Use of AnteoBind technology for evaluating SARS-CoV-2 variants in a Multiplex ACE2-RBD inhibition assay (RBDCoV-ACE2) performed at the NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany.

Background

As vaccination campaigns against SARS-CoV-2 proceeded internationally, interest in both the longevity and quality of immune protection increased, especially as new variants of concern continually emerged with the potential of evading existing immune responses. Neutralising antibodies (Nabs) prevent infection of the cells with pathogens by neutralising them, rendering the pathogen or particle harmless. The longevity of a Nab response against SARS-CoV-2 is thought to have important implications for both immune protection and in directing potential future vaccination strategies (1).

For SARS-CoV-2, Nabs primarily block the interaction of the receptor binding domain (RBD) with the human cell receptor angiotensin converting enzyme 2 (ACE2) – the mechanism by which the virus enters and infects cells. Currently, virus neutralization tests are the gold-standard for detecting Nabs, but these assays require live and infectious virus strains, which can only be handled in a biosafety level 3 laboratory. The study performed at the NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany, investigates the ACE2 binding inhibition of serum from COVID-19 patients using RBDCoV-ACE2 - an in-house developed high throughput bead-based multiplex ACE2 RBD inhibition assay. This serological assay allows the simultaneous analysis of ACE2 binding inhibition to the RBDs of all SARS-CoV-2 variants of concern and interest (VOCs and VOIs) in a single well. The ACE2 binding inhibition is noticeably reduced for ten out of eleven observed variants compared to wild-type(WT), as demonstrated in Figure 1.

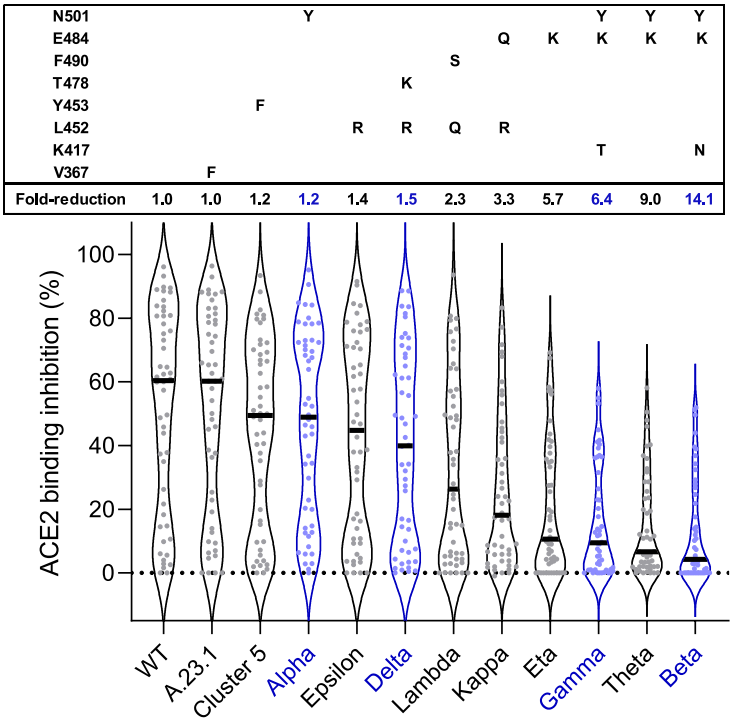

Figure 1: Serum samples from COVID-19 patients (n=50) were analyzed with RBDCoV-ACE2. Fold-reduction of ACE2 binding inhibition in comparison to wild-type corresponds to the ratio between the medians of wild-type and the respective RBD mutant (1).

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How AnteoBind has helped with the Study?

RBDCoV-ACE2 is fast, suitable for high throughput assays and highly reproducible. In instances where the standard covalent conjugation strategies, such as EDC/sNHS chemistry, are susceptible to specific mutations within the RBD region, AnteoTech's Activation Kit for Multiplex Microspheres (A-LMPAKMM-400) containing AnteoBind efficiently binds mutant RBD proteins from 11 different SARS-CoV-2 variants, including the beta strain, as illustrated in Figure 2.

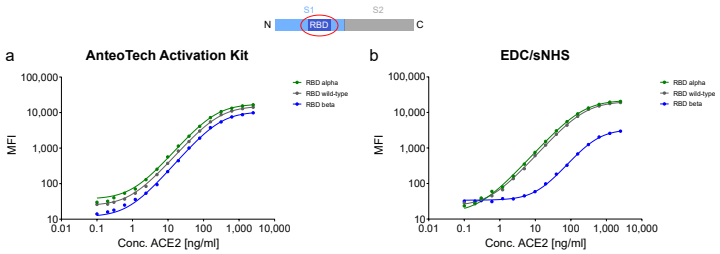


Figure 2: ACE2-RBD binding comparison of beads with different conjugation strategies. RBD proteins from wild-type, alpha and beta variants conjugated to MagPlex beads using AnteoTech's Activation Kit (a) and EDC/sNHS chemistry (b) were incubated with biotinylated ACE2 ranging from 0.1 ng/mL to 2.5 µg/mL.

During the assay, all beads are pooled to produce a bead mix, which is then incubated with patient serum diluted in assay buffer containing biotinylated ACE2. RBD-bound ACE2 is then detected using phycoerythrin-labelled streptavidin and MFI values are normalized. RBDCoV-ACE2 generates highly reproducible data with minimal variation (as determined by low intra and inter assay variation, %CVs, Figure 3).

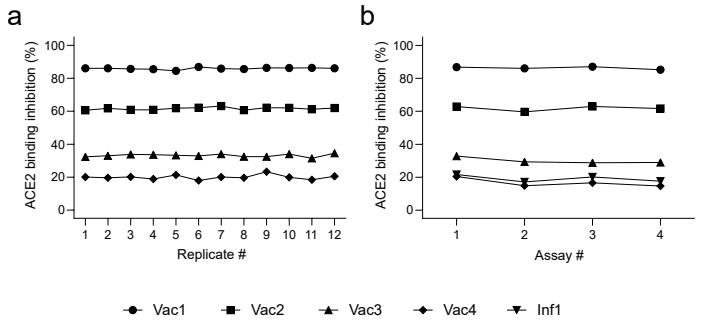


Figure 3: Figure 3: Results of intra assay precision (a) and inter assay precision (b) of RBDCoV-ACE2 analyzing ACE2 binding inhibition to wild-type RBD (in %) of five serum samples (vaccinated (n=4, Vac1-Vac4), infected (n=1, Inf1)). Adapted from (1).

Why use AnteoBind as an alternative technology?

From a clinical perspective, the speed and ease of incorporating new variants into the assay compared to the standard virus neutralization tests make RBDCoV-ACE2 ideal for screening how ACE2 binding inhibition changes for emerging variants. The use of AnteoBind containing Activation kit for Multiplex Microspheres illustrates good batch-to-batch reproducibility for serum ACE2 binding inhibition on the Luminex platform.

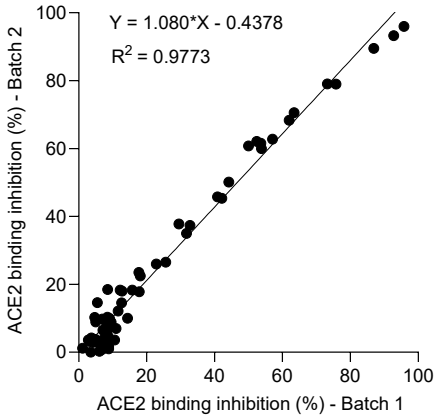
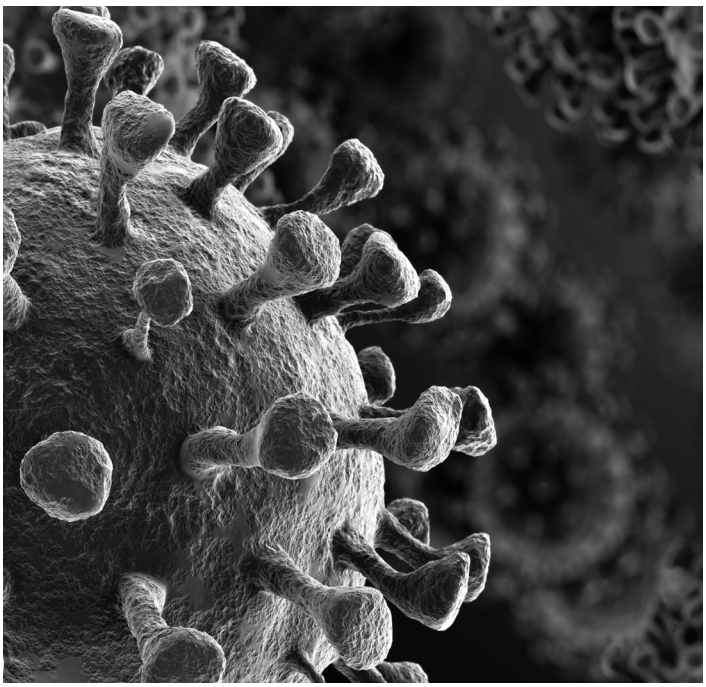


Figure 4: The use of AnteoBind illustrates a high degree of batch-to-batch reproducibility. Two batches (batch 1 and 2) of beads were conjugated with wild-type RBD using AnteoTech's Activation Kit for Multiplex Microspheres. Correlated are the ACE2 binding inhibitions (in %) of serum samples from COVID-19 patients (n=62) with varying disease severity for both bead batches.



AnteoBind Offers Flexible Solutions to Time Consuming Problems

AnteoTech recognises some key protein conjugation roadblocks in the Life Science industry. We believe resources and on-time delivery of products are critical to the success of healthcare products during and beyond the pandemic.

Please contact us at sales@anteotech.com for further information on our AnteoBind products.

For further information on RBDCoV-ACE2, please contact: Alex.Dulovic@nmi.de

References

1. Junker, D., Dulovic, A., Becker, M. et al. COVID-19 patient serum less potently inhibits ACE2-RBD binding for various SARS-CoV-2 RBD mutants. Sci Rep 12, 7168 (2022). https://doi.org/10.1038/s41598-022-10987-2

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