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**Monoclonal Antibodies based ELISA for detection of SARS CoV-2 antigen and antibodies in multiple hosts**

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**Abstract:**

**Background/Objective:** The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the coronavirus disease 2019 (COVID-19) lead to a global health crisis of deep concern. The nucleocapsid protein (NP) of SARS-CoV-2 being conserved, immunogenic, and abundant, proves to be a good marker for viral infection in the infected human/animal, while correlating well with the acute phase of COVID-19. In this study, monoclonal antibodies were developed against the NP to develop assays for detection of antigen in the infected cell culture and antibody in convalescent sera of animals/animals.

**Methods:** In this study, we expressed the SARS-CoV-2 NP in insect cells using baculovirus expression system and purified it using nickel affinity chromatography. The recombinant NP was validated by exhibition of its reactivity with convalescent lion sera in western blot and was utilized to generate a panel of 16 murine mAbs. A select antibody was characterized by isotyping, indirect ELISA, western blot and immunoperoxidase monolayer assay (IPMA) in delta (B.1.617.2) and Omicron BA.1 (B.1.1.529.1) SARS-CoV-2 infected cells. The antibody was thus evaluated for its diagnostic utility in sandwich ELISA for virus detection in infected cell culture supernatant as well as in a blocking ELISA to detect antibodies from convalescent sera from multiple species.

**Results:** Out of the 16 mAbs produced, the mAb 7H9 of IgG1 isotype showed strong reactivity towards the recombinant NP in western blot as well as indirect ELISA. The mAb also showed cross-variant reactivity with virus in IPMA. The cross-variant reactivity was reciprocated in sandwich ELISA with the assay being able to detect 104 pfu/ml of virus in cell culture supernatant. However, the sensitivity of the assay was lower for the omicron BA.1 variant when compared to delta variant of virus. The blocking ELISA was used to screen dog, cat and lion sera samples, out of which 19/427, 0/6 and 10/18 sera samples, respectively tested positive for anti-NP antibody.

**Conclusion:** The sandwich ELISA has potential application in the antigen detection in the virus harvests as this offers cheaper and quicker alternative to virus detection. The blocking ELISA for antibody detection can be applied for detection of infection antibodies in the convalescent subjects and for sero-surveillance studies for detection of antibodies against NP of SARS-CoV-2 in animals or human population.

**Keywords:** SARS-CoV-2, nucleocapsid, sandwich ELISA, monoclonal antibodies, blocking ELISA

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| 1 | Date/Location/Venue of the symposium/seminar/conference etc.  to be organized | ISAH & SEAOHUN International Conference 2024  One Health in Action – Innovations in Health, Welfare and Environment for a Sustainable Animal Production  16-20 September 2024 at Thailand |
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