Comparison of a novel surface plasmon resonance –based and conventional ELISA assay for determination of SARS-CoV-2 antibodies in serum samples

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Introduction

In this study, SARS-CoV-2 antibodies of Covid-19 patients were measured with commercial ELISA IgG assay kit and with the method based on Multi-Parametric Surface Plasmon Resonance (MP-SPR) technology. The principle of SPR technology is shown in Figure 1. The ELISA kit assayed antibodies formed against spike protein of the SARS-CoV-2 virus whereas MP-SPR technology enables parallel quantitation of SARS-CoV-2 specific antibodies against three SARS-CoV-2 antigens. The sensor surface coated with avidin was functionalized with biotinylated proteins: spike S1, nucleocapsid (NC) and spike receptor binding domain (RBD) along with negative control HSA protein.

Aims

To demonstrate the functionality of MP-SPR assay in providing quantitative data of patient's Covid-19 related immune response and to compare the Covid-19 patients' antibody levels measured with a conventional immunoassay to those measured by the MP-SPR technology.

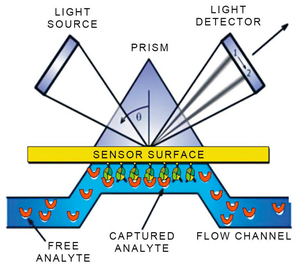


Figure 1.

Principle of surface plasmon resonance (SPR) technology. The specific binding of measured analyte onto the active gold sensing surface of the SPR device induces a refractive index change that can be monitored. SPR technology allows on-line and nearly real-time measurements.

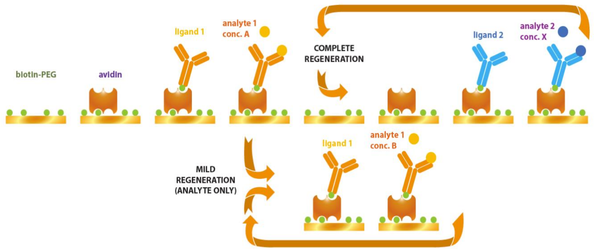


Figure 2.

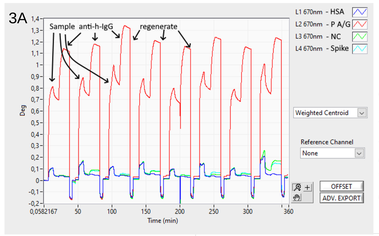
The regenerable avidin sensor. Biotinylated proteins used to bind analyte molecules themselves bind to avidin. After the sample is measured, sensor surface can be regenerated completely or mildly.

Methods

Serum samples were collected from Covid-19 patients who had consented to a study monitoring SARS-CoV-2 antibody levels 3, 6 and 12 months after the infection. Blood samples were collected to vacuum tubes, sera were separated and stored in -20 °C until analyzed. SARS-CoV-2 antibodies were assayed by Vircell Covid-19 ELISA IgG -kit according to manufacturer´s instructions at the Unit of Measurement Technology laboratory. The aliquoted samples were also analyzed with MP-SPR Covid-19 diagnostic kit by BioNavis Ltd. Measurements were performed using 4-channel MP-SPR Navi™ 420A ILVES instrument equipped with lasers at 670 nm wavelength. The Covid-19 diagnostic kit was composed of regenerable avidin sensor functionalized with biotinylated proteins: spike S1, nucleocapsid (NC) and spike receptor binding domain (RBD) along with negative control HSA protein. The principle of avidin sensor is shown in Figure 2. Patient samples were measured with certified reference serum materials EURM-17 and EURM-18 (Joint Research Centre, European Commission), and negative serum samples (Octaplas®).

Results

Use of regenerable avidin kit and sensor surface chemistry based on PEG molecules ensured low level of non-specific binding from serum. The responses of samples are shown in Figure 3 A and B. Combination of 3 different antigens on the sensor allowed parallel detection of antibodies against three specific antigens. The sandwich assay format, based on final recognition by anti-human IgG antibody, ensured determination of positive SARS-CoV-2 antibodies -containing samples which resulted in high level of correlation (R>0.8) to ELISA measurements (Figure 4.). The vaccination against Covid-19 gives boost to levels of specific antibodies against the antigen used in vaccination whereas new infection of SARS-CoV-2 coronavirus gives different signature to selected virus antigens.



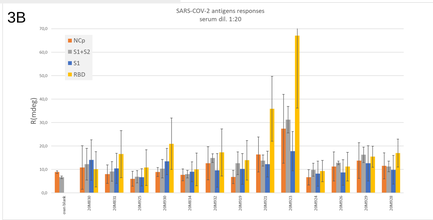


Figure 3 A and B

SPR sensogram of the serum samples A and analysed SPR results B. In this assay series HSA was replaced with S1-S2 combination.

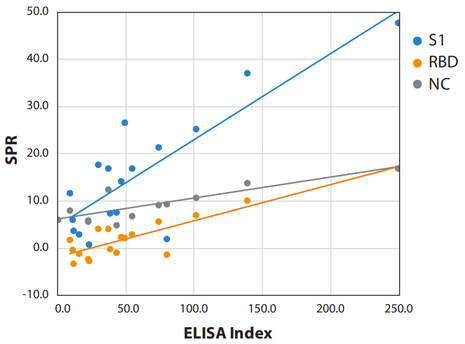


Figure 4.

Comparison of Covid-19 serological testing performed MP-SPR and ELISA on the same set of seropositive samples.Correlation for S1, RBD and NC response are 0.84, 0.89 and 0.84, respectively.

Conclusions

Human serum samples from Covid-19 positive patients were collected after 1-3 months from confirmed positive Covid-19 test. Combination of spike S1 protein, RBD domain and nucleocapsid (NC) on the sensor made possible to recognize if study patients had antibodies generated as a response on Covid-19 diseases or vaccination or on both stimulus. This suggests together with high interpersonal variation in antibody levels further significance for pathogen antibody assays in the next pandemics.

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