

(B-087) A Novel Ultrasensitive, High-throughput, and Quantitative SARS-CoV-2 Neutralizing Antibody Titer Measurement Assay in Dried Blood Spot Samples

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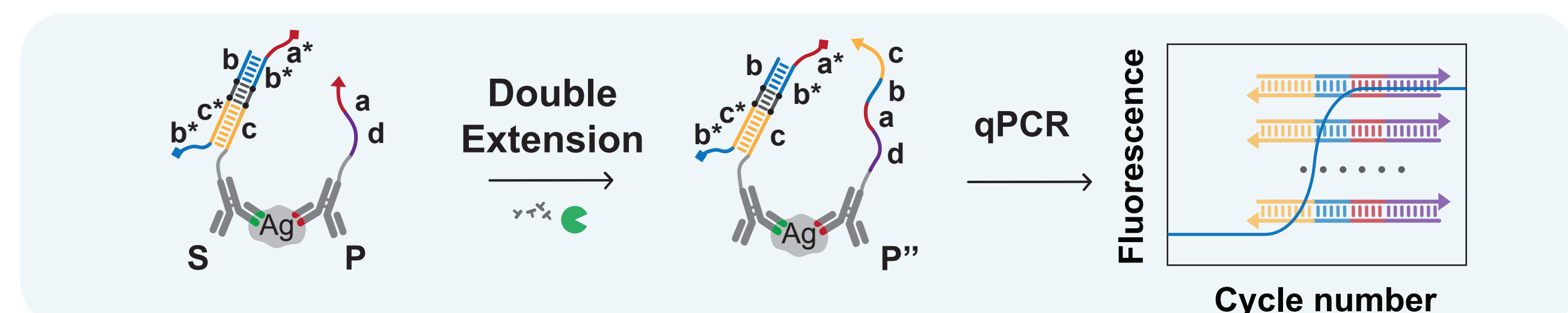
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NEED: a Quantitative Neutralizing Antibody Detection Assay that enables AT HOME SAMPLING & COMMUNITY SURVEILLANCE

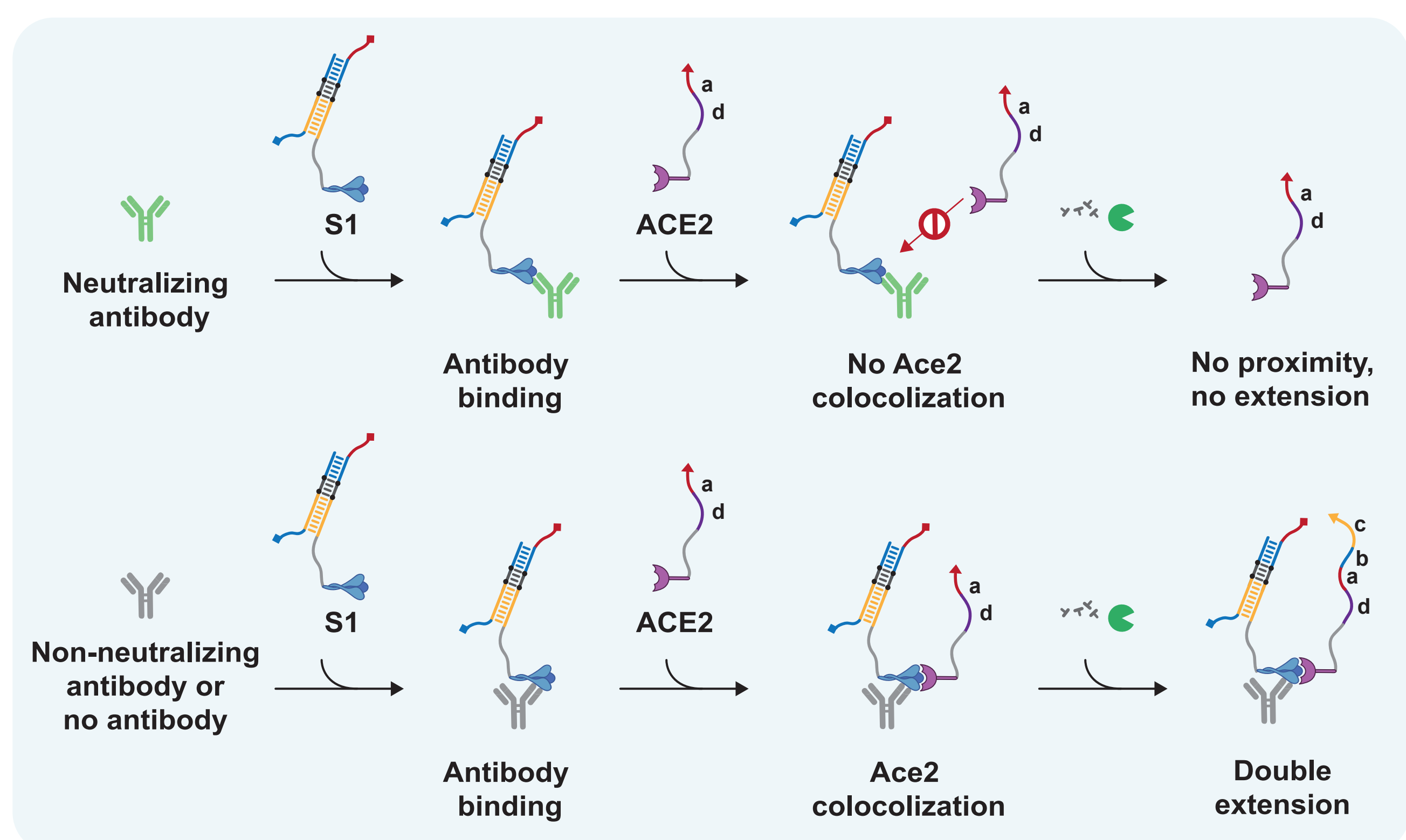
The COVID-19 Pandemic has emphasized the need for sensitive technologies with the capacity to measure the immune status of a population with low cost and high efficiency. In particular, Neutralizing Antibody (NAB) titers have been shown to correlate with protection against viral infection. Traditional neutralizing antibody quantification uses cell-based methods, such as plaque reduction neutralization assay (PRNT) and pseudotyped virus neutralization assay (PsVNA), which are time-consuming, require BSL-2 or above laboratories and show high variation in results. Here, we developed a breakthrough neutralization assay, NAB-Sure™, for ultrasensitive and high-throughput (compatible with 384-well plate and automation) NAb detection and quantification. The NAB-Sure assay can be completed within 6 hours, requires only simple lab equipment, and shows high result precision across different laboratories. More importantly, the ultrasensitivity of NAB-Sure enables NAb quantification in Dried Blood Spot (DBS) samples, which is minimally invasive and makes at home sampling and community surveillance possible.

NAB-Sure™ Technology

NAB-Sure is built on our proprietary SPEAR (Successive Proximity Extension Amplification Reaction) technology. It is an ultrasensitive homogenous immunoassay platform in which the colocalization of two specially designed DNA probes will generate full sequences (i.e., P'') with extremely low background through a 2-step polymerase extension. The generated full sequences then can be quantified using qPCR.



NAB-Sure is a surrogate virus neutralization assay, a synthetic recombinant protein system that utilizes the competition between NABs and human cell receptor proteins (e.g., ACE2) for viral spike proteins (e.g., S1). In the case of Non-neutralizing antibody or no antibody, the interaction between S1 and ACE2 is not inhibited and can produce full sequences. In the case of NABs, the interaction between S1 and ACE2 is blocked by the antibody, and therefore no full sequence is generated.

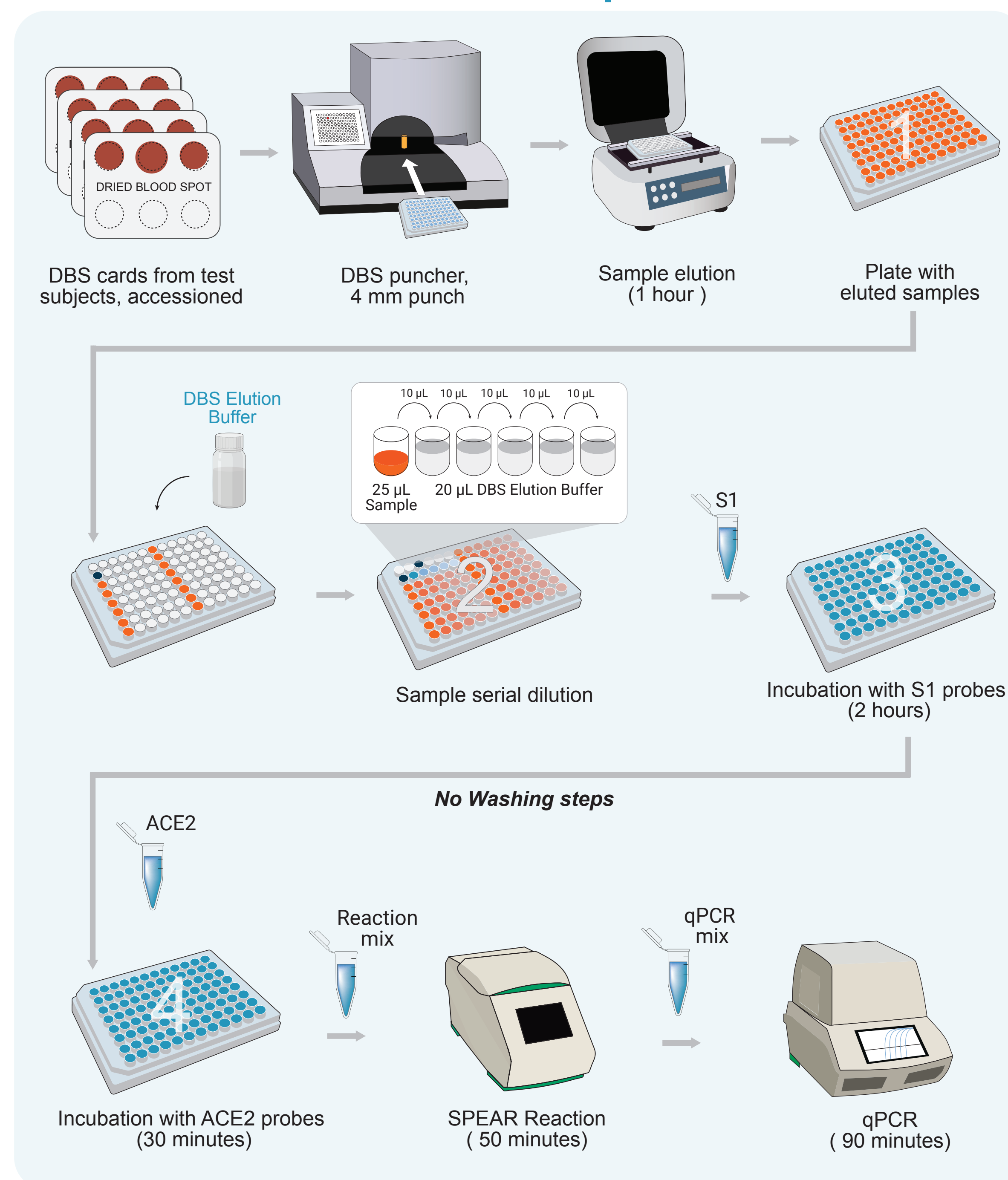


The higher the neutralization capability the sample has, the less copies of full sequences generated. Using a negative sample without antibodies as a reference, the inhibition can be calculated as the percentage of full sequence copy reduction compared to the negative sample reference. For quantitative neutralization antibody titer measurement, the sample is serially diluted and the neutralization titer at 50% inhibition (NT50) is interpolated as the dilution fold at which 50% of inhibition is reached.

Summary

- ★ NAB-Sure™ enables high-sensitivity and high-throughput neutralizing antibody measurement in serum, plasma, and DBS samples with excellent lab-to-lab consistency.
- ★ NAB-Sure™ results correlate well with PRNT results.
- ★ NAB-Sure™ does not require BSL-2 or above laboratories and the total assay time is within 6 hours.
- ★ NAB-Sure™ enables community profiling for neutralizing antibodies with at-home finger-stick DBS sampling.

NAB-Sure Workflow for DBS samples



NAB-Sure Performance and Applications

Detection sensitivity and specificity

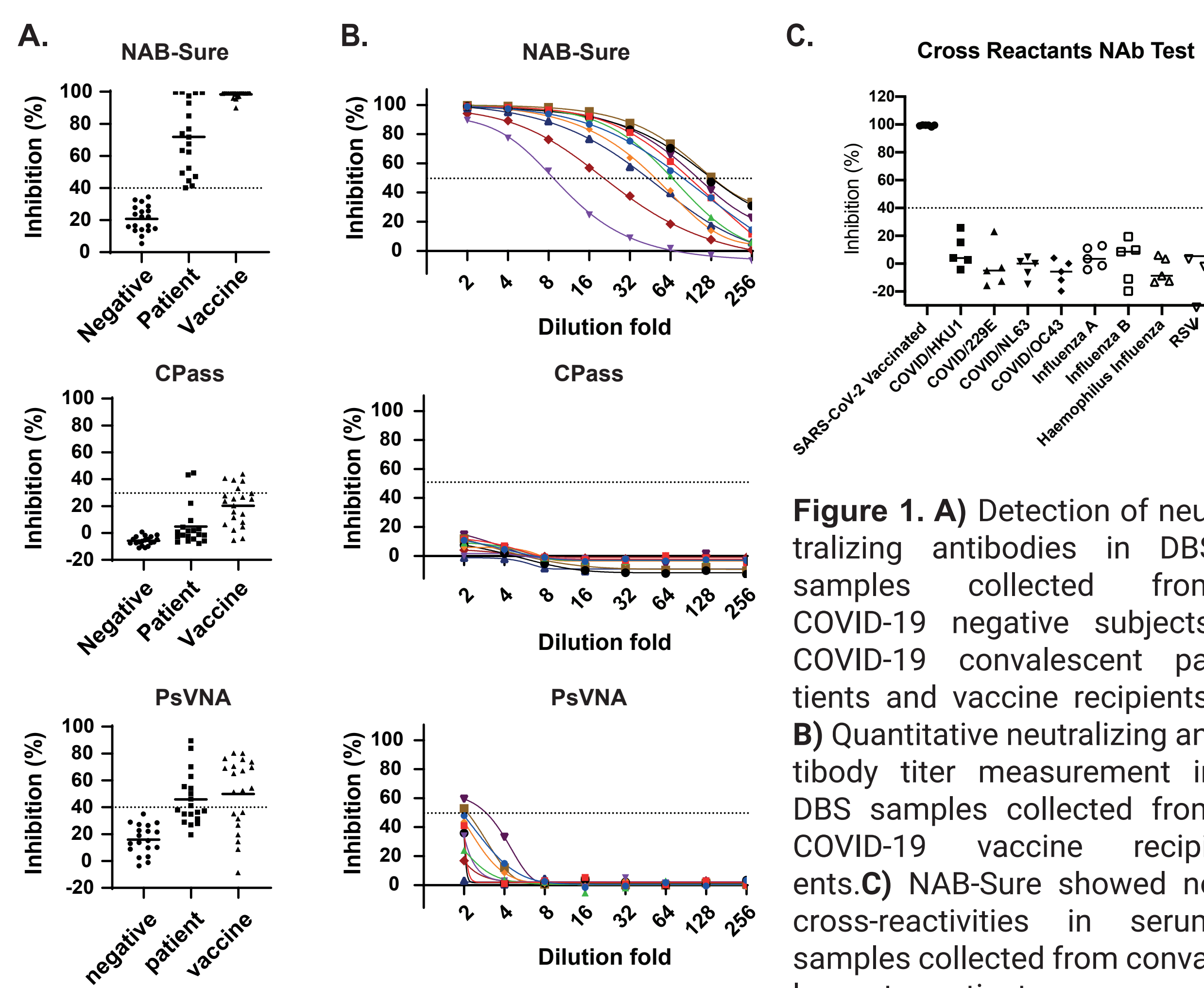


Figure 1. A) Detection of neutralizing antibodies in DBS samples collected from COVID-19 negative subjects, COVID-19 convalescent patients and vaccine recipients. **B)** Quantitative neutralizing antibody titer measurement in DBS samples collected from COVID-19 vaccine recipients. **C)** NAB-Sure showed no cross-reactivities in serum samples collected from convalescent patients recovered from eight other viruses.

Detection accuracy and precision

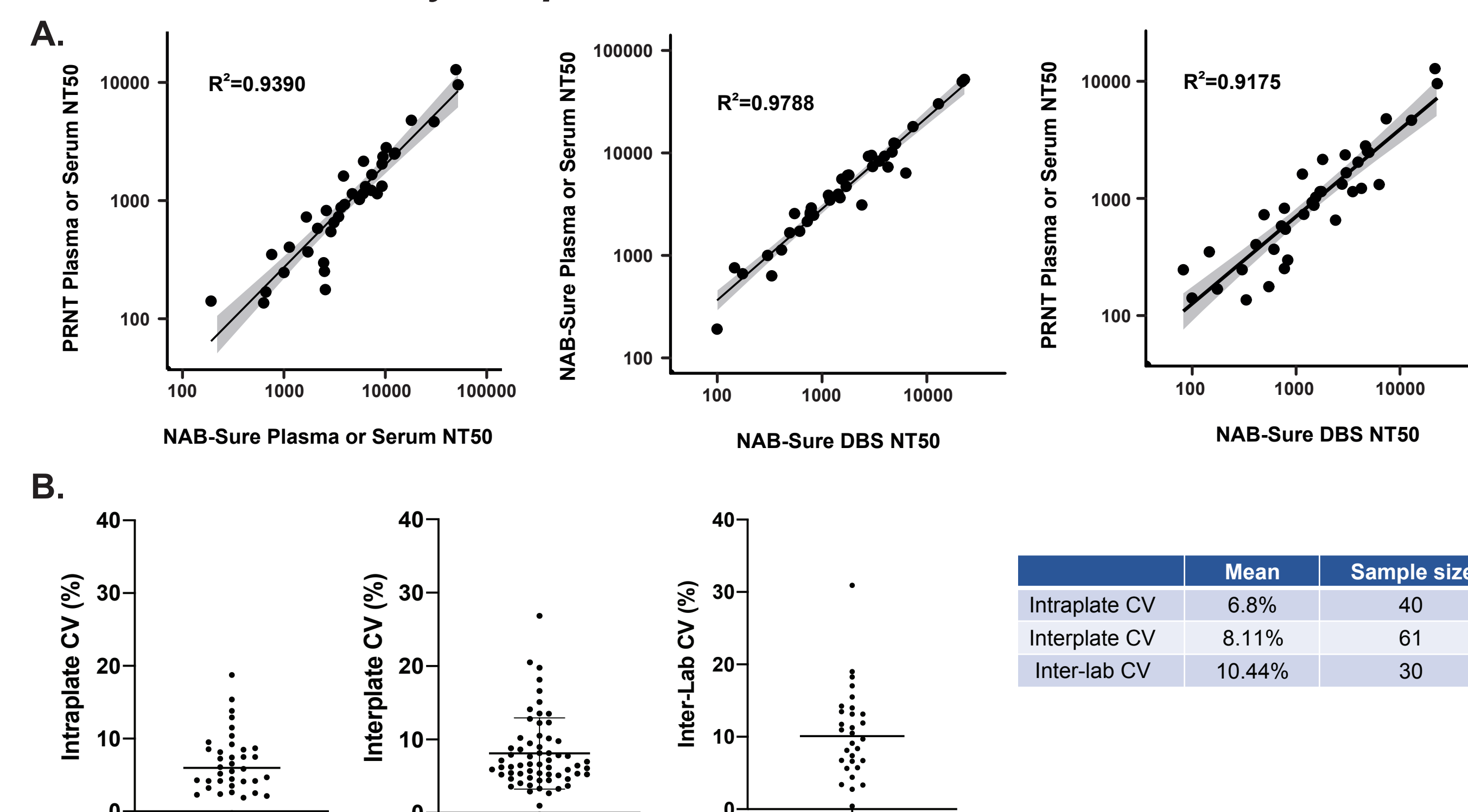


Figure 2. A) NAB-Sure results in DBS samples were highly concordant with PRNT results obtained from serum/plasma samples from the same subjects. **B)** NAB-Sure results showed low variation within run, between runs, and between laboratories (CV: coefficient of variation).

Longitudinal analysis of neutralization against WT and variant viruses

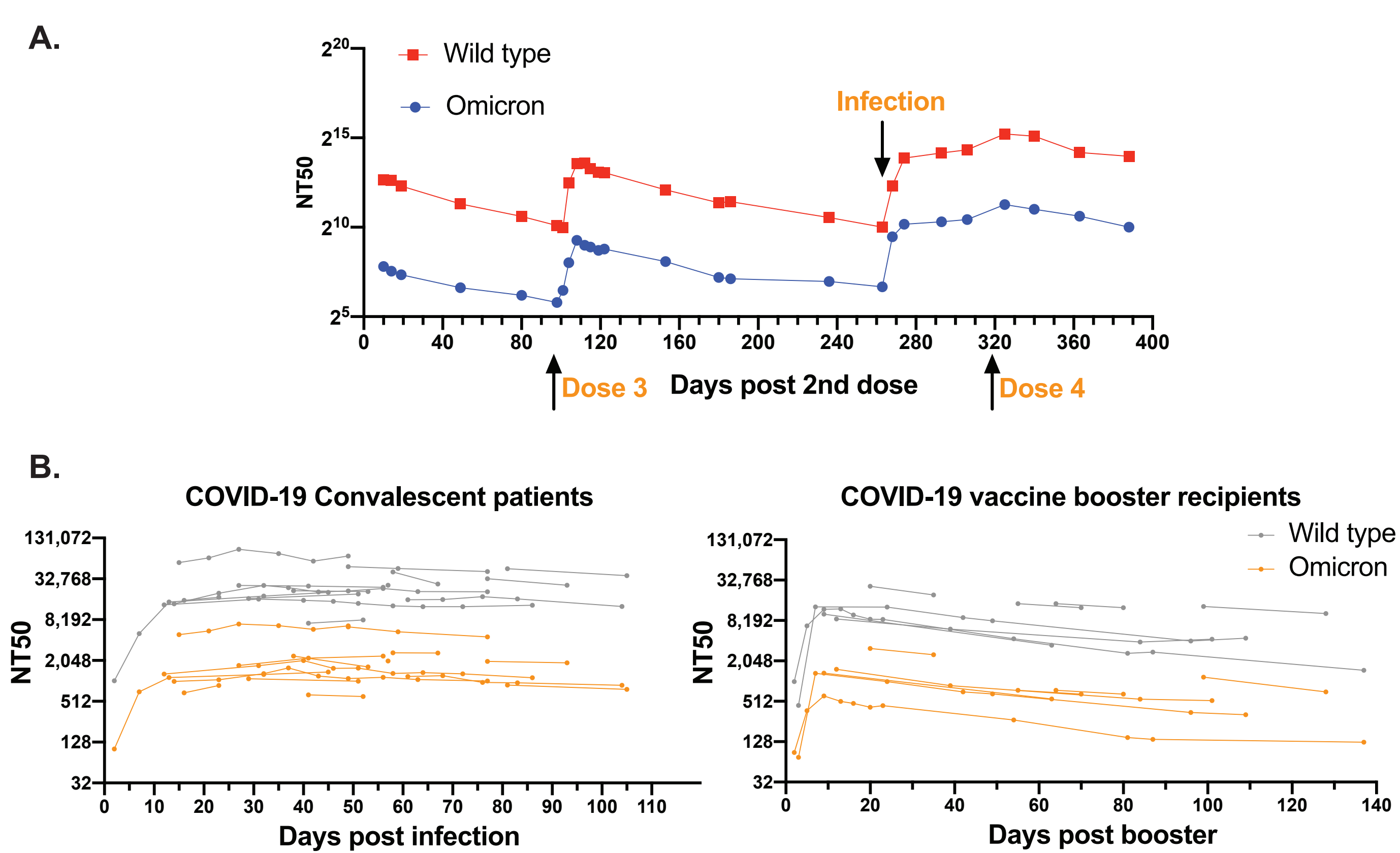


Figure 3. A) Neutralizing antibody titers were measured using NAB-Sure in DBS samples collected from a subject who received vaccine booster doses and got infected. **B)** Comparison of neutralizing antibody durability in COVID-19 convalescent patients (vaccinated) and COVID-19 vaccine recipients (no infection history).

Multiplexed longitudinal analysis of COVID-19 vaccine response

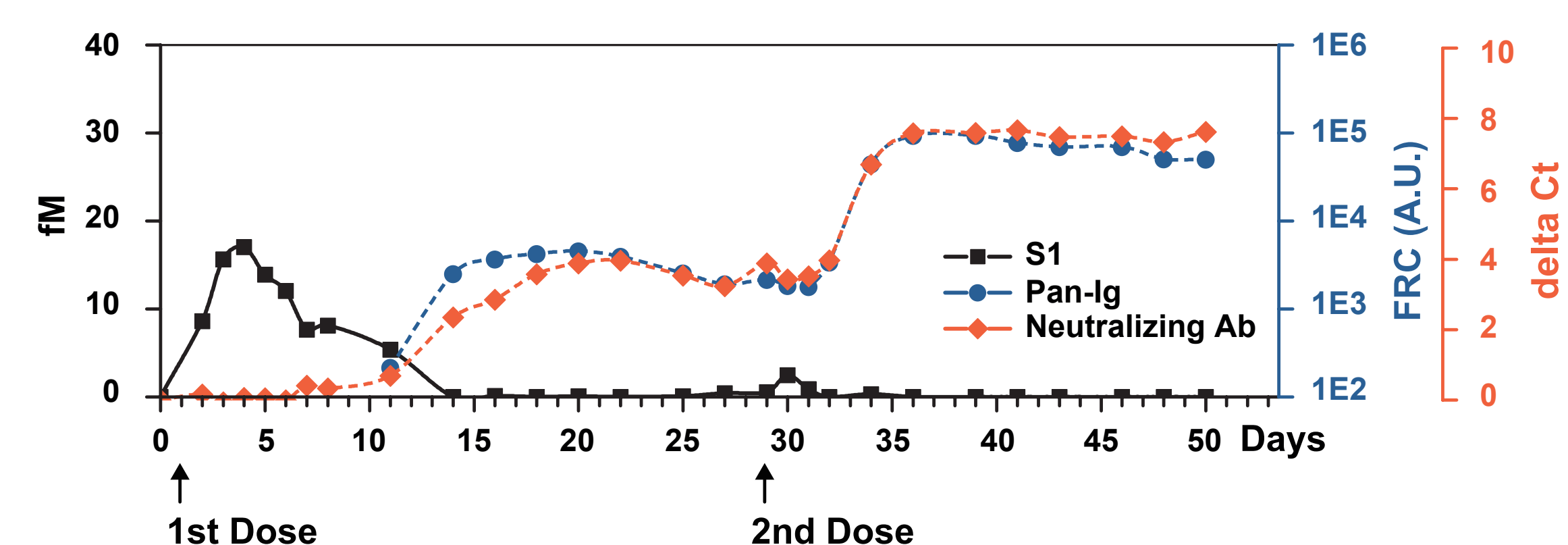


Figure 4. SARS-CoV-2 spike protein, anti-SARS-CoV-2 pan-immunoglobulin and anti-SARS-CoV-2 neutralizing antibodies were detected using SPEAR (S1 & Pan-Ig) and NAB-Sure in DBS samples that were collected after COVID-19 vaccine administration.