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INgezim COVID 19 S VET

A multi-species ELISA for detection of specific antibodies against S protein of the SARS-CoV-2 VIRUS

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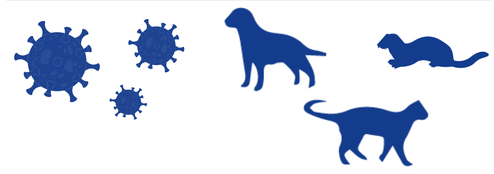
INTRODUCTION

Coronavirus disease (COVID-19) is a zoonotic disease caused by the SARS-CoV-2 coronavirus. It was declared a public health emergency outbreak by WHO in January 2020. Since then, cases of natural infection have been reported in companion (cats, dogs and hamsters), production (mink), wildlife (white-tailed deer) and zoo (monkeys, gorillas, tigers and lions) animals, showing different degree of susceptibility to infection and a wide range of clinical signs, from none to very severe signs.

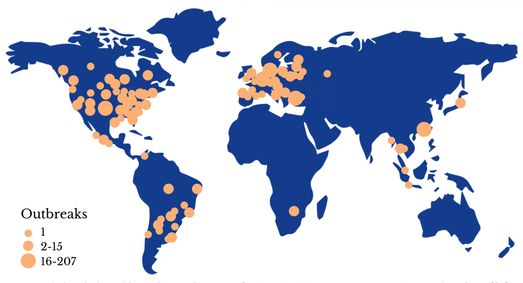
To date, some cases of human-to-animal, animal-to-animal and animal-to-human transmission have been reported. Although animals do not seem to play an important role in the spread of the virus among humans, all these facts have led the OIE to promote studies on the prevalence of infection in animals, and the European Food Safety Authority (EFSA) to issue instructions for surveillance of mink farms.

AIM

The development of a commercial kit able to detect specific IgG to SARS-CoV-2 in multi-species assay.



COVID 19 incidence in animals



Worldwide distribution of SARS-CoV-2 outbreaks in fifteen animal species reported to the OIE (as of 28 February 2022).

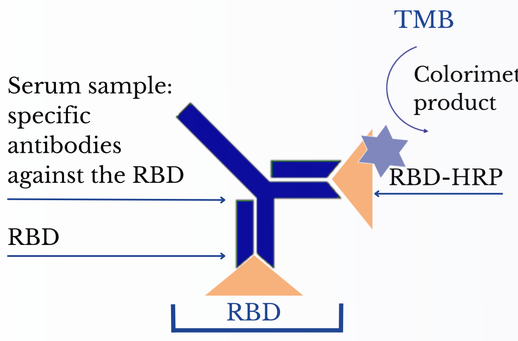
Region \ Species	Cat	Dog	Mink	Otter	Pet ferret	Lion	Tiger	Puma	Snow leopard	Gorilla	White-tailed deer	Fishing cat	Binturong	Coatimundi	Spotted hyena	Eurasian lynx	Canada lynx	Hippo	Hamster
Africa						Yes		Yes											
Americas	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		Yes		
Asia	Yes	Yes				Yes	Yes												Yes
Europe	Yes	Yes	Yes		Yes	Yes	Yes									Yes		Yes	

Number of outbreaks (n=663) reported worldwide, by species and region (as of 28 February 2022).

MATERIALS AND METHODS

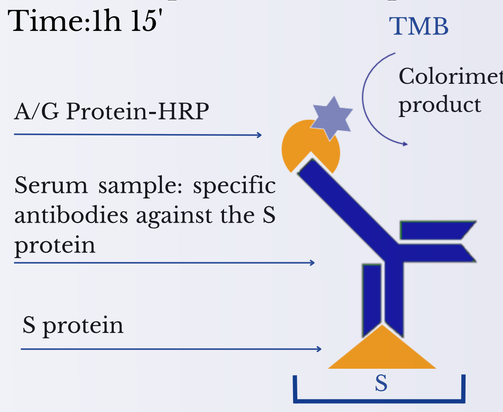
Double recognition enzymatic immunoassay (DR): MICROARRAY and ELISA

Detection of N and RBD specific Ig simultaneously. For mink sera.
Time: 1h 15'



Indirect enzymatic immunoassay: ELISA

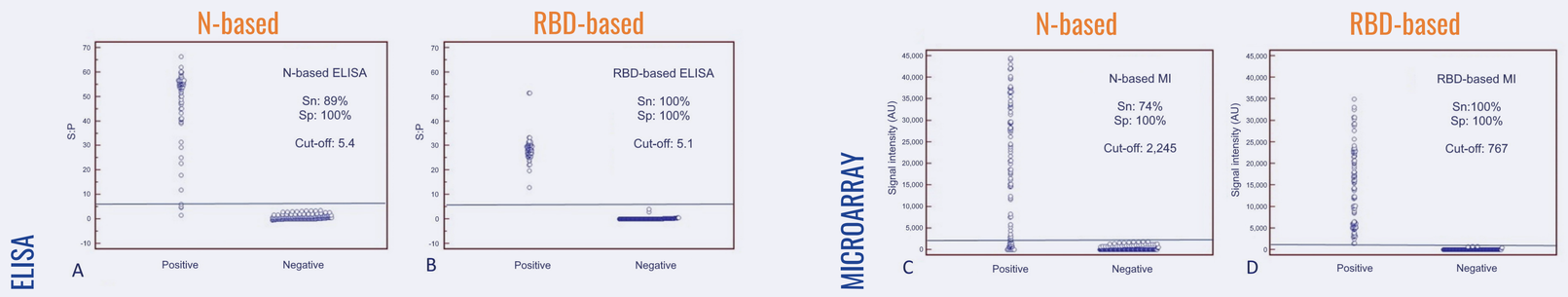
Based on S protein. Multi-species assay.
Time:1h 15'



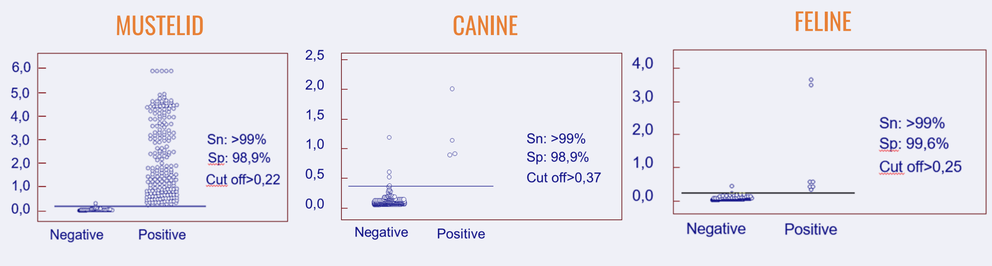
ASSAYS AND RESULTS

Comparison between N and RBD as antigenic markers in DR format in ELISA and MICROARRAY

In this work, we have evaluated the performance of the nucleoprotein (N) and the receptor binding domain (RBD) of the S protein of SARS-CoV-2, as antigenic markers for veterinary serological diagnosis, using mink sera as proof of concept. To achieve this goal, we developed two ELISAs and a duplex immunoassay microarray all in double recognition (DR) format, to detect N-specific and RBD-specific antibodies in serum. Both RBD-based ELISA and MI had a sensitivity and specificity of 100%, discriminating accurately between farmed mink exposed (n=101) and unexposed (n=163) to SARS-CoV-2. In contrast, we found a worse performance of N in DR-ELISA, not only for mink, but also, for feline and canine sera.



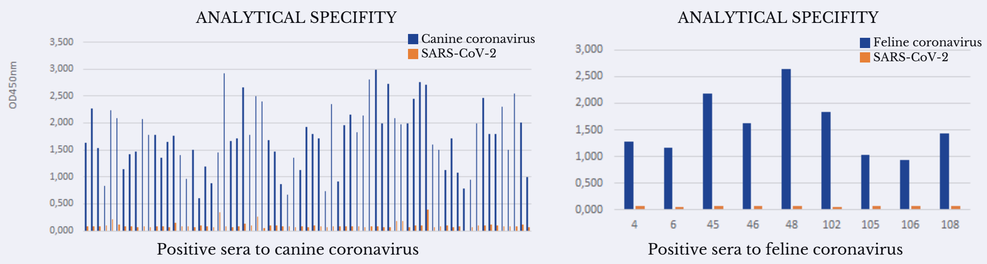
Indirect ELISA based on S protein shows high sensitivity and specifity



Based on results shown above, we developed and commercialized INgezim COVID 19 S VET, a multispecie indirect ELISA kit for detection of S-specific antibodies in plasma and serum samples. Our test showed a sensitivity and specificity higher than 98.1% in mustelid (n=454), feline (n=234) and canine (n=362) sera.

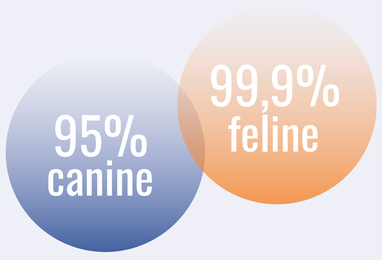
No cross-reactivity with canine or feline coronavirus

In addition, our test shows no cross-reactivity with dog and cat sera positive for antibodies to canine coronavirus and feline coronavirus, respectively.



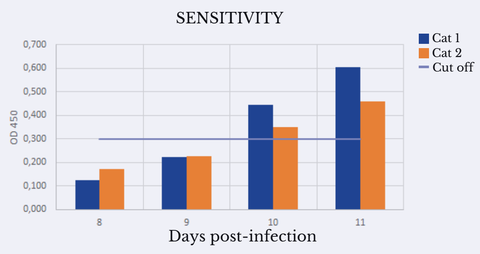
Optimal concordance with seroneutralization assays

INgezim COVID 19 S VET also showed an optimal concordance with seroneutralization assays for 21 canine (95% concordance) and 17 feline (99.9%) samples.



Sensitivity: detection day 10 post-infection

INgezim COVID 19 S VET was able to detect specific antibodies in two sera of experimentally infected cats from day 10 post-infection.



CONCLUSIONS

S protein and RBD have a better performance than N protein as antigenic markers in ELISA and microarray immunoassays for Ig detection specific to SARS-CoV-2 in serum samples.

Our commercial kit COVID 19 S VET based on S protein, shows a sensitivity and specifity higher than 98%.

COVID 19 S VET is an useful tool for serosurveillance of pets and farmed mink .

ACKNOWLEDGEMENT

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