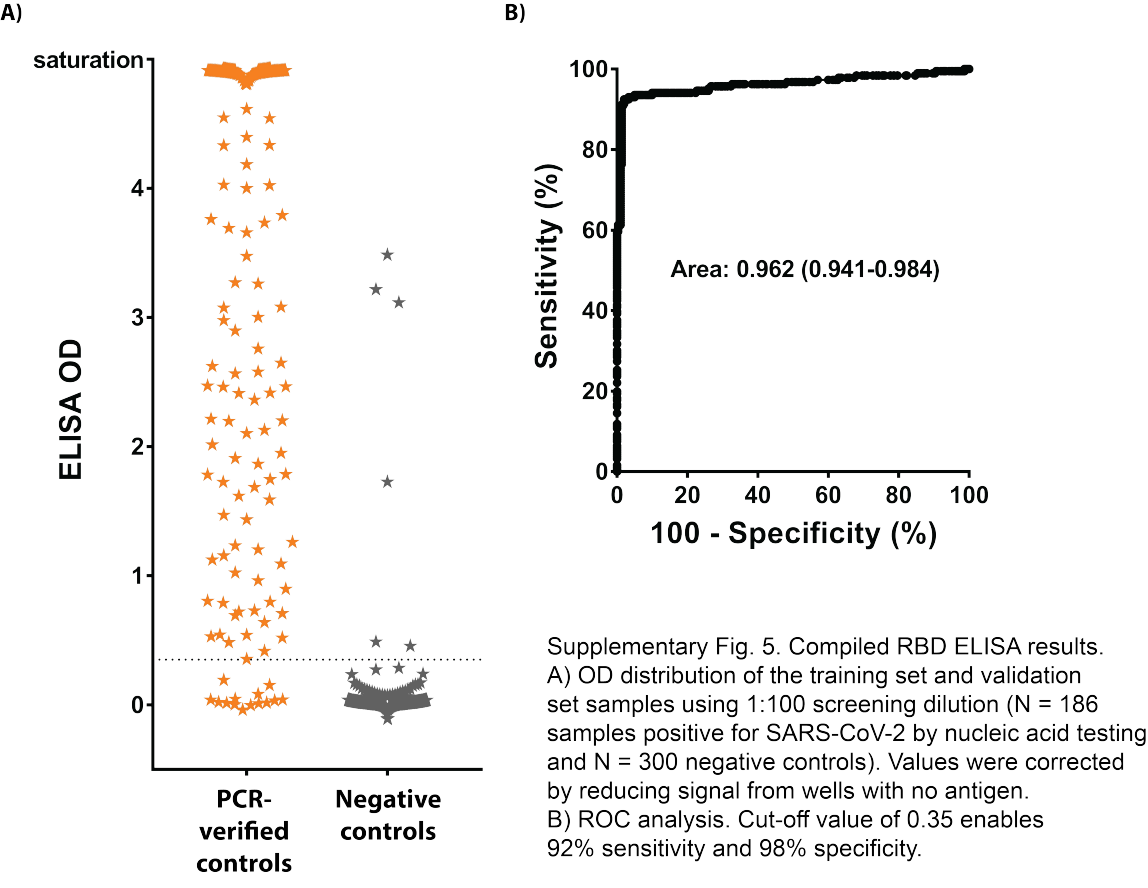
Supplementary Fig. 2: IgG and IgM antibodies purified from pooled sera of convalescent Covid-19 patients or from a control serum pool. The purification products (10 µg/lane) were separated by SDS-PAGE on a 4–12% Bis-Tris gel with MES running buffer and stained with InstantBlue Coomassie stain.



Supplementary Fig. 3: Comparing serum and plasma as sample matrices; analyzing mouse serum.
A) Paired human serum and EDTA plasma samples from 8 SARS-CoV-2-positive and 8 negative individuals were obtained from commercial sources and tested in parallel by the mix-and-read assay. Orange: samples from individuals with history of PCR-confirmed SARS-CoV-2 infection. Gray: negative control samples.
B) Analyzing mouse serum. Five serum samples from mice that were repeatedly immunized in order to generate antibodies against SARS-CoV-2 spike domains were analyzed as positive controls, and negative control serum was from an untreated mouse.



Supplementary Fig. 4: Run-to-run reproducibility of quantification controls. 3A2 mAb was spiked into normal human serum in 3 different concentrations, or serum was left without mAb for a negative control. Aliquots were stored frozen and run together with samples of interest to monitor day-to-day and run-to-run performance of the assay. Data from 9 independent runs presented.
CV; coefficient of variation.



Supplementary Fig. 5. Compiled RBD ELISA results. A) OD distribution of the training set and validation set samples using 1:100 screening dilution (N = 186 samples positive for SARS-CoV-2 by nucleic acid testing and N = 300 negative controls). Values were corrected by reducing signal from wells with no antigen. B) ROC analysis. Cut-off value of 0.35 enables 92% sensitivity and 98% specificity.