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PRODUCTION OF HIGH-QUALITY SARS-COV-2 ANTIGENS FOR VACCINE DEVELOPMENT AND SEROLOGICAL ASSAYS IMPLEMENTATION

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The development of novel and/or improved vaccines as well as the establishment of tools to monitor vaccine responses are two key factors to control COVID-19 pandemic, often requiring the manufacturing of significant amounts of high-quality SARS-CoV-2 antigens.

In this work, we produced SARS-CoV-2 Spike (S) and the receptor binding domain (RBD) proteins in human or insect cell lines to be further used in (i) the implementation of serological assays for detection of antibodies against SARS-CoV-2 virus in the Portuguese population, and (ii) the development of a virosome-based COVID-19 vaccine candidate.

For serological assays implementation, we explored different scalable cell culture systems (i.e. stirred-tank and wave bioreactors, from 1-30 L scale) and cell hosts (i.e. HEK293 and Expi293F) for S and RBD production. Production yields of S protein were close to 2 mg/L of culture independently of the bioprocessing approach and cell host. RBD production yields were significantly higher, with 90 mg/L of culture being commonly achieved. Noteworthy, the produced antigens showed excellent performance in serologic ELISA tests as denoted by the high specificity and sensitivity observed. In addition, S and RBD performance in serologic assays could be maintained after storage at different temperatures (i.e. 14 days at 4 ºC and 1 day at RT).

For vaccine development, we explored different bioprocess engineering strategies (i.e. signal peptides, baculovirus transfer vectors, cell lines, infection strategies and formulation buffers) for S production to include in a virosome-based vaccine candidate. Using the insect cells-baculovirus expression vector system (IC-BEVS), we were able to obtain approximately 4 mg/L of S protein. In addition, S protein exhibited mid-term stability upon storage (i.e. up to 90 days at -80°C and 4 ºC or after 5 freeze-thaw cycles). Noteworthy, antigenicity of S protein, either as single antigen or displayed on the surface of virosomes, was confirmed by ELISA with binding of S protein to ACE2 receptor, pan-SARS antibody CR3022 and neutralizing antibodies to the various epitope clusters. Binding capacity was also maintained on S-virosomes stored at 4ºC for 1 month.

In summary, we established scalable production protocols to obtain high-quality SARS-CoV-2 S and RBD proteins to be used as vaccine candidates and/or as antigens in COVID-19 serological assays.

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