

VAC_12 - SARS-CoV-2 S1, S2, M & N antigens expressed in *Pichia pastoris*: Affordable, safe & effective vaccine for developing countries

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Introduction: SARS-CoV-2 encodes multiple structural proteins viz., S, N, M, and E that could potentially serve as immunogens for an anti SARS-CoV-2 vaccine. The virus uses spike glycoprotein (S protein) containing subunits, S1 and S2, mediating attachment and membrane fusion, respectively and generates neutralizing antibodies. The highly immunogenic S1 subunit and the highly conserved S2 subunit are key targets for vaccines. The virus surface membrane protein (M protein) is conserved and immunogenic eliciting strong cellular immune response. Nucleocapsid protein (N protein) is representative antigen for T-cell response in human body. These antigens induce potent and stable immune responses, both humoral and cellular, that presents the idea of a multivalent vaccine against SARS-COV-2 viral infections. There is an urgency to address and respond to Gavi's call and pursue safe, low-cost, easily administered and rapidly scalable approaches for low-and-middle income countries. The selected host *Pichia pastoris*, provides for high expression and post-translational modifications to produce cost effective product with a scalable process.

Objective: To develop multivalent SARS-CoV-2 vaccine comprising spike protein subunit S1 and S2, M protein and N protein individually expressed in clinically validated yeast-based *Pichia pastoris* platform as a vaccine candidate against Covid-19 infections.

Methodology: The gene sequences responsible for S1 subunit & S2 subunit of spike protein, M protein and N protein in SARS-COV-2 virus were transformed and expressed in *Pichia pastoris*, grown in fermentor and using methanol as an inducer to express the proteins. Mechanical cell disruption followed by various purification steps including chaotropic treatment using polymer & salt, adsorption/desorption, centrifugation, chromatography, ultrafiltration, ultracentrifugation and salt treatment were followed for desired level of purity. Samples analyses at different stages is underway for purity and impurity levels. Formulations are being developed using alum salt as adjuvant.

Results: S1 & S2, M and N proteins have been cloned separately in *Pichia pastoris* and successfully expressed in small fermentor. Preliminary characterization confirms the expression of these proteins. Further characterization, purification and formulation of antigens using alum adjuvant with suitable dose regime are expected to be completed in due course. Fermentation scale-up and analytical method development is underway.

Conclusion: This technology identifies and fills the gaps while addressing the challenges in vaccine design by providing economic and effective option for preventing SARS-CoV-2 infections in developing countries. The platform used to develop the technology has the advantage of not requiring dedicated or specialized facility making it an affordable option using existing manufacturing facilities without significant additional capital investments.

Keywords: SARS-CoV-2; Vaccine; Protein