IVD_13 - Production and characterizarion of recombinant nucleocapsid protein for its application on covid-19 diagnosis

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Introduction: COVID-19 pandemic was caused by the severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2). Nucleocapsid (N) protein is the most abundant protein in virion, and it is a highly immunogenic antigen. Several studies have demonstrated N protein as one of the best markers for diagnosis, as much molecular as serological assays.

Objectives: In this study, we produce recombinant SARS-CoV-2 N protein (r2N), performed its biochemical and biophysical characterization and immunological evaluation by serological methods.

Methodology: r2N was produced in *Escherichia coli* and purified by metal affinity chromatography. Characterization was performed through circular dichroism spectroscopy, intrinsic tryptophan fluorescence, denaturant electrophoresis, western blotting, Ethylene glycol bis (succinimidyl succinate) (EGS) crosslinking assay and size exclusion chromatography. The immunological performance was evaluated by enzyme-linked immunosorbent assay (ELISA) and beads-based array immunoassay.

Results: The r2N protein was obtained with high yield using scalable method and homogeneity over 97%. The identity of r2N protein was confirmed by commercial anti-N antibodies. r2N protein oligomers were observed and related to N protein association with nucleic acid. Structural analysis revealed secondary and tertiary structures of r2N protein starting to modify over 40 oC revealing that nucleic acid did not interfere with thermal stability. Interestingly, nucleic acid was able to prevent r2N protein aggregation even with increasing temperature while benzonase treated protein begin aggregation process above 55oC. Immunological characterization performed by ELISA with 233 serum samples presented a sensitivity of 97.44% (95% Confidence Interval, CI, 91.04%, 99.69%) and a specificity of 98.71% (95% CI, 95.42%, 99.84%) while beads-based array immunoassay carried out with 217 samples showed 100% sensitivity and 98.6% specificity.

Conclusion: The results exhibited an excellent immunological performance of r2N protein in serologic assays showing that, even in presence of nucleic acid, it can be used as a component of an immunoassay for sensitive and specific detection of SARS-CoV-2 antibodies.

Keywords: COVID-19, Immunological test, Intrinsic tryptophan fluorescence, ELISA