Production of high-quality SARS-CoV-2 antigens: impact of bioprocess and storage on glycosylation, biophysical attributes, and ELISA serologic tests performance

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Abstract

SARS-CoV-2 is an RNA coronavirus that causes severe acute pneumonia, also known as COVID 19 disease. The World Health Organization declared the COVID-19 outbreak in January 2020 and a pandemic 2 months later. Serological assays are valuable tools to study virus spread among the population and, importantly, to identify individuals that were already infected and would be potentially immune to a virus re-infection. SARS-CoV-2 Spike protein and its Receptor Binding Domain (RBD) are the antigens with higher potential to develop SARS-CoV-2 serological assays. Moreover, structural studies of these antigens are key to understand the molecular basis for Spike interaction with angiotensin converting enzyme 2 receptor, hopefully enabling the discovery and development of COVID-19 therapeutics. Thus, it is urgent that significant amounts of this protein became available at the highest quality. In this work we evaluated the impact of different and scalable bioprocessing approaches on Spike and RBD production yields and, more importantly, in these antigens' quality attributes. Using negative and positive sera collected from human donors, we show an excellent performance of the produced antigens, assessed in serologic ELISA tests, as denoted by the high specificity and sensitivity of the test. We have shown that, despite of the human cell host and the cell culture strategy used, for production scales ranging from 1 L to up to 30 L, final yields of approx. 2 mg and 90 mg per liter of purified bulk for Spike and RBD, respectively, could be obtained. To the best of our knowledge these are the highest yields for RBD production reported to date. An in-depth characterization of SARS CoV-2 Spike and RBD proteins was also performed, namely the antigens oligomeric state, glycosylation profiles and thermal stability during storage. The correlation of these quality attributes with ELISA performance show equivalent reactivity to SARS CoV 2 positive serum, for all Spike and RBD produced, and for all the storage conditions tested. Overall, we provide herein straightforward protocols to produce high-quality SARS CoV-2 Spike and RBD antigens, that can be easily adapted to both academic and industrial settings; and integrate, for the first time, studies on the impact of bioprocess with an in-deep characterization of these proteins, correlating antigens glycosylation and biophysical attributes to performance of COVID-19 serologic tests. We strongly believe that our work will contribute to advance the current and recent knowledge on SARS-CoV-2 proteins and support the scientific society that is persistently searching for solutions for COVID-19 pandemics.

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