An Alternative Bio-Conjugation Chemistry to Bind Complex Proteins to Luminex® MagPlex® Microspheres

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Introduction

The Activation Kit for Multiplex Microspheres contains the novel AnteoBind™ chemistry designed to bind proteins, e.g. antibodies or antigens to Luminex® MagPlex® or MicroPlex® microspheres. AnteoBind is a patented proprietary metal ion chelation coordination chemistry that interacts with electron donating groups on proteins such as carboxyl or hydroxyl groups. AnteoBind helps achieve a key objective to attach proteins strongly via multiple chelation binding, while maintaining their active tertiary structure.

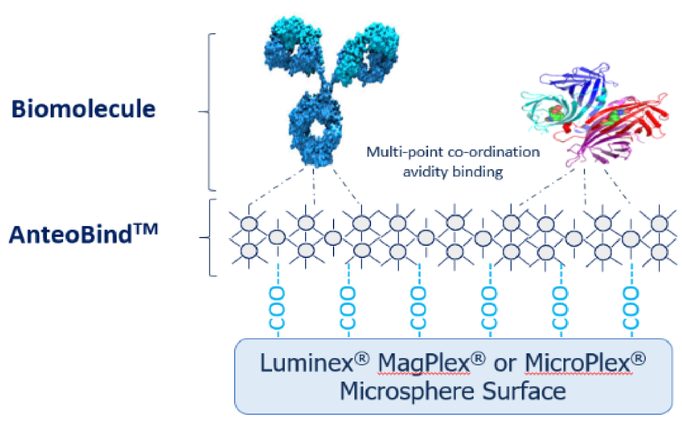

Figure 1 – AnteoBind Functional Mechanism

Using AnteoBind technology for evaluating SARS-CoV-2 variants

Multiplexing platforms by Luminex that use microspheres for simultaneous detection of antibodies against several analytes are effective tools to analyse complex serological responses to pathogens such as SARS-CoV-2. The aim of the study performed at the NMI Natural and Medical Sciences Institute at the University of Tübingen, Germany, was to investigate the ACE2 binding inhibition of serum from COVID-19 convalescent individuals using RBDCoV-ACE2 - a high throughput bead-based multiplex ACE2 (Angiotensin Converting Enzyme 2) RBD (Receptor Binding Domain) inhibition assay.

RBDCoV-ACE2 is a fast, reproducible, high-throughput competitive inhibition assay. In instances where the standard covalent conjugation chemistries such as EDC/NHS are susceptible to specific mutations within the RBD region, AnteoTech's Activation Kit for Multiplex Microspheres (A-LMPAKMM) efficiently conjugates proteins from different SARS-CoV-2 variants of concern in 3 simple steps as described in Figure 2.

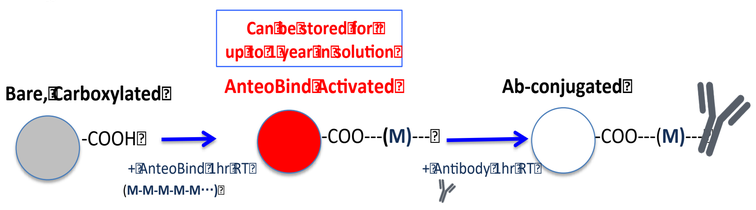

Figure 2 – Activation Kit Simple Workflow

Figure 3 compares the different conjugation chemistries emphasizing the use of AnteoBind technology for proteins of SARS-CoV-2 variants such as RBD beta.

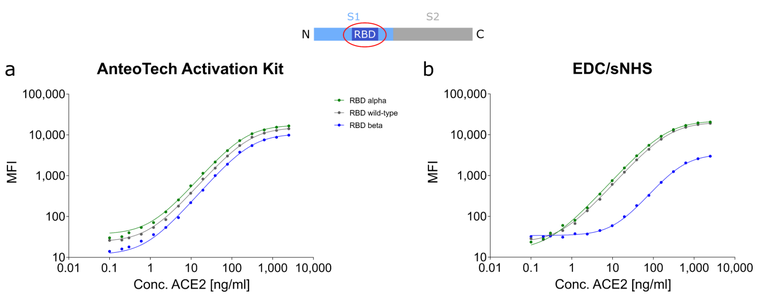

Figure 3 – Comparison of AnteoBind (a) and EDC/NHS (b) Chemistries. ACE2-RBD binding curves are shown using beads conjugated with RBD proteins from SARS-CoV-2 wild-type, alpha and beta.

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 traditional virus neutralisation 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 for the technical development of this assay illustrates good batch-to-batch reproducibility (Figure 4), stability and robustness for serum ACE2 binding inhibition on the Luminex platform.

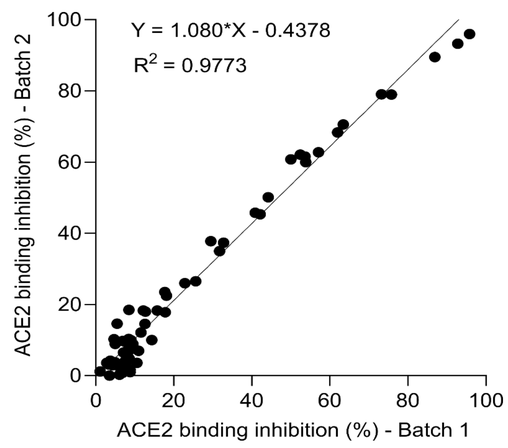

*Figure 4 - The use of AnteoBind illustrates a high degree of **batch-to-batch reproducibility**. Two batches (batch 1 and 2) of beads were conjugated with wild-type RBD using AnteoTech's Activation Kit for Multiplex Microspheres. Correlated are the ACE2 binding inhibitions (in %) of serum samples from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

To demonstrate the long-term stability of AnteoBind, IgG binding against the receptor binding domain (RBD) of the SARS-CoV-2 mu variant was analyzed with 24 serum samples of pre-pandemic, SARS-CoV-2 infected and vaccinated donors at three time points (2, 6 and 12 months after conjugation). As illustrated in Figure 5, MFI signals remained stable over the entire period, with 90 % of the infected/vaccinated samples showing percentage Coefficient of Variation (CVs) less than 10 % (60 % of samples ≤ 5%). This high reproducibility illustrates that AnteoBind conjugated beads can be used in serological assays for at least one year without sacrificing performance.

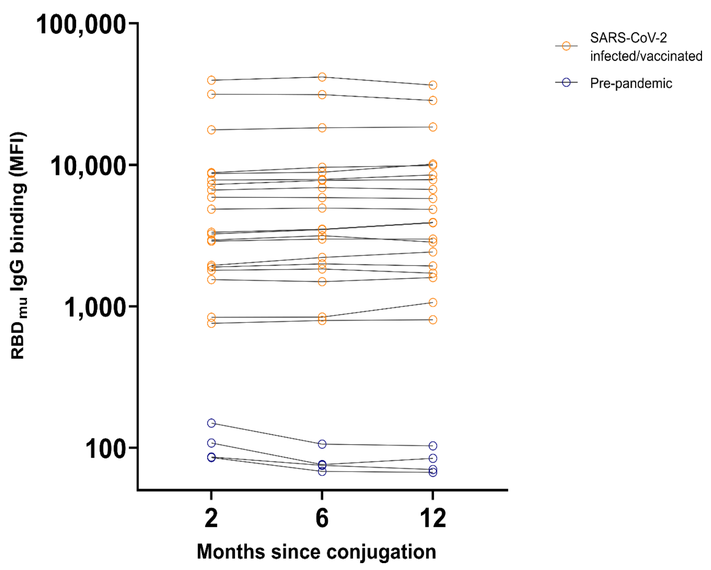

Figure 5 - Long-term stability of MagPlex beads conjugated with AnteoBind. IgG binding of serum samples (n=24) from pre-pandemic (n=4), SARS-CoV-2 infected (n=8) and vaccinated (n=12) individuals against the receptor binding domain (RBD) of the SARS-CoV-2 mu variant using the SARS-CoV-2 antibody assay MULTICOV-AB [1]. Measurements were made 2, 6 and 12 months after the RBD was conjugated to the beads using AnteoBind.

The stability and robustness of AnteoBind conjugated beads can be further demonstrated by comparing multiplex and singleplex measurements of the same samples. In Figure 6, MagPlex Beads conjugated with RBDs from SARS-CoV-2 wild-type (WT) and delta were used in singleplex and multiplex (12 analytes) measurements to determine ACE2 binding inhibitions of sera from COVID-19 patients as well as healthy donors (pre-pandemic).

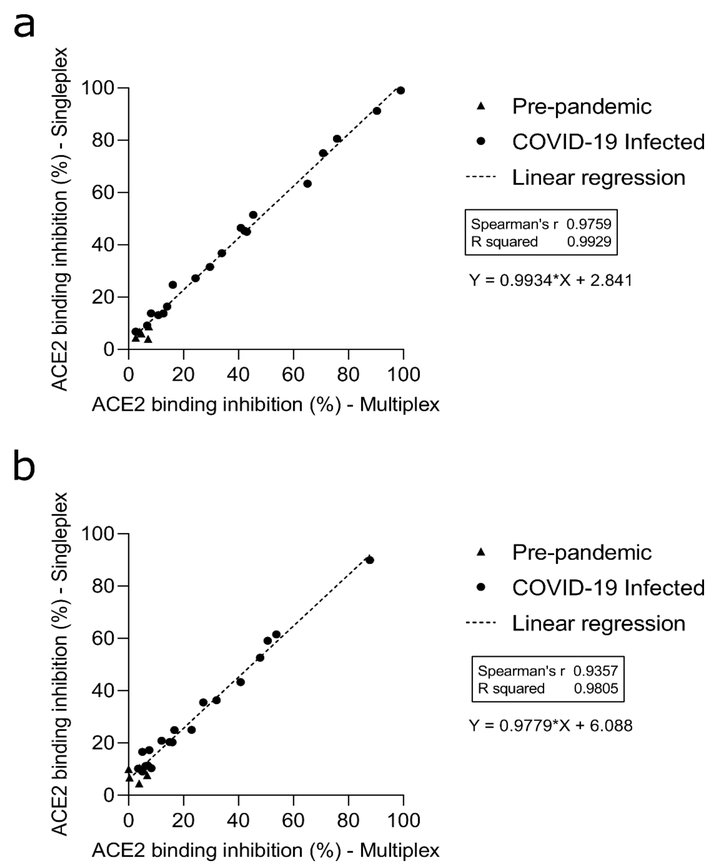

*Figure 6 - Beads conjugated with AnteoBind are comparable in multiplex and singleplex measurements demonstrating **Robustness**. Figure 6 - Beads conjugated with AnteoBind are comparable in multiplex and singleplex measurements. Linear regression analysis between ACE2 binding inhibition (%) values of samples from pre-pandemic (n=5) and COVID-19 infected (n=19) individuals analyzed in both multiplex and singleplex for RBD WT (a) and RBD delta (b). ACE2 binding inhibition (%) was determined with RBDCoV-ACE2 [2]. Correlation analysis was performed after Spearman and the correlation coefficient r is shown. Adapted from [2].*

Summary and Conclusion

AnteoBind technology has been applied by NMI to evaluate SARS-CoV-2 variants using Luminex multiplex immunoassay system show consistent batch-to-batch reproducibility and storage stability.

The results also demonstrate AnteoBind's technical advantage as an alternative bio-conjugation tool when compared to the conventional EDC/NHS method when dealing with difficult to conjugate proteins.

Reference

[1] Becker, M., Strengert, M., Junker, D. et al. Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity. Nature communications. **12**, 1152 (2021). https://doi.org/10.1038/s41467-021-20973-3

[2] 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. Scientific Reports. **12**, 7168 (2022). https://doi.org/10.1038/s41598-022-10987-2, licensed under http://creativecommons.org/licenses/by/4.0/.

Contact

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

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