High sensitivity COVID-19 detection using a molecularly imprinted polymer-based sensor

A.K. Thomson, R.E. Johnson, F. Canfarotta, A.J. Groves, J.D. Czulak, A. Guerreiro
(MIP Diagnostics Limited, Sharnbrook, United Kingdom)

M. Peeters, J. McClements, B. Payne
(University Of Newcastle, United Kingdom)

A. Strapazon
(University Of São Paolo, Brazil)

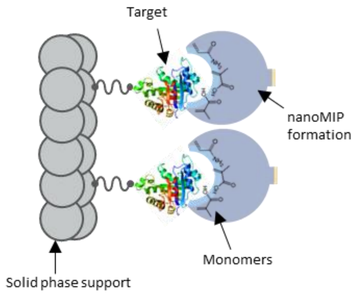
N. Bhalla
(University Of Ulster, United Kingdom)

Background

MIP Diagnostics produces nanoMIPs™ (nano-sized molecularly imprinted polymers) that are suitable for use in clinical diagnostic systems as a synthetic alternative to antibodies. They have unique properties compared to traditional binding molecules, not least the ability to be autoclaved and still retain functionality.

Methodology

nanoMIPs™ specific to the spike protein of SARS-CoV-2 (COVID-19) were synthesized using proprietary methodology, whereby a portion of the receptor binding domain (RBD) region of the spike glycoprotein molecule was immobilised on a solid phase, monomers and cross-linker were added, controlled polymerisation was initiated and, ultimately, nanoMIPs™ with high affinity for the spike protein were eluted. The affinity of the nanoMIPs™ for the spike glycoprotein was assessed using SPR (surface plasmon resonance), and selectivity was proven using dot blot.



MIP production

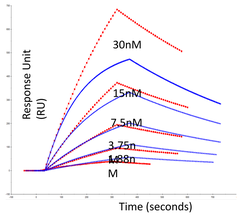
nanoMIP™ imprinting demonstrating the polymerisation step of the production process.

Subsequently the nanoMIPs™ were incorporated into a sensor platform by Marloes Peeters' Group at the University of Newcastle (UK). Screen printed electrodes were activated and nanoMIPs™ were coupled to the surface of the electrodes via EDC/NHS chemistry. The functionalised electrodes were then placed into flow cells, such that solutions of analyte could be passed over the electrodes. The flow cells were connected to a heat-transfer device, and the thermal resistance (R_{th}) was determined by dividing the temperature gradient (T_1-T_2) over the power required to keep the heat sink at 37°C.

Results

Thermal Stability by SPR (below, left)

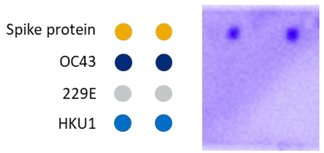
COVID-19 nanoMIPs™ have excellent stability. The SPR data below is from a lot of COVID-19 nanoMIPs™ pre- and post-autoclave (121°C, 15 minutes approx.) as analysed by SPR using the BiaCore. As evidenced, the affinity values are consistent (within experimental error) and this level of stability will lead to a very long shelf-life.



K_D pre-autoclave = 7nM

K_D post-autoclave = 3nM

NB: two chips / runs

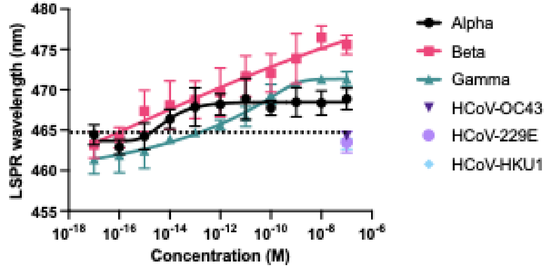


Selectivity by Dot Blot (above, right)

SARS-CoV-2 spike protein and other human coronaviruses; OC43 protein, 229E protein and HKU1 protein were spotted down and exposed to fluorescent tagged nanoMIPs™ in 2 parallel runs. The dot blot results demonstrated selectivity to SARS-CoV-2 spike protein and no cross reactivity to the other human coronaviruses.

Advanced SPR Sensor Analysis

The COVID-19 nanoMIPs™ were immobilized onto an advanced SPR sensor platform by the group of Nikhil Bhalla at University of Ulster (UK). Detection of spike protein from multiple SARS-CoV-2 variants was observed at sub-pM concentrations, and the Dot Blot selectivity above was re-confirmed.

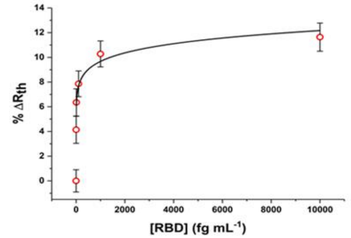


Sensitivity by Sensor Analysis

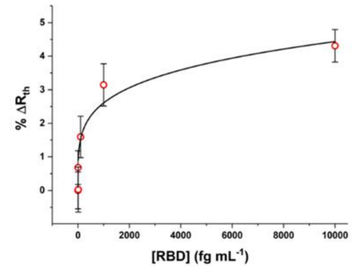
The COVID-19 nanoMIPs™ and a commercially available antibody to the COVID-19 spike protein from a leading antibody supplier were immobilized on separate electrode surfaces and integrated into a sensor platform that relies on monitoring the thermal resistance (R_{th}) at the solid-liquid interface by Marloes Peeters Group, Newcastle University.

Analytical sensitivity was tested in PBS buffer spiked with SARS-CoV-2 receptor binding domain protein. The dose response curves show an analytical sensitivity of < 5 fg/ml with the COVID-19 nanoMIP and a sensitivity of circa 105 fg/ml with the commercial antibody.

Dose response curve using COVID-19 nanoMIPs demonstrating an analytical sensitivity of <5 fg/ml



Dose response curve using COVID-19 antibodies demonstrating an analytical sensitivity of circa 105 fg/ml



Conclusions

MIP Diagnostics proprietary nanoMIPs™ specific for SARS-COV-2 (COVID-19) spike protein have been shown to have high affinity and specificity for the SARS-COV-2 spike glycoprotein with outstanding thermal stability. The MIPs have then been successfully integrated into multiple sensor systems demonstrating a limit of detection of <5 fg/ml, circa 20 times lower than the limit of detection achieved with an antibody from a leading commercial supplier. This level of sensitivity should facilitate a new wave of antigen-detecting COVID-19 rapid tests with reduced false negatives.

MIP Diagnostics

To discuss collaboration opportunities, please contact Alan Thomson at enquiries@mip-dx.com