

Genomic SPR biosensor for the detection and monitoring of coronaviruses in wild and farm animals

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During the past two decades, coronaviruses (CoVs) have caused three epidemics and pandemics with a severe impact on global public health and the global economy. CoVs are sorted into four main genera based on their phylogenetic relationship and genomic structures: Alpha-, Beta-, Delta-, and Gamma-CoV. Alphacoronavirus and Betacoronavirus, also known as PanCoV, can infect a wide range of mammals, including humans, while Gammacoronavirus and Deltacoronavirus are mostly found in avian species^[1]. In fact, the causal agent behind COVID-19, SARS-CoV-2, potentially originated from a coronavirus found in Chinese horseshoe bats, which initially crossed into an unidentified intermediate animal host.

Veterinary medicine has recorded several instances of emerging diseases resulting from coronaviruses crossing the species barrier, including the ongoing pandemic^[2]. Due to this potential transmission risk from wild, farm, and domestic animals to humans, a better understanding of the CoVs circulation is needed to prevent and predict future epidemics.

However, current analytical methods, mainly based on Polymerase Chain Reaction (PCR) processes and other centralized laboratory techniques, are costly, laborious, and time-consuming, which greatly hampers an efficient routine screening of animal populations.

We propose a genomic Surface Plasmon Resonance (SPR) biosensor^[3] for rapid detection, identification, and monitoring of CoV RNA (Alpha-, Beta-, Gamma-CoV) in samples from wild and farm animals. Our SPR biosensor is integrated in a small and portable device with a user-friendly operation, ideal for decentralized application at the point of need by non-specialized technicians.

We have designed and implemented a direct hybridization assay, using single-stranded DNA probes specific to the two viral RNA targets (panCoV and avianCoV). Sensor biofunctionalization was optimized to provide a stable probe immobilization with suitable grafting density to minimize possible steric hindrance effects. Also, hybridization conditions and parameters were studied to enhance

the detection efficacy and ensure maximum specificity and selectivity. The limits of detection (LOD) and limits of quantification (LOQ) determined for the direct assay are in the pM-nM range, proving the high sensitivity of the biosensor. Furthermore, we have evaluated different signal amplification strategies, reaching detection limits for Alpha/Beta-coronaviruses and Gamma-coronaviruses were achieved at 129 pM and 1.49 nM, respectively. Finally, it is important to mention that the biosensor assay can be completed in less than 20 min sample-to-result turnaround time, and using a minimum volume of sample (100 – 150 μ L), which is especially relevant for the screening of small animals.

Our genomic SPR biosensor introduces a rapid technique for identification of zoonotic coronavirus in different types of wild and farm animals, offering the opportunity to perform routine and cost-effective screening of the populations. The implementation of this type of biosensors in veterinary research and practice could aid in the control of viral infection transmission and in the prevention of future outbreaks.

References

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Figures

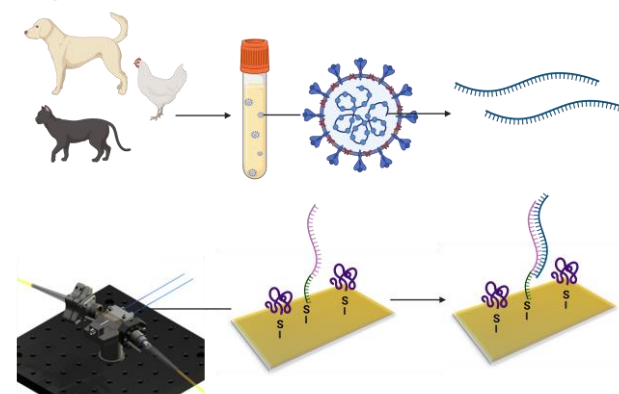


Figure 1. Biosensor for viral RNA detection in animal serum samples.