UNIVERSITÀ CATTOLICA del Sacro Cuore

Dipartimento di scienze biotecnologiche di base, cliniche intensivologiche e perioperatorie

Gemelli

Proteomics and Metabolomics for Personalized Medicine

XV ITALIAN PROTEOMICS ASSOCIATION ANNUAL MEETING

ItPA
Italian Proteomics Association

IN PARTNERSHIP WITH
HELLENIC PROTEOMICS SOCIETY

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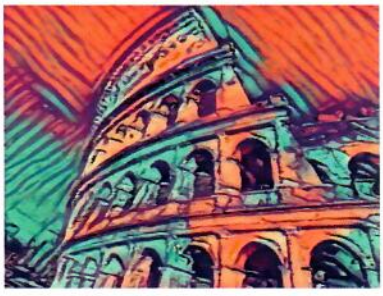

CATHOLIC UNIVERSITY OF THE SACRED HEART

ROMA, ITALY

AUDITORIUM

2021
September
8th-10th

P-07

Proteomic and immunological characterization of recombinantly expressed nucleocapsid SARS-CoV2 protein fragment in *E. Coli*

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Serological testing is important method for diagnosis of severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) infection and for assessment of immunological response after the vaccination. Nucleocapsid (N) protein is the most abundant virus protein and strong immunogen. The aim was develop efficient, low-cost production of N protein large fragment and to characterize it with bottom-up, high-resolution tandem mass spectrometry and immunologically. SARS-CoV-2 recombinant fragment of nucleocapsid protein (rfNP; 58–419 aa) was expressed in *E. coli* in soluble form and purified by several chromatographic steps and was subjected to SDS-PAGE and in-gel digested with trypsin. 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. Identity of rfNP was confirmed with high scores and peptide coverage above 80%. Estimation from the value of areas under ion extracted chromatographic curves is that only up to 0,03% of the total band protein quantity belongs to host proteins, while rfNP share is well above 99,9%, resulting in highly pure nucleocapsid protein preparation. Cost-effective approach for soluble recombinant N protein fragment production was developed, with reliable IgG and IgM antibodies detection of SARS-CoV-2 infection [1].

Funding: MESTD of Republic of Serbia No. 451-03-9/2021-14/200168, Horizon2020, FoodEnTwin project, GA No.810752, USAID GA No LVP-BPA UNDP and the Science Fund of Serbia, project Covid-19- CAPSIDO GA No.7542203.

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[1] Djukic, T., Mladenovic, M., Stanic-Vucinic, D., Radosavljevic, J., Smiljanic, K., Sabljic, L., Devic, M., Cujic, D., Vasovic, T., Simovic, A., Radomirovic, M., & Cirkovic Velickovic, T. (2021). Expression, purification and immunological characterization of recombinant nucleocapsid protein fragment from SARS-CoV-2. *Virology*, *557*, 15–22. https://doi.org/10.1016/j.virol.2021.01.004