

# Development of a novel competitive ELISA to investigate exposure of animals to SARS-CoV-2

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## Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the COVID19-causing virus, is a zoonotic pathogen. There is concern that it may spillover into wildlife species which may then serve as reservoirs for future infection of humans, domestic animals, or other wildlife species. Furthermore, impacts of the virus on potentially susceptible wildlife species are currently unknown. There is, therefore, an urgent need to develop a single test that could be used for the serosurveillance of multiple wildlife species for exposure to SARS-CoV-2. While serological tests to detect antibodies in SARS-CoV-2 infected and/or exposed human patients have been developed, few assays have the capacity to detect antibodies in a wide variety of species. Here, we describe the development of a competitive enzyme-linked immunosorbent assay (cELISA) to detect SARS-CoV-2 antibodies in animals for which species-specific reagents are not available. This cELISA was developed to detect SARS-CoV-2 spike 1 (S1), spike 2 (S2) and nucleocapsid (N) specific antibodies and was validated using sera from experimentally infected hamsters. We further validated our cELISA by comparing it with results obtained from the surrogate virus neutralization test (cPASS, GenScript) and indirect ELISA using anti-hamster horse radish peroxidase (HRP) conjugated reagents. This cELISA will have broad applications in screening potential animal reservoirs for SARS-CoV-2, and uses multiple targets, including more conserved structural proteins which are subjected to less selective immunological pressure. These would allow detection of exposure to variants missed by conventional assays that target antibodies against the viral receptor binding domain. This assay will be a valuable tool which can be implemented in surveillance programs investigating evidence of exposure to SARS-CoV-2 in multiple domestic, captive, or wild animal species, and in studies investigating impacts of SARS-CoV-2 on wildlife populations.

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