Development of SARS-CoV-2 N-protein specific capture ELISA

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Introduction

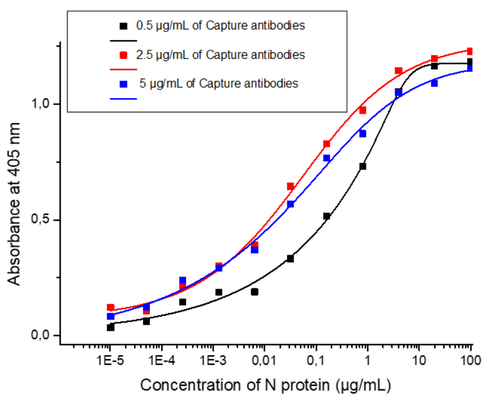
- The accurate diagnosis of people with suspected infection with the SARS-CoV-2 is essential to curb the global spread of COVID-19.
- The presence of SARS-CoV-2 can be detected by RT-PCR (it detects RNA of the virus) or by the presence of viral antigens in biological fluids in ELISA or similar techniques using antibodies developed in animals.
- The aim of the study was the establishment of a quantitative polyclonal sera-based test for routine measurement of the concentration of SARS CoV-2 nucleocapsid protein using absorbance measurement in a standard 96-well microtiter plate.

Methods

- For the purposes of the test development, recombinant N protein was produced and used for the production of mice and rabbit antisera.
- Produced antisera were purified and titer was determined.
- High-affinity polyclonal N-protein specific antisera were used for N-protein specific ELISA test development.
- The test is based on mice polyclonal sera adhered to microtiter plate bottom for the capture of the N-protein from the specimen.
- Various concentrations of the recombinant N protein were used to generate a standard curve for protein quantification.
- The N-protein bound to the mice antibodies was detected with rabbit polyclonal sera and anti-rabbit antibody coupled to an enzyme that provides spectrophotometric measurement.

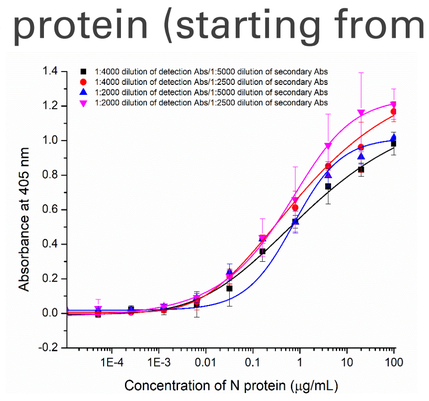
Results

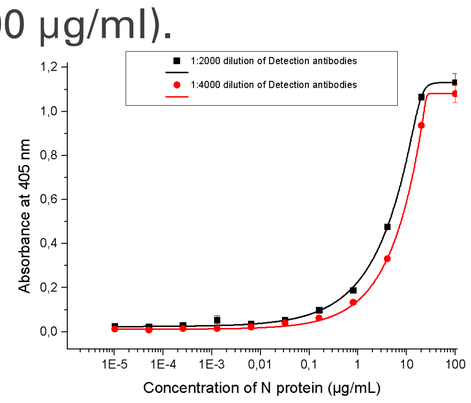
- Initially, the Capture antibodies (mouse anti-N-protein) with the high concentrations of detection (rabbit anti-N-protein) and secondary antibodies (anti-rabbit-biotin antibodies) were tested at concentrations of 0.5, 2.5 and 5 µg/ml



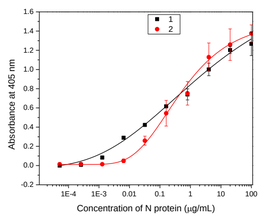
Results

- Optimization of the detection and secondary antibodies included 1:2000 and 1:4000 dilutions of detection antibodies and 1:2500 and 1:5000 dilutions of secondary in duplicates for each of eleven 5-fold serial dilutions of N- protein (starting from 100 µg/ml).





- **Optimized antibody concentrations, i.e. 2.5 µg/ml for capture antibodies, 1: 2000 dilution for detection antibodies, and 1:2500 dilution of secondary antibodies were used in in-house developed prototype ELISA.**



The average LOD value for the prototype ELISA was determined to be 9.2 ng/mL, while the average LOQ value was 10.2 ng/mL.

- Validation: the serum substitute was spiked with recombinant N-protein to achieve the final concentrations of 32, 160 and 800 ng/mL. The recovery of the N-protein in the prototype ELISA was calculated as the determined concentration in the serum substitute samples expressed as a percentage of the spiked concentration of N-protein in the samples.

Spiked concentration (ng/mL)	Average recovery (%)
32	138
160	96
800	66

Conslusion

- We have demonstrated that produced polyclonal antisera are suitable for the detection of N-protein with affinity and specificity similar to, or better than commercial antibodies.
- We have successfully developed the prototype ELISA for the quantification of N-protein with the detection limit being in the range of ng/mL.
- Furthermore, the prototype ELISA can be used with satisfactory confidence for quantification of the N-protein in protein-rich samples, similar to human sera.

Acknowledgement

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