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SUPERIOR SARS-COV-2 RBD ANTIGEN DESIGNS FOR HIGHLY SPECIFIC, QUANTITATIVE SEROTESTS

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Quantitative high-quality SARS-CoV-2 serotests that are easy-to-implement have been gaining great importance as means to characterize and monitor the magnitude of infection- or vaccine-induced immunity over time and are of particular interest for academic laboratories doing COVID-19 research or small diagnostic laboratories with basic equipment.

In the present work, we developed and extensively validated two (now commercially available) quantitative, enzyme-linked immunosorbent assay (ELISA)-based serotests relying on the SARS-CoV-2 receptor-binding domain (RBD) and nucleocapsid protein (NP) of superior design and quality. For the production of glycosylated RBD, we screened five eukaryotic expression systems and compared them for their capacity to support transient high-quantity and high-quality expression of the protein. Our quality attributes covered activity in a functional binding assay, protein integrity and glycosylation as well as manufacturability. Our results indicate that serotest performance is already influenced as early as by the choice of the antigen production system and stresses out the importance of protein purity on specific antibody detection.

Already at an early stage of its structural and functional characterization we observed that RBD (aa 319-541) tends to form dimers and we demonstrated that even small amounts of residual dimeric RBD can strongly affect kinetic studies of its interactions with ACE2 or other ligands. Furthermore, naturally formed dimeric RBD appears structurally impaired and its removal for the generation of homogeneous monomeric RBD preparations not only necessitates more extensive downstream procedures, but it also results in a high percentage of the recombinant protein yield to be discarded. We therefore evaluated novel RBD designs and could show that deletion or substitution of the unpaired cysteine residue C538 reduces the intrinsic propensity of RBD to form oligomeric aggregates, thereby increasing the yield of the monomeric form of the protein. These optimized RBD variants displayed excellent performance for the specific detection of even low levels of SARS-CoV-2 antibodies in convalescent sera. Validation studies with unprecedented large and heterogeneous multi-centric specificity and sensitivity cohorts revealed that the simple ELISA-based antibody tests relying on optimized antigen designs performed equally well or even better than fully automated CE-marked test platforms and results correlate well with that from neutralization assays using authentic SARS-CoV-2 virus. While our data originally stem from our effort in producing highly qualitative antigens for diagnostic purpose early in the pandemic, they may not only be instrumental for the further development of SARS-CoV-2 diagnostics, they also inform the design of RBD-based protein vaccine candidates.