Application Note # 169

Diagnostic method for serological testing of Covid-19 antibodies

Considering the indisputable need for Covid-19 related diagnostic tools, we have developed a dedicated serological testing method based on MP-SPR technology. The combination of MP-SPR instrument and optimized sensor format functionalized with virus proteins enabled rapid detection of SARS-CoV-2 antigen-specific antibodies in human serum. The measurement accuracy has been proven with seropositive patient samples and validated with certified reference serum materials. As alternative to currently existing methods, BioNavis will launch a Covid-19 diagnostic kit to be used for immunity surveys across populations and screening the efficacy of treatments developed against SARS-CoV-2.

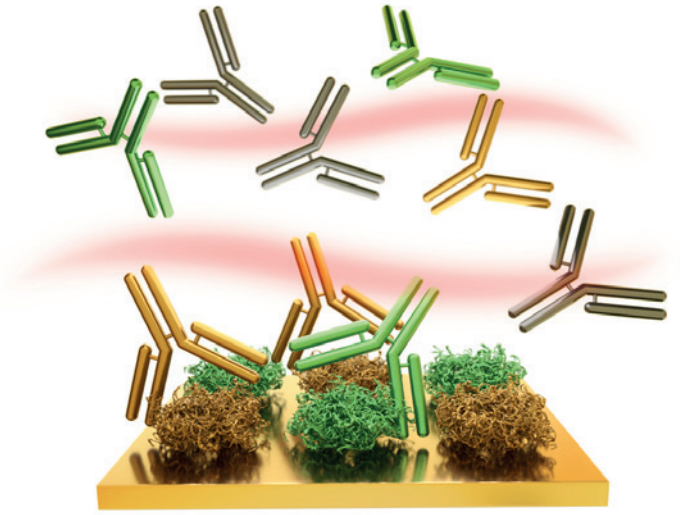

Figure 1. Schematic representation of Covid-19 diagnostic method: SARS-CoV-2 specific antibodies from human serum are detected on MP-SPR sensor functionalized with virus proteins.

Introduction

The unprecedented threat from the SARS-CoV-2 coronavirus, causing a global pandemic with several million casualties worldwide, brought the need of new diagnostic tools to fight the virus spread and ensure long-term control of infections. In this matter, serological testing to evaluate the level of virus antigen-specific antibodies in patient samples is of great importance. Such testing is mostly performed by time- and labour- consuming immunoassays, such as ELISA. Also, hundreds of lateral flow tests have been developed offering only binary results and often lacking detection specificity. Therefore, we introduce an alternative diagnostic assay for serological testing of Covid-19 antibodies based on MP-SPR.

Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a well-established technique capable of monitoring biomolecular interactions and characterization of layer properties in a real-time and label-free manner. MP-SPR instrumentation underpins assay development in a wide range of applications from biosensors on food safety up to tests for clinical use. Indeed, the technology has a proven track of references in the detection of specific antibodies and other biomarkers, even in complex media, such as serum or plasma. The MP-SPR based diagnostic method is successfully applied to detection of SARS-CoV-2 specific antibodies in human serum samples and provides more comprehensive quantitative data of a patient's serological signature of the Covid-19 related immune response.

Materials and methods

Measurements were performed using 4-channel MP-SPR Navi™ 420A ILVES instrument equipped with lasers at 670nm wavelength. The running buffer was PBS + 0.05% Tween 20 (pH 7.4) provided at a flow rate of 30µl/min and temperature of 22°C. The Covid-19 diagnostic kit was composed of regenerable avidin sensor (BioNavis) functionalized with biotinylated proteins according to previously reported instructions (cf. Application Note #166). SARS-CoV-2 proteins (Sino Biological Inc.): spike S1, nucleocapsid (NC) and spike RBD, along with negative control HSA protein (Sigma-Aldrich) were biotinylated using a fast biotin conjugation kit (Abcam). S1, RBD, NC, HSA proteins were individually captured onto 4 channels prior to loading the serum samples for analysis. Covid-19 seropositive samples from 23 patients in Kainuu region (Finland) were obtained from University of Oulu, Kajaani University Consortium (KUC)*. Patient samples were sequentially loaded along with certified reference serum materials EURM-17 and EURM-18 (Joint Research Centre, European Commission), and negative serum samples (Octaplas®). Each sample was prepared from as little as 15µl serum (20-fold dilution in running buffer), introduced onto the sensor in 8min injection followed by a 5min dissociation step. Secondary anti-human-IgG antibody (Jackson ImmunoResearch) was subsequently applied (5min injection and 5min dissociation time) and final signal readout processed with MP-SPR Navi™ Navi Data Viewer. The SPR sensor was regenerated between the samples with 2min injection of 10mM glycine-HCl (pH=2.1). Data analysis was performed on averaged signals from at least duplicate injections and normalized to EURM-17 standard sample signal.

AN_169.2104.a

Excellence in Surface Plasmon Resonance
BioNavis

e-mail : info@bionavis.com
www.bionavis.com

Results and discussion

MP-SPR instrument combined with dedicated sensor functionalized with SARS-CoV-2 antigens enabled successful characterization of serological patient samples. Human serum samples from Covid-19 positive patients were collected after 1-2 months from manifestation of symptoms and confirmation by PCR testing. Use of regenerable avidin kit and sensor surface chemistry based on PEG molecules ensured low level of non-specific binding from such complex samples like serum. Combination of 3 different antigens on the sensor (spike S1 protein, RBD domain and nucleocapsid (NC) protein) provided a patient-specific serological signature of the Covid-19 immune response. The sandwich assay format, based on final recognition by anti-human IgG antibody, ensured determination of positive samples, which resulted in high level of correlation (R>0.8) to ELISA measurements performed at KUC* (Fig.2). The responses from serological testing have been confirmed and validated with certified reference materials (EURM-017 and EURM-018) containing SARS-CoV-2 antibodies. The assay enabled the distinction of different levels of immunity in tested samples, which could be divided into groups: from low level of antibodies (group A) to high immune response (group B, C; Fig.3). Testing was performed from only 15µL volume of patient sample. A 20min assay combined with 4-channel instrument configuration enabled analysis of up to 72 patient samples a day.

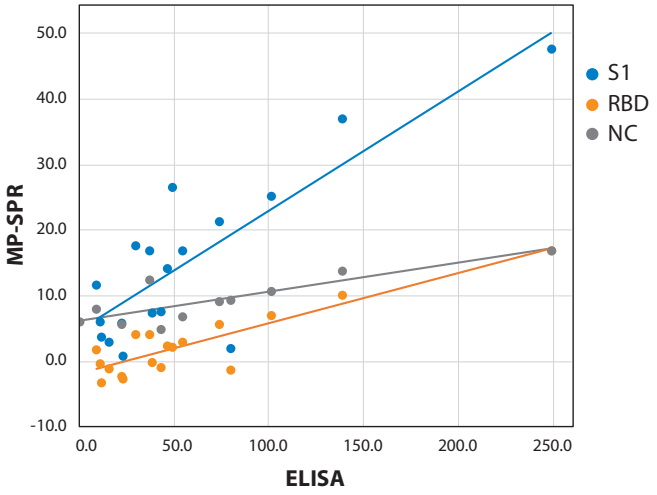

Figure 2. Comparison of Covid-19 serological testing performed MP-SPR and ELISA on the same set of seropositive samples. Correlation coefficients for S1, RBD and NC response are 0.84, 0.89 and 0.84, respectively.

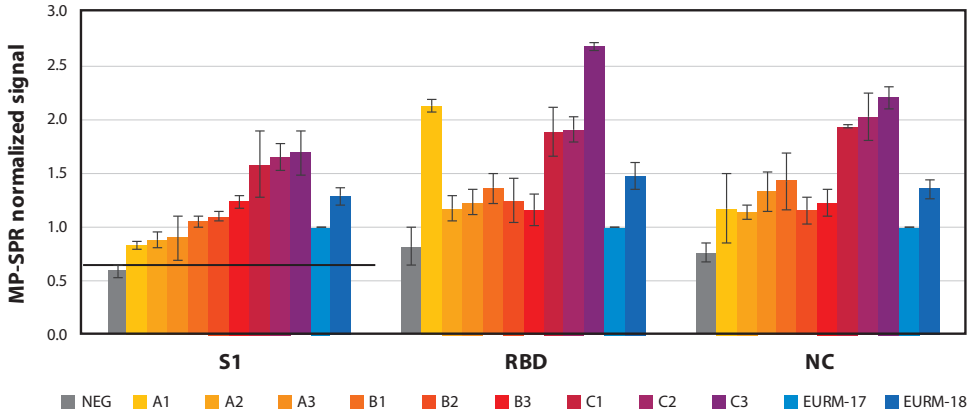

Figure 3. Results of Covid-19 serological testing using MP-SPR based sensor functionalized with spike (S1), RBD and nucleocapsid (NC) proteins. Signals were averaged from at least duplicate serum runs and referenced by subtraction of negative HSA control signal. Normalization was done against EURM-17 certified reference serum sample signal. The threshold line indicates the S1 signal above which a sample was categorized as positive.

Conclusions

MP-SPR based Covid-19 diagnostic tool presented here enabled the detection of SARS-CoV-2 specific antibodies in positive serum samples. Based on 3 antigens, the assay provides complete quantitative data on Covid-19 patient immune response, with an accuracy that was validated with certified serum materials. The kit exceeds performances offered by lateral flow testing or immunoassays based on ELISA or chemiluminescence. The developed solution can be immediately implemented in existing MP-SPR instrumentation for urgent and long-term monitoring of Covid-19 related immunity across populations. Besides serodiagnostic surveys, the kit can be also used to study the efficacy of vaccines and future treatments against SARS-CoV-2. The regenerable format of the method makes it a versatile diagnostic tool whose use can be extended to new and re-emerging pathogens (e.g. influenza, Zika, Ebola), given the availability of virus antigenic proteins.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 410A KAURIS, 420A ILVES
Sensor surfaces: regenerable avidin kit
Software: MP-SPR Navi™ Control, DataViewer, TraceDrawer™ for MP-SPR Navi™

* Kajaani University Consortium (KUC), University of Oulu, Unit of Measurement Technology & Kainuu Social and Health Care Joint Authority

AN_169.2104.a