A rapid and quantitative serum test for SARS-CoV-2 antibodies with portable surface plasmon resonance sensing

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Abstract

We report a surface plasmon resonance (SPR) sensor detecting nucleocapsid antibodies specific against the novel coronavirus 2019 (SARS-CoV-2) in undiluted human serum. When exposed to SARS-CoV-2, the immune system responds by expressing antibodies at levels that can be detected and monitored to identify the patient population immunized against SARD-CoV-2 and support efforts to deploy a vaccine strategically. A SPR sensor coated with a peptide monolayer and functionalized with SARS-CoV-2 nucleocapsid recombinant protein detected anti-SARS-CoV-2 antibodies in the nanomolar range. This bioassay was performed on a portable SPR instrument in undiluted

human serum and results were collected within 15 minutes of sample/sensor contact. This strategy paves the way to point-of-care and label-free rapid testing for antibodies.

Keywords: Surface plasmon resonance, Antibody detection, coronavirus, serum analysis, biosensor

Main text

In the event of a viral outbreak, it is of utmost importance to rapidly test populations that are actively infectious, thereby offering the capacity to limit widespread contagion. Identification of individuals who are actively infected with SARS-CoV-2 mainly relies on real-time reverse transcription polymerase chain reaction (rRT-PCR) amplification of the viral genetic material collected in nasopharyngeal swabs (Chen, Gao et al. 2020, Vashist 2020). These assays show high sensitivity and can be highly specific. Due to high workload and to reagent shortages during the epidemic stage, PCR tests are mainly performed on patients displaying COVID-19-related symptoms. Detailed studies are underway to better understand disease progression following infection with SARS-CoV-2 [2]. Although hard numbers are only beginning to come to light [3], a significant fraction of infected individuals remain asymptomatic [4]. Asymptomatic, contagious individuals often go undetected and are thought to contribute to the spread of the disease. To address this global pandemic, significant efforts are being rapidly deployed to adapt diagnostics tests, identify effective therapeutics and develop vaccines against SARS-CoV-2 [5].

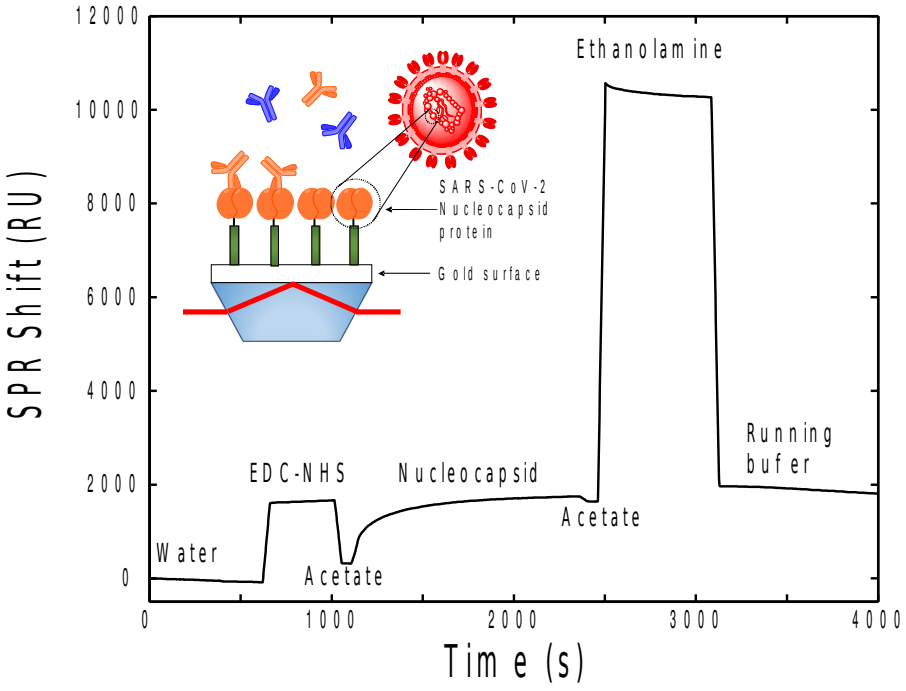
The immune system produces antibodies to SARS-CoV-2 within days to a few weeks following viral infection [6]. Antibodies are expected to remain at a high level for months, perhaps years, following infection, as previously shown following the 2003 outbreak of SARS-CoV-1 [7]. The immune reaction to coronaviruses generally provides innate immunity via neutralizing antibodies [8] in the event of a second exposure to the

virus and also provides the basis for vaccine development. Early stages of vaccine development and clinical trials will require assessing antibody titers or concentrations in animal and human subjects, as is currently underway for SARS-CoV-2 [9]. As such, serological antibody testing is essential to assess the fraction of the population that is immune to a virus [10].

Antibody detection is typically performed using ELISA assays. While ELISA has high-throughput capability, it requires several hour-long steps that lengthen assay time. Alternatively, faster and portable sensing technologies can decrease assay time and be employed at the point-of-care for infectious diseases [11]. Lateral flow assays have often been proposed for antibody detection, but suffer from reliability issues and are not quantitative. Alternatively, surface plasmon resonance (SPR) sensing is a label-free sensing technique that is highly sensitive [12], especially for large biomolecules such as antibodies. SPR sensing has been reported in the detection of antibodies to the first SARS-CoV [13], albeit in phosphate-buffered saline solution (PBS). Since then, SPR sensors have been reported to work in crude biofluids [14], illustrating their applicability to direct detection in clinical samples [15], such as for the analysis of antibodies in allergic reactions [16], for blood grouping [17] or for infection/viruses (Chagas disease [18], Dengue [19], Epstein-Barr virus [20], hepatitis [21], Syphilis [22], or typhoid fever [23]). SPR sensing is thus well suited for quantitative analysis of antibodies associated to SARS-CoV-2 in biofluids.

A portable SPR instrument [24] (Affinité Instruments, Canada) and a SPR surface modified with a monolayer of 3-mercaptopropionic-Leu-His-Asp-Leu-His-Asp-COOH [25] (3-MPA-LHDLHD-COOH, AffiCoat, Affinité Instruments, Canada) were employed here in the construction of the SPR sensor (Scheme 1). After stabilization of the SPR signal in water, the surface was modified with a 1:1 aqueous solution of 100 mM N-hydroxysuccinimide (NHS) and 400 mM N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) for 2 minutes, rinsed for 20

seconds with the immobilization buffer (described below) and reacted for 20 minutes with SARS-CoV-2 nucleocapsid recombinant (rN) protein (MBS569934, MyBioSource, USA). rN protein was selected due to commercial availability and recent reports showing its performance in an ELISA test for detection of SARS-CoV-2 antibodies [26]. The sensor was rinsed for 20 seconds with the immobilization buffer and passivated with 1M ethanolamine pH 8.5 for 10 minutes. The sensor was then equilibrated in the running buffer composed of PBS (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) supplemented with 0.1% bovine serum albumin (BSA) and 0.005% Tween 20 (Figure 1). SARS-CoV-2 anti-nucleocapsid antibodies (MBS569903, MyBioSource, USA) were diluted in the running buffer or in undiluted human serum from human male AB plasma (cat. no. H4522, Sigma Aldrich) and 300 µL was injected with a syringe and measured for 15 minutes on the SPR sensor. The SPR shift was calculated from the RU difference between the beginning and the end of the measurement. The sensor could be regenerated at least three times with 10 mM glycine pH 2.2, where the response for 10 µg/mL anti-rN yielded a response of 221, 220 and 258 RU for each run.



Scheme 1. SPR sensorgram of the surface functionalization. After EDC-NHS activation of the AffiCoat surface, the nucleocapsid protein of SARC-CoV-2 (rN) was bound to the surface of the SPR chip and remaining activated sites were passivated with ethanolamine.

Composition of the immobilization buffer was investigated to optimize the SPR response for antibody detection at 10 μg/mL, a concentration around the mid-detection range, as shown below. Acetate buffers (pH 4.4 and 5.5) and PBS (pH 6.5 and 7.4) are commonly used SPR immobilization buffers in the context of EDC-NHS chemistry. Immobilization of the rN protein led to shifts between 534 and 1240 RU (Table 1, 1 RU is approximately equal to 1 pg/mm2). More importantly, antibody detection led to shifts of approximately 200 RU in all immobilization buffers, with the exception of PBS pH 6.5, which was significantly lower. As the acetate buffer led to the largest concentration of rN protein bound to the surface among the better immobilization buffers for anti-rN detection, it was selected for all further studies.

A linear relationship was observed between the concentration of rN protein applied and that immobilized on the surface (Table 1). The highest response for anti-rN antibody detection was 226 RU, obtained upon immobilization of 10 μg/mL rN protein. Increasing the concentration of the rN protein on the surface led to a significant decrease in the detection of the antibody, suggesting that steric hindrance reduced access to the binding site on the rN protein at higher concentrations. Thus, 10 μg/mL rN protein was used for the remaining experiments.

Table 1. Optimization of the SPR conditions for the immobilization of rN protein

Buffer optimization	**Concentration optimization**

Buffer	rN shift (RU)	Ab shift (RU)	rN conc. (µg/mL)	rN shift (RU)	Ab shift (RU)
Acetate pH 4.4	689 ± 64	230 ± 23	5	263 ± 120	192 ± 22
Acetate pH 5.5	866 ± 86	226 ± 23	10	734 ± 236	226 ± 23
PBS pH 6.5	1240 ± 162	162 ± 41	20	1871 ± 128	183 ± 22
PBS pH 7.4	534 ± 144	225 ± 31	40	3111 ± 70	80 ± 30

Control experiments showed the selectivity of the interaction between the antibody and the protein. The immobilization of a protein unrelated to SARS-CoV-2, the lac repressor (LacI), led to minimal interaction of the anti-rN antibody with the sensor. In another control, a rN-modified SPR sensor led to no response to anti-PSA (anti-prostate-specific antigen) for concentrations as high as 50 µg/mL. Calibration of the sensor was performed with sequential injections of increasing concentrations of the anti-rN antibody in PBS solution, for antibody concentrations between 5 and 75 µg/mL (approximately 30 to 500 nM). This was repeated in undiluted serum, where we observed even greater sensitivity of the sensor (Figure 1). The typical error on anti-rN measurements were between 5 and 20% (n=3) for the SPR shift in undiluted serum, where larger deviations were found for lower concentrations. The limit of detection was estimated to be near 1 µg/mL.

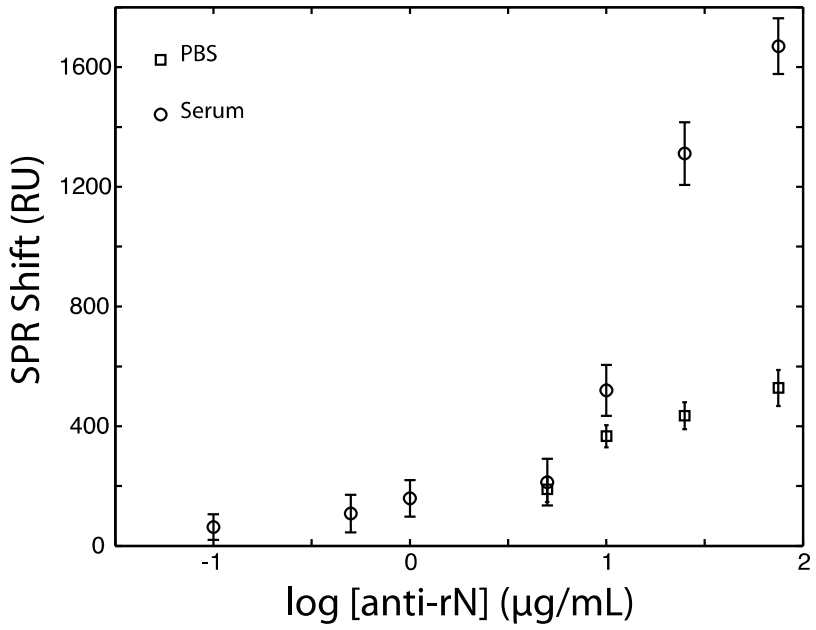


Figure 1. Comparison of SPR response in PBS (squares) and undiluted serum (circles) for the detection of SARS-CoV-2 anti-nucleocapsid (anti-rN) antibody at low concentrations.

Enhanced assay sensitivity in serum was also observed at higher anti-rN antibody concentrations. For example, the shift observed over the range of 10 to 75 µg/mL, the highest concentration tested, was generally three times larger in serum than in PBS (Figure 2). The nonspecific adsorption or bulk refractive index effect of serum can be ruled out. First, the nonspecific adsorption observed with undiluted human serum gave a signal of approximately 300 RU for the AffiCoat surface, significantly less than the increase in performance of the sensor in serum relative to PBS (Figure 1). All serum measurements were conducted on a surface passivated with a blank serum (serum containing no anti-rN antibody) prior to analysis, further minimizing nonspecific adsorption. Finally, blank serum was injected into the reference channel of the SPR instrument at the same time as samples and subtracted from the measurement channels (Figure 2), confirming the enhancement of sensitivity in serum relative to PBS. We

hypothesize that the enhancement results from adsorption of serum proteins on the captured antibodies, increasing their mass and refractive index shift. We note that the any remaining nonspecific adsorption on the SPR surface, albeit minimized, may help stabilize the surface-bound rN protein and improve binding of its cognate antibodies.

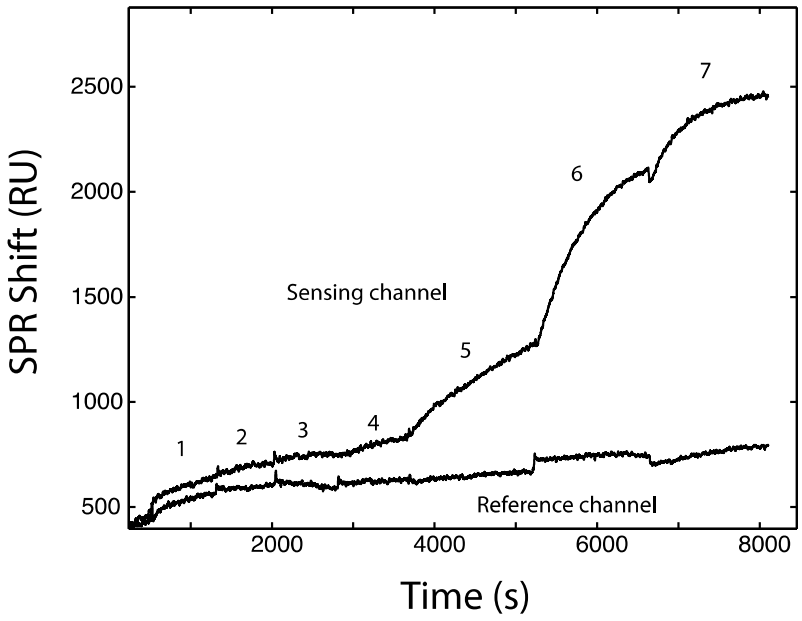


Figure 2. SPR sensorgram for the detection of SARS-CoV-2 anti-rN antibody in serum. Concentrations of anti-rN antibody: (1) 100 ng/mL, (2) 500 ng/mL, (3) 1 μg/mL, (4) 5 μg/mL, (5) 10 μg/mL, (6) 25 μg/mL, and (7) 75 μg/mL. The reference channel confirms that nonspecific adsorption is minimal.

In conclusion, we demonstrate a sensing platform suited for the rapid detection of SARS-CoV-2-associated antibodies. Antibody detection will be urgently needed to assist vaccine development and to evaluate the fraction of the population that has become immune to SARS-CoV-2. Since this detection method is generically applicable to other SARS-CoV-2 antigens, the current report provides the blueprint for development of a

series of antibody sensors for this virus and others, toward analysis of clinical samples. The portability of the SPR instrument will allow deployment of the method in the field for rapid on-site measurements.

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