SELECTION OF THE ANTIGENIC TARGET FOR THE MEASUREMENT OF ANTI-SARS-COV-2 ANTIBODIES IN CORONAVIRUS DISEASE (COVID-19) PATIENTS

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33rd AMLI Annual Scientific Meeting
August 2020

KEY MESSAGES

- The nucleocapsid protein (NP) and Spike (S) of SARS-CoV-2 showed optimal clinical performance on the chemiluminescent immunoassay platform
- NP/S is targeted at the early onset of disease

INTRODUCTION

Several methods have been developed for the detection of antibodies to SARS-CoV-2 utilizing a wide range of antigenic constructs. Among the antigens, the spike glycoprotein, specifically its receptor binding domain (SpRBD), which plays a key role during viral entry due to its ability to interacting with the human host angiotensin converting enzyme 2 (ACE2), is believed to be one of the suitable targets for antibody assays. Consequently, several assays are based on SpRBD. However, recent studies describe the nucleocapsid protein (NP) of SARS-CoV-2 as the most conserved and predominant virus derived protein throughout the infection.

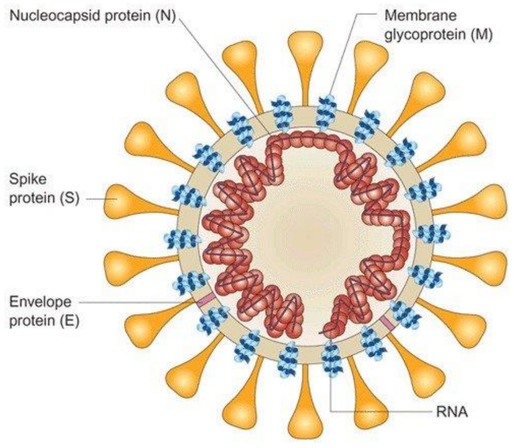

Figure 1 Structural overview of SARS-CoV-2. Image depicting the Nucleocapisid (NP) and Spike proteins which were the focus of this study.

NP template mRNA is the most abundant subgenomic RNA, which makes it a suitable target for molecular and antibody-based diagnostics. In addition, several recent studies indicate that NP-based immunoassays might exhibit higher sensitivity in the early phase of infection. Unfortunately, most of the studies compared this aspect using different diagnostic platforms which might introduce confounding factors (such as the assay architecture). Therefore, the objective of this study is to compare the antigen constructs independently in comparison of being combined on the same diagnostic platform.

METHODS

A total of 113 samples were included in a Spearman's correlation analysis, consisting of 36 samples from COVID-19 patients confirmed to have the disease by polymerase chain reaction (PCR) test, as well as 77 samples from patients that tested positive for anti-SARS-CoV-2 antibodies. For 33 samples, information on the duration between PCR and blood sampling was available (median 26 days, 10-43 days). A total of 174 samples were used for ROC curve analysis using 36 COVID-19, PCR confirmed samples and 138 control samples. All samples were tested by fully automated chemiluminescent immunoassays (CIAs) on the BIO-FLASH instrument (Research use only, Inova Diagnostics, USA) using three different antigenic constructs [NP full-length/Spike (NP-FL/S), NP-RBD, SpRBD].

RESULTS

Using all SARS-CoV-2 samples, a high degree of quantitative agreement by Spearman's correlation was observed between the different antigens (all Spearman's rho> 0.7): NP-FL/S *vs.* NP-RBD *rho*=0.80 [95% confidence interval (CI) 0.72-0.86], NP-FL/S *vs.* SpRBD *rho*=0.79 (95% CI 0.71-0.85), NP-RBD vs. SpRBD *rho*=0.86 (95% CI 0.80-0.90). When analyzing samples that had detailed information available (n=33), NP-FL/S showed the highest levels of antibodies in the early phase of COVID-19 (see figure 2). Lastly, all antigens showed good diagnostic performance, with NP-FL/S yielding the highest area under the ROC curve (Figure 3).

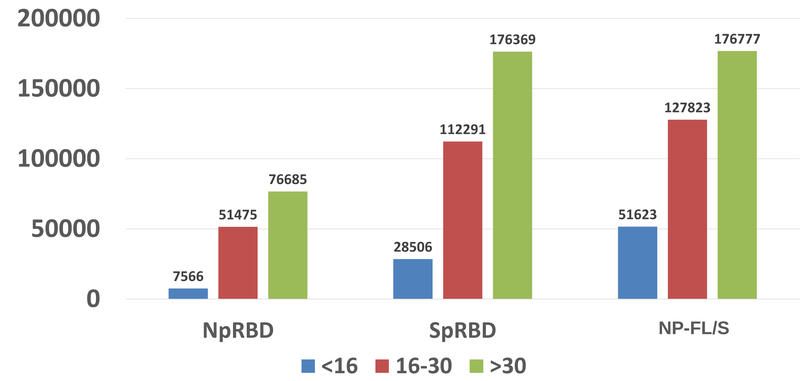

Figure 2 Median signal intensities by antigen and duration between PCR and blood sampling.

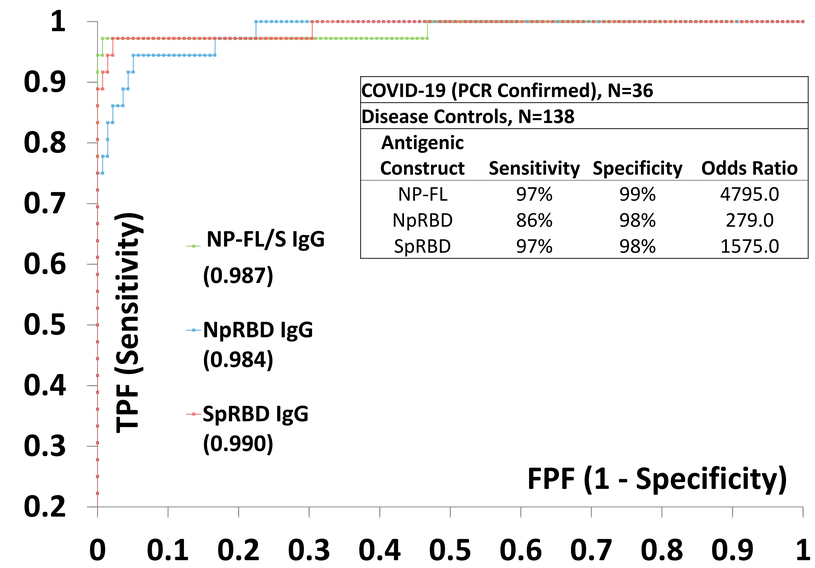

Figure 3 ROC Curve analysis with nucleocapsid constructs NP-FL/S & NpRBD, and the Spike construct SpRBD. Area under the curve notated in parenthesis in the legend.

CONCLUSION

Using a CIA platform, our study provides evidence that NP-FL/S provides higher sensitivity within the first days post infection.

REFERENCES

1. Stadler, K. et al. SARS-beginning to understand a new virus. *Nat. Rev. Microbial*. 1, 209-218 (2003).
2. Liu W, Liu L, Kou G, et al. Evaluation of Nucelocapsid and Spike Protein-Based Enzyme-Linked Immunosorbent Assays for Detecting Antibodies against SARS-CoV-2. J Clin Microbial. 2020;58(6):e00461-20. Published 2020 May 26. doi:10.1128/JCM.00461-20