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EXPRESSION, PURIFICATION AND IMMUNOLOGICAL CHARACTERIZATION OF RECOMBINANT PROTEIN FRAGMENT FROM SARS-CoV-2

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Background: Serological testing is important method for diagnosis of severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) infection. Nucleocapsid (N) protein is the most abundant virus derived protein and strong immunogen. We aimed to find its efficient, low-cost production, suitable for serological diagnosis.
Methods: SARS-CoV-2 recombinant fragment of nucleocapsid protein (rfNP; 58–419 aa) was expressed in E. coli in soluble form, purified by immobilized metal ion affinity chromatography and strong cation exchange chromatography after which it was analyzed by Mass and CD spectrometry and characterized biochemically and immunologically.
Results: Purified rfNP has secondary structure of full-length recombinant N protein, with high percentage of disordered structure (34.2%) and of β-sheet (40.7%). rfNP was tested in immunoblot using sera of COVID-19 convalescent patients. ELISA was optimized with sera of RT-PCR confirmed positive symptomatic patients and healthy individuals. IgG detection sensitivity was 96% (47/50) and specificity 97% (67/68), while IgM detection was slightly lower (94% and 96.5%, respectively).
Conclusion: Cost-effective approach for soluble recombinant N protein fragment production was developed, with reliable IgG and IgM antibodies detection of SARS-CoV-2 infection.

Keywords: Recombinant nucleocapsid protein, COVID-19, SARS-CoV-2, Prokaryotic expression, serological assay

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