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THE EFFECT OF THE PRODUCTION METHOD ON THE QUALITY OF CORONAVIRUS SPIKE PROTEIN RBD VARIANTS OF CONCERN: IMPLICATIONS ON THE SPECIFICITY AND SENSITIVITY OF SEROLOGICAL ASSAYS

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The high circulation and replication of SARS-CoV-2 in the human population has generated variants of concern. To determine the magnitude of the immune response generated against the different coronavirus variants, the development of specific and sensitive serological assays is needed. In this study, we determined if the specificity and sensitivity of these assays are affected by several factors, such as the antigen's glycosylation profile, purity, and structure. The Receptor Binding Domain (RBD) of the spike protein of the parental SARS-CoV-2 and the variants Alpha and Delta were produced in two mammalian cell systems. HEK-293T cells were cultured in T-flasks and CHO cells were cultured at different scales: 0.2 L (shake flasks), 5 L and 50 L (bioreactors). RBDs with a purity of at least 90 % were obtained after three purification steps. As the culture scale in CHO cells increased, the glycosylation profile of the parental variant changed. Differences in high-mannose and sialic acid content were observed between the RBD variants. RBDs were lyophilized or stored in liquid form. An ELISA assay was developed and validated, with a sensibility and specificity above 90%. The effect of the characteristics of each RBD on the determination of IgG and IgM in serum samples of people vaccinated or previously infected with SARS-CoV-2 were determined. The results that will be presented are crucial for accurately determining the prevalence of SARS-CoV-2.