PRODUCTION OF HIGH-QUALITY SARS-COV-2 ANTIGENS: IMPACT OF BIOPROCESS AND STORAGE ON GLYCOSYLATION, BIOPHYSICAL ATTRIBUTES, AND ELISA SEROLOGICAL TESTS PERFORMANCE

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INTRODUCTION

Serological assays are valuable tools to study SARS-CoV-2 spread and, importantly, to evaluate the efficacy of COVID-19 vaccines. Spike protein and its Receptor Binding Domain (RBD) are the antigens with higher potential to develop SARS-CoV-2 serological assays. Thus, to respond to COVID-19 pandemic, it is crucial that significant amounts of Spike protein became available at the highest quality.

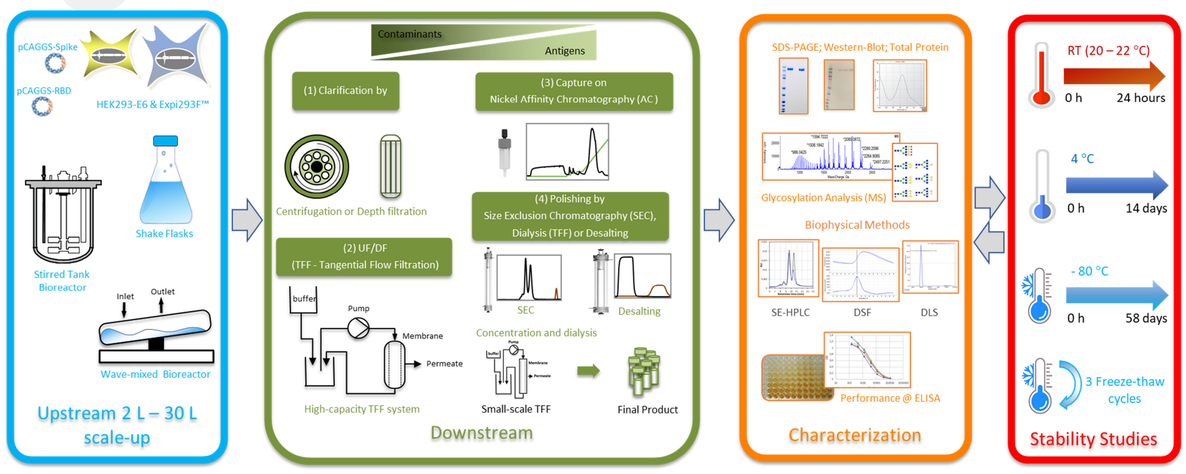
In this work, we investigated the production process for Spike and RBD antigens, using two human cell hosts, HEK293-E6 [1] and Expi293F™. Different cell culturing approaches and production scales were evaluated. The impact of downstream processing (DSP) steps and distinct storage temperature conditions were also assessed. An in-depth characterization of the antigens was performed correlating oligomeric state, glycosylation profile, and thermal stability with the bioprocess set-up and the storage conditions. Finally, the quality of the antigens was assessed in enzyme-linked immunosorbent assay (ELISA) serological tests using human serum control samples.

METHODS

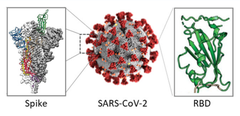
Detailed description of the methods used can be found in Castro *et al.* 2021 [2].

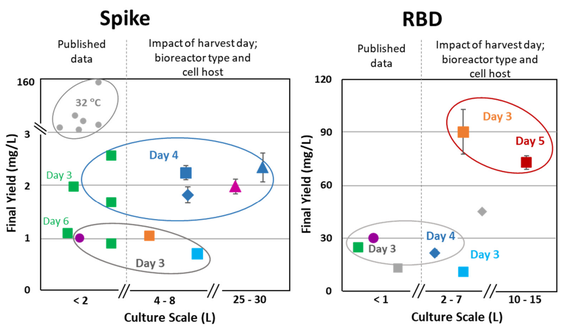
Cell lines and culture conditions. HEK293-E6 and Expi293F™ cells were cultivated in suspension in Freestyle F17 expression medium and Expi293™ Expression Medium (Thermo Fisher Scientific), respectively, at 37 °C. **Spike and RBD production.** Plasmid DNA for the expression of SARS-CoV-2 Spike and Spike's receptor binding domain (RBD) was kindly provided by Prof. Florian Krammer (ISMMS, NY, USA). Exponentially growing HEK293-E6 and Expi293F™ cells were transiently transfected with Spike or RBD plasmid DNA [3] and cultures were harvested 3 to 5 days post-transfection. The following cell culturing strategies were tested: 2.5 L culture volume in shake flasks (Corning, NY, USA), stirred tank bioreactors (STB, Sartorius, Germany) and wave-mixed bioreactors (Biostat Cultibag RM, Sartorius). **Spike and RBD purification.** Antigen purification was based on nickel affinity chromatography followed by size exclusion chromatography or protein desalting/dialysis. **Antigen characterization.** Spike and RBD characterization was performed by SDS-PAGE, size exclusion HPLC, Differential Scanning Fluorimetry, Mass spectrometry analysis of glycosylation pattern and Enzyme-linked immunosorbent assay (ELISA).

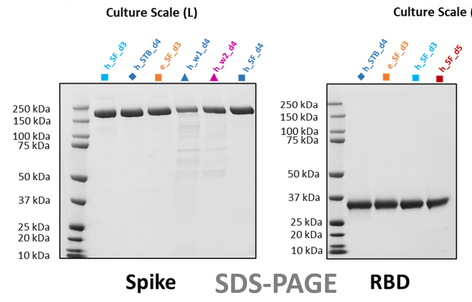
STRATEGY



ANTIGEN PRODUCTION





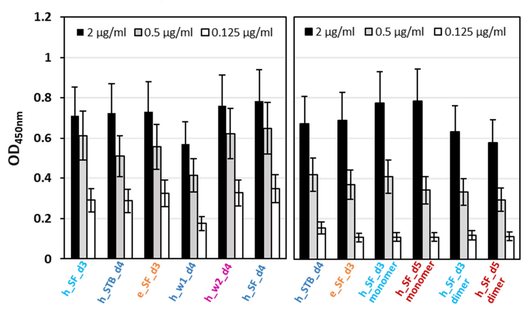


HEK293-E6, shake flask: impact of harvest day
- Harvest day 3 (h_SF_d3)
- Harvest day 4 (h_SF_d4)
- Harvest day 5 (h_SF_d5)

HEK293-E6, harvest at day 4: impact of bioreactor type
- HEK293-E6, wave-mixed bioreactor, run 1 (h_w1_d4)
- HEK293-E6, wave-mixed bioreactor, run 2 (h_w2_d4)
- HEK293-E6, stirred tank bioreactor (h_STB_d4)

Shake flask, harvest day 3: impact of cell host
- Expi293F™ (e_SF_d3)
- HEK293-E6 (h_SF_d3)

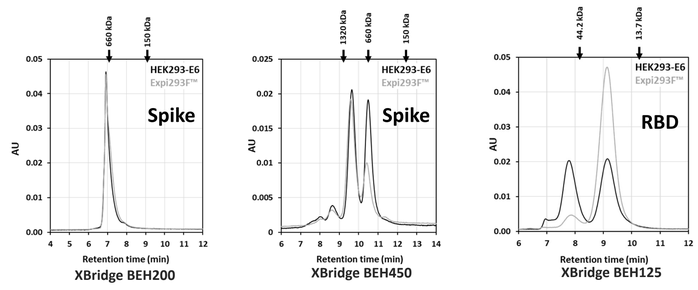
Published data: different culture systems and cell host
Expi293F™ ● Insect cells ● CHO cells ■ ◆ *Pichia pastoris*



✓ **Different culturing approaches and scale for Spike and RBD production do not impact performance of ELISA serologic COVID-19 tests or antigen quality.**

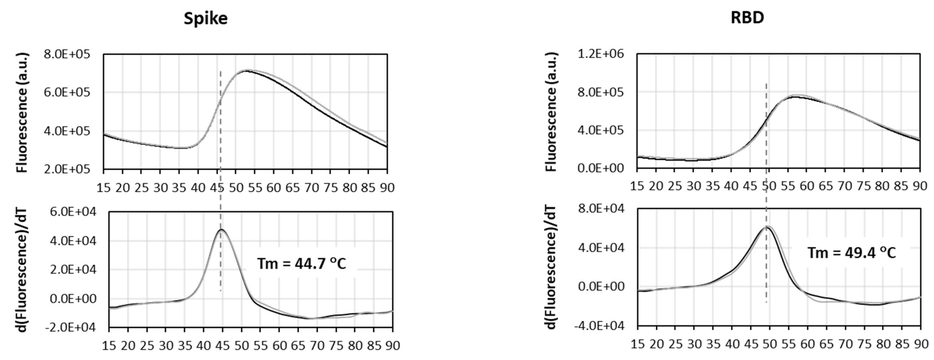
BIOPHYSICAL CHARACTERIZATION

Analysis of Spike and RBD oligomeric state by SE-HPLC

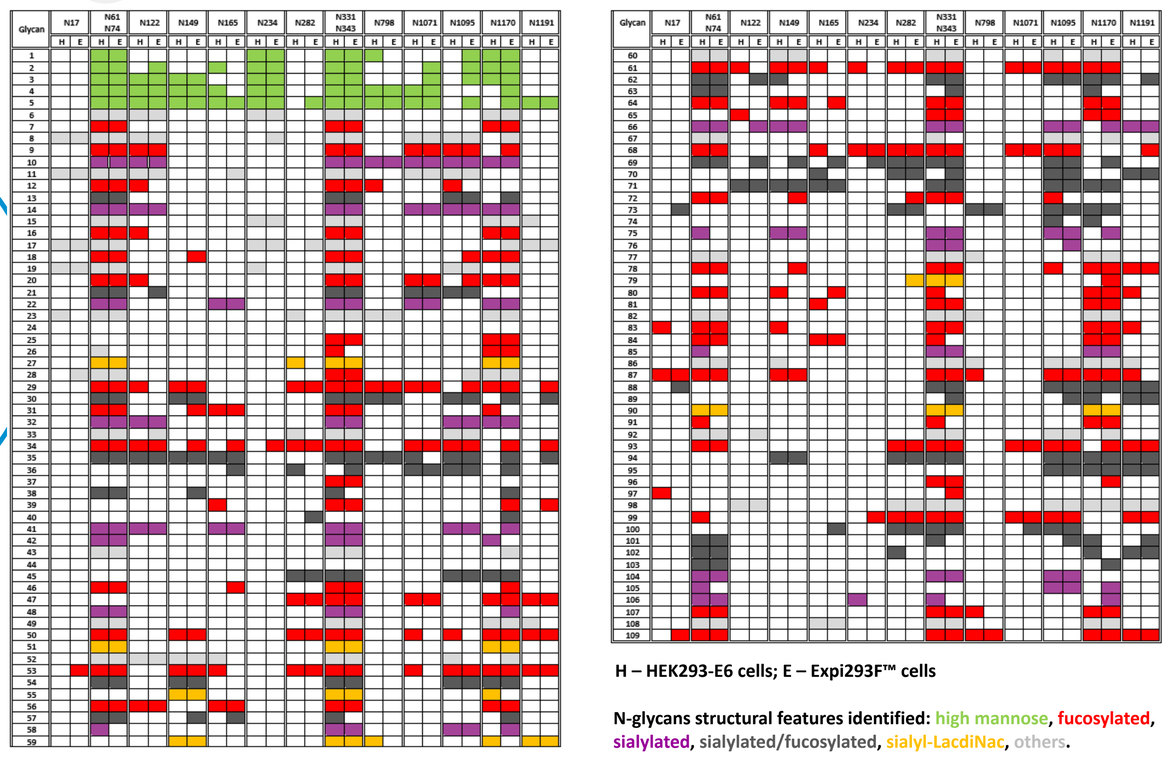


✓ **Trimeric conformation of Spike is confirmed with BEH200 column, however analysis with BEH450 column suggests higher molecular weight; RBD monomer and dimer are detected with BEH125 column.**

DSF analysis revealed lower thermal stability for Spike as compared to RBD



SPIKE GLYCOSYLATION PATTERN ANALYSIS BY LC-MS

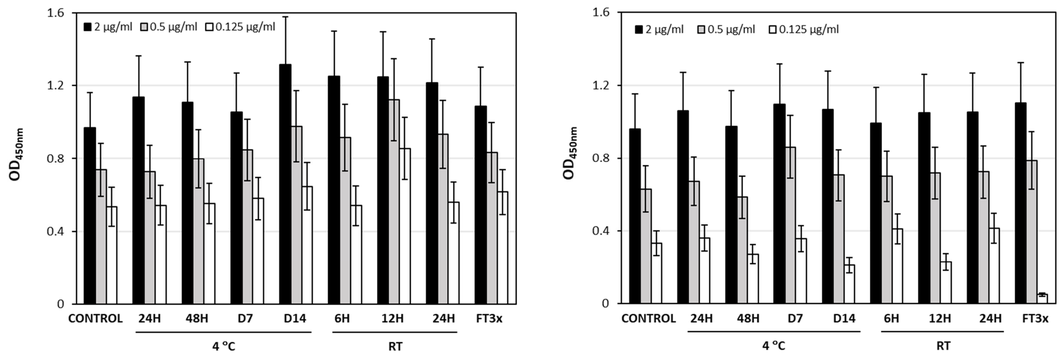


H – HEK293-E6 cells; E – Expi293F™ cells

N-glycans structural features identified: high mannose, fucosylated, sialylated, sialylated/fucosylated, sialyl-LacdiNac, others.

✓ **Similar site-specific glycosylation pattern of Spike produced in HEK293-E6 and Expi293F™ cells**

ANTIGEN PERFORMANCE IN SEROLOGICAL ASSAYS



✓ **Spike and RBD performance in serologic assays is mantained after storage at different temperatures.**

CONCLUSIONS

- **Similar yields for Spike and RBD production in HEK293-E6 and Expi293F™ cells.**
- **Development of scalable Spike and RBD production protocols, that are compatible with industrial production settings.**

ACKNOWLEDGMENTS

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REFERENCES

[1] Durocher, Y., *et al.*(2002) *Nucleic Acids Research*, 30: E9. [2] Castro, R., *et al.* (2021) *Biotechnology and Bioengineering*, 118: 2202-2219. [3] Amanat, F., (2020) *Nature Medicine*, 26: 1033-1036.