

[DOI: 10.1002/eji.202048715]

## LIPS method for the detection of SARS-CoV-2 antibodies to spike and nucleocapsid proteins

Detection of antibodies to SARS-CoV-2 proteins can be used to study immune responses in COVID-19 patients [1]. Spike (S) and nucleocapsid (N) are main immunogenic proteins in SARS-CoV-2 [2]. Several ELISA based methods using SARS-CoV-2 proteins have been developed to detect the seroconversion of COVID-19 patients, confirming the presence of antibodies in circulating blood of over 90% of COVID-19 patients by day 14 [3].

We developed a simple and sensitive luciferase IP system (LIPS) assay to detect antibodies to S and N proteins in COVID-19 patients. LIPS works as a liquid-phase immunoassay, uses target antigens in their native conformation and is suitable to detect antibodies directed against linear and conformational epitopes [4].

Due to low natural background of bioluminescence, soluble crude cell lysates or culture media of the luciferase enzyme tagged recombinant proteins can be extracted from transfected cells and directly used, making it easy and fast method to produce antigens. Furthermore, the output of LIPS measurement is quantitative enabling to measure antibody levels

over time during disease or in response to treatments. We used the light-producing NanoLuc enzyme that is a small ca 20-kDa protein and enables the secretion of fusion proteins into cell culture media, which can be used to detect antibodies to SARS-CoV-2 S and N proteins in COVID-19 patients.

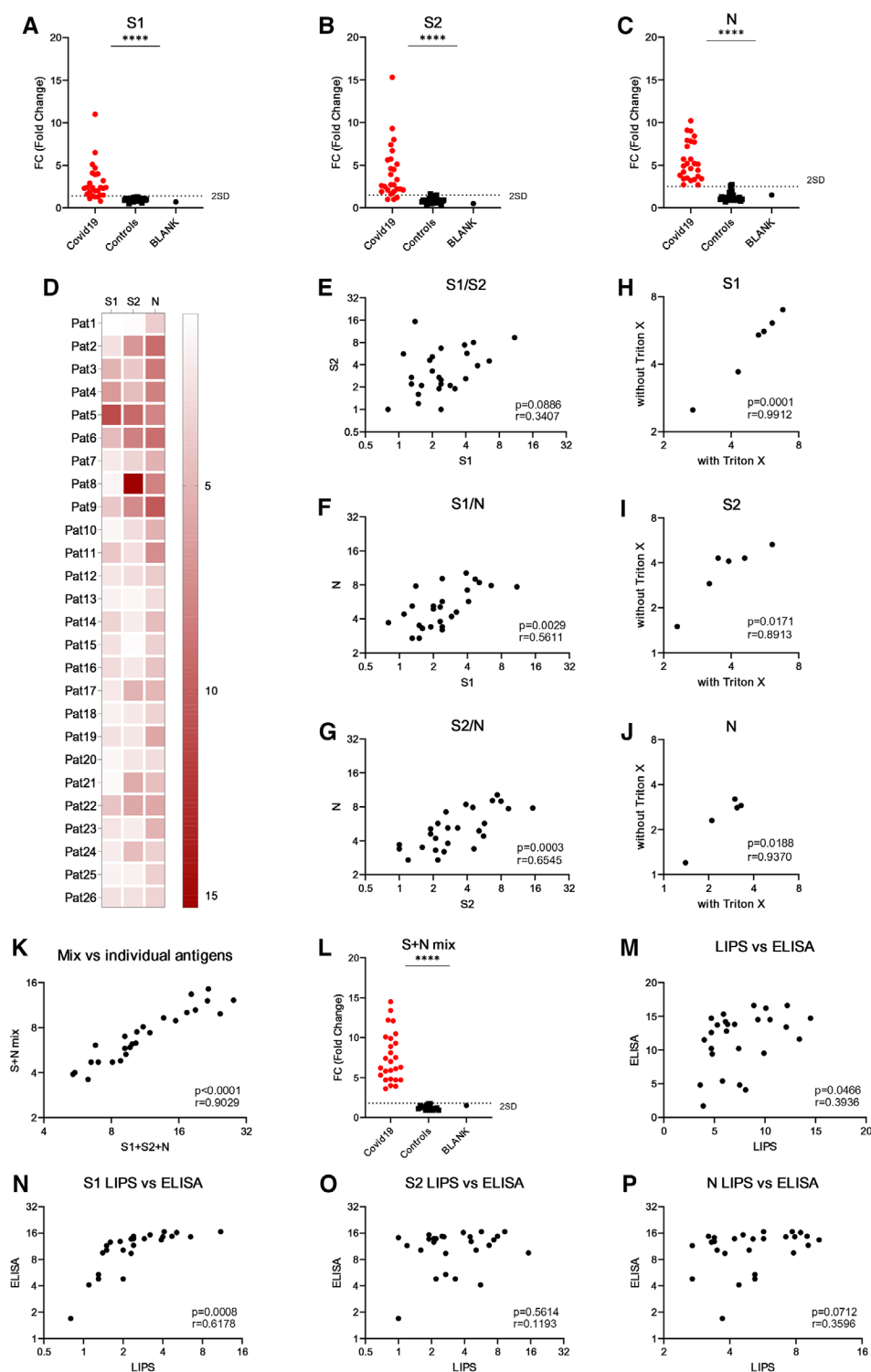
We carried out LIPS as previously reported [5]. We expressed S (S1 aa 1–680 and S2 aa 820–1211) and N (aa 2–419) protein fragments of SARS-CoV-2 in fusion with NanoLuc luciferase in HEK293 cells and confirmed the expression of the proteins by Western blotting (Supporting Information Fig. S1). After the transfection for 72 h, the cell lysates containing antigens were used in LIPS assay with COVID-19 patients (see Supporting Information for the methods).

We tested plasma samples collected at 8–37 days after the infection from 26 COVID-19 patients (age range 33–91 years). The COVID-19 diagnosis was confirmed by PCR analysis for SARS-CoV-2 virus. In addition, we studied 26 healthy controls (age range 23–54 years) without recent infection or COVID-19 symptoms (fever or cough) for past month. LIPS profiling detected a robust seropositive response to viral protein fragments. Using NanoLuc plasmid without the SARS-CoV-2 gene inserts gave no specific signal in the assay (Supporting Information Fig. S2). The statistical analysis showed highly significant reactivities to S1 (Fig. 1A), S2 (Fig. 1B), and N (Fig. 1C) antigens in the patients (Supporting Information Table S1). We found antibodies to S1 and S2 in 21 and 23 out of 26 patients, respectively, whereas all 26 patients had antibodies to the N antigen. The experimental replication of the assays gave 100% concordance of the patient positivity with high

correlation coefficient in luciferase values ( $r = 0.98$  for S1,  $r = 0.97$  for S2, and  $r = 0.91$  for N). Overall, the antibody responses were present to all three proteins in COVID-19 patients (Fig. 1D), suggesting that the reactivity is not limited to a single viral epitope or antigen. Interestingly, we found no significant correlation between S1 and S2 values (Fig. 1E), but the correlation was present between S1 and N ( $r = 0.56$ ; Fig. 1F) and S2 and N ( $r = 0.65$ ; Fig. 1G), most likely because of the higher seropositivity for N protein.

The analysis of COVID-19 blood samples by PCR has raised concerns that the patients with severe disease may have viral RNAemia at the acute infection phase. We therefore tested whether nonionic surfactant such as 1% Triton X, which can be used to neutralize potentially infectious plasma samples for routine laboratory handling, has an impact on the viral antigen detection in LIPS assay. The treatment of patient samples with Triton X did not affect the results as we saw high concordance of the results between samples, indicating that low concentration of mild detergent has no negative impact on assay performance (Fig. 1H–J).

LIPS is a convenient method as it allows to pool multiple antigens to a single reaction for screening purposes. To see whether the mixing of three antigens together gives an increased signal values, we combined S1, S2, and N cell lysates and studied for COVID-19 antibody reactivity. The combination of three viral antigens gave overall increased signals and 100% assay sensitivity with 26 patients (Fig. 1L) and correlated highly ( $r = 0.90$ ) with the sum of each individual antigen values tested separately (Fig. 1K). This result



**Figure 1.** LIPS analysis of antibodies to SARS-CoV-2 antigens. S1, S2, and N genes were cloned in fusion with NanoLuc (Promega) gene and expressed in HEK293 cells. The cell lysates were incubated with plasma samples (in 1:40 dilution) and bound to Protein G Sepharose to capture antibody complexes with viral proteins. After the washing, luciferase substrate Nano-Glo™ (Promega) was added and luminescence was measured in VICTOR X multilabel reader (PerkinElmer Life Sciences). Results are expressed as fold changes (FC) of luminescence units (LU) (FC = LU sample/average LU of five healthy control samples). The positive/negative discrimination level was set to the mean plus two standard deviations of the healthy control samples. The LIPS experiments were performed three times in three experimental replicates with 26 patients and 26 controls per experiment. The heatmap (D) shows average reactivities to three antigens in individual patients. The correlation of LIPS values between S1 and S2 (E), S1 and N (F), and S2 and N (G) antibody values. Triton X was added to the assays to test its impact on LIPS performance with S1 (H), S2 (I), and N (J) antigens. Mixing three antigens (S+N mix) in a single LIPS assay was correlated with the sum of the values from the three different assays (S1+S2+N) (K) and compared between patients and controls (L). The anti-SARS-CoV-2 IgG ELISA (Euroimmun) was performed according to the manufacturer's instructions. Statistics were performed using unpaired Student's *t*-test and Pearson correlation analysis in Graphpad Prism. \*\*\*\**p* < 0.0001.

shows that a relatively simple combination of antigens in the LIPS assay gives the highest performance to detect SARS-CoV-2 antibodies. Finally, we compared our results to the ELISA-based test (EUROIMMUN) that measures antibodies to S protein and found a good correlation with

our LIPS assay where three antigens were combined together (Fig. 1M, Supporting Information Table S1). We further compared the ELISA test for each individual antigen in LIPS method and, interestingly, found a highly significant correlation with S1 (Fig. 1N), but not with S2 (Fig. 1O) or N



(Fig. 1P), suggesting that the ELISA mainly detects S1 fragment of SARS-CoV-2.

Previous reports have suggested earlier coronavirus infections to affect the performance of serological assays [6], however, none of our negative control samples were positive for the SARS-CoV-2 antibodies.

We should note that our sample size of controls was relatively small and the analysis of more individuals would provide additional information on the utility of LIPS to differentiate the individuals with previous coronavirus from the positive responses to SARS-CoV-2.

Our results confirm the recent study [7], which used LIPS method to detect antibodies to S and N proteins in COVID-19 patients and showed that antibodies to N protein are more sensitive for the detection of early infection. To address the safety of COVID-19 blood samples, the study found no significant difference in antibody levels between heated and unheated plasma samples. Thus, the LIPS method is useful to study SARS-CoV-2 antibody levels in heat-inactivated or Triton X treated COVID-19 blood samples.

Taken together, we have developed sensitive and specific LIPS method combining different SARS-CoV-2 antigens for research screening purposes. LIPS assay with S and N antigen fragments may give useful information about the immune response in COVID-19 patients with different clinical courses.

Liis Haljasmägi<sup>1</sup>, Anu Remm<sup>1</sup>,  
Anna Pauliina Rumm<sup>1</sup>,  
Ekaterina Krassohhina<sup>1</sup>, Hanna Sein<sup>1</sup>,  
Anu Tamm<sup>2</sup>, Kai Kisand<sup>1</sup>   
and Pärt Peterson<sup>1</sup> 

<sup>1</sup> Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

<sup>2</sup> United Laboratories, Tartu University Hospital, Tartu, Estonia

**Acknowledgments:** We thank Drs Andres Männik and Mart Ustav for providing the SARS-CoV-2 S and N cDNA, and Liilija Verev for technical help. The study was supported by the Estonian Research Council grants PRG377 (PP) and PUT1367 (K.K.).

**Conflict of interest:** The authors declare no commercial or financial conflict of interests.

## References

- 1 Petherick, A., *Lancet* 2020. 395: 1101–1102.
- 2 Liu, W. et al., *J Clin. Microbiol.* 2020. 58: e00461-20.
- 3 Amanat, F. et al., *Nat. Med.* 2020. <https://doi.org/10.1038/s41591-020-0913-5>.
- 4 Burbelo, P. D. et al., *J. Vis. Exp.* 2009. (32): 1549.
- 5 Fishman, D. et al., *Front. Immunol.* 2017. 8: 976.
- 6 Guo, L. et al., *Clin. Infect. Dis.* 2020.
- 7 Burbelo, P. D. et al., *J. Infect. Dis.* 2020.

**Abbreviations:** LIPS: luciferase IP system · N: nucleocapsid · S: spike

**Keywords:** antibodies · COVID-19 · LIPS · luciferase immunoprecipitation · SARS-CoV-2

**Full correspondence:** Pärt Peterson, PhD, Institute of Biomedicine and Translational Medicine, University of Tartu, 50411, Tartu, Estonia  
e-mail: part.peterson@ut.ee

The peer review history for this article is available at <https://publons.com/publon/10.1002/eji.202048715>

Received: 22/4/2020

Revised: 10/6/2020

Accepted: 24/6/2020

Accepted article online: 25/6/2020



The detailed *Materials and methods* for Technical comments are available online in the Supporting information