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Development and evaluation of an indirect ELISA for detecting sialodacryoadenitis virus antibodies in laboratory rats

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Abstract
Sialodacryoadenitis virus (SDAV), a coronavirus that commonly infects laboratory rats, causes respiratory illness and inflammation of the salivary and lacrimal glands. This study focuses on the development and evaluation of an indirect enzyme-linked immunosorbent assay (ELISA) for detecting antibodies against SDAV. The ELISA demonstrated promising results for rapid and reliable diagnosis of SDAV infection. The assay was applied to a panel of positive and negative serum against SDAV and has demonstrated a 100% positive and negative predictive value. The clear distinction between negative and positive samples suggests high sensitivity and specificity of the assay. This indirect ELISA shows potential as a valuable tool for SDAV diagnosis in laboratory settings, offering rapid and reliable results that could significantly enhance disease monitoring and control strategies in animal facilities. In conclusion, the developed indirect ELISA for SDAV antibody detection demonstrates promising performance and could contribute to improved animal welfare and research quality in laboratory settings.

Keywords: Sialodacryoadenitis virus (SDAV), coronavirus infection in rats, indirect ELISA for SDAV detection

1. Introduction
Sialodacryoadenitis virus (SDAV) is a coronavirus that commonly infects laboratory rats, causing respiratory illness and inflammation of the salivary and lacrimal glands ((Bartak *et al*., 2021; Yoo *et al*., 2000) [1, 10]. SDAV infection leads to a range of clinical manifestations, including lesions in the nasal cavity, respiratory epithelium, and olfactory epithelium (Bihun & Percy, 1995) [3]. The virus can replicate in various cell types, including Clara cells, ciliated cells in the bronchial airway, and alveolar type I and II cells in the lung parenchyma (Funk *et al*., 2009) [5]. Interestingly, SDAV can also infect mice, causing interstitial pneumonia and antibody responses, despite the animals remaining asymptomatic (Bhatt *et al*., 1977) [2]. This cross-species infection potential highlights the importance of understanding SDAV's characteristics and zoonotic potential, especially given the ongoing pandemic and the role of the Coronaviridae family in human zoonoses (Bartak *et al*., 2021) [1]. The study of SDAV has been challenging due to the lack of a widely available continuous cell line for virus propagation. However, recent advancements have shown that SDAV can be propagated in LBC and L-2 cell lines, facilitating further research and comparison with other murine coronaviruses (D. H. Percy *et al*., 1991) [6]. Understanding SDAV infection in laboratory rats is crucial for maintaining the health of research animals and preventing potential confounding effects in experimental studies (Kemp *et al*., 1991; Yu *et al*., 2011) [6, 12].
Enzyme-linked immunosorbent assay (ELISA) has revolutionized the detection of various pathogens, including viruses, bacteria, and parasites (Ellens, 1981; Richardson & Warnock, 1983) [4, 8]. This versatile technique can be adapted for the detection of sialodacryoadenitis virus (SDAV) infection, a coronavirus that affects rodents. ELISA offers several advantages over traditional diagnostic methods, including high sensitivity, specificity, and the ability to process multiple samples simultaneously (Suzuki *et al*., 1982; Young *et al*., 2021) [9, 11]. In this study we have developed an indirect ELISA for the detection of antibodies against SDAV.

This assay would enable rapid and reliable diagnosis of SDAV infection, facilitating better management and control of the disease in laboratory animal facilities.

2. Materials and Methods

2.1 Cells

Rat lung epithelial cell line (L2P) cells were cultured at 37 ℃ in high-glucose Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin.

2.2 Virus culture and antigen preparation

The SDAV culture was a kind gift from Dr. Nagendra Hegde, National Institute of Biotechnology, Hyderabad. SDAV was propogated on L2P cells. Experiments were performed at a Biosafety Level-2 according to the institutional guidelines. For antigen preparation, the infected cell supernatant was centrifuged at 20000 rpm on a 15-45% sucrose density gradient and the pure antigen interface ring was collected and stored at -80 ℃ until use

2.3 Serum

Rat serum samples used in this study were collected from Laboratory animal medicine, Tamil Nadu veterinary and animal sciences university, Chennai and Central animal facility, Indian institute of science, Bengaluru. The serum panel comprised of 10 known positive serum and 20 negative serum. The serum samples were inactivated for 30 min in a heat block at 56 ℃ and stored at − 20 ℃ until use.

2.4 ELISA

ELISA plates (Nunc, USA) were coated with SDAV antigen (200ng per well) in carbonate bicarbonate buffer (pH 9.6) and incubated overnight at 4 ℃, and blocked with 0.1% Bovine serum albumin. After washing three times with PBS containing 0.05% Tween20, serum samples were added at a dilution of 1:200. The antibodies against SDAV present in the serum was detected using goat anti-rat conjugated to HRP (1:1000; Sigma), with TMB as the substrate followed by addition of. The optical density was measured at 450nm (Fig 1).

3. Results and Discussion

The format of the ELISA is shown in Fig 1. Following a checkerboard titration the best coating concentration of to be 0.20 µg/well. The best serum dilution concentration was arrived at 1: 100, and the determination results of 20 negative sera are shown in Table1.

The optical density values of the negative sample ranged between 0.123 - 0.289. The mean optical density value of the negative samples was 0.209. The cut off value was calculated by the formula ODNeg + 3*SD and was arrived as 0.348. The assay was then used on twenty number of serum samples positive for RCV (Table 1). The assay was able to detect all the positive samples as strongly positive. The mean optical density value of the positive samples was 2.906 (Range 1.868 - 3.267).

This study successfully developed an indirect ELISA for detecting antibodies against Sialodacryoadenitis virus (SDAV) in laboratory rats. The assay demonstrates promising results for rapid and reliable diagnosis of SDAV infection, which could significantly improve disease management and control in laboratory animal facilities. When applied to 20 serum samples positive for Rat Coronavirus (RCV), the assay successfully identified all samples as strongly positive. The OD values for these samples were substantially higher than the defined cut-off, with a mean of 2.906 (Range 1.868 - 3.267). This clear distinction between negative and positive samples suggests high sensitivity and specificity of the assay. These results indicate that our indirect ELISA method is effective in differentiating between SDAV-positive and SDAV-negative samples. The wide gap between the cut-off value (0.348) and the lowest positive sample OD (1.868) further supports the robustness of the assay.

In conclusion, this indirect ELISA shows promise as a valuable tool for SDAV diagnosis in laboratory settings. Its ability to provide rapid and reliable results could significantly enhance disease monitoring and control strategies in animal facilities, ultimately improving animal welfare and research quality.

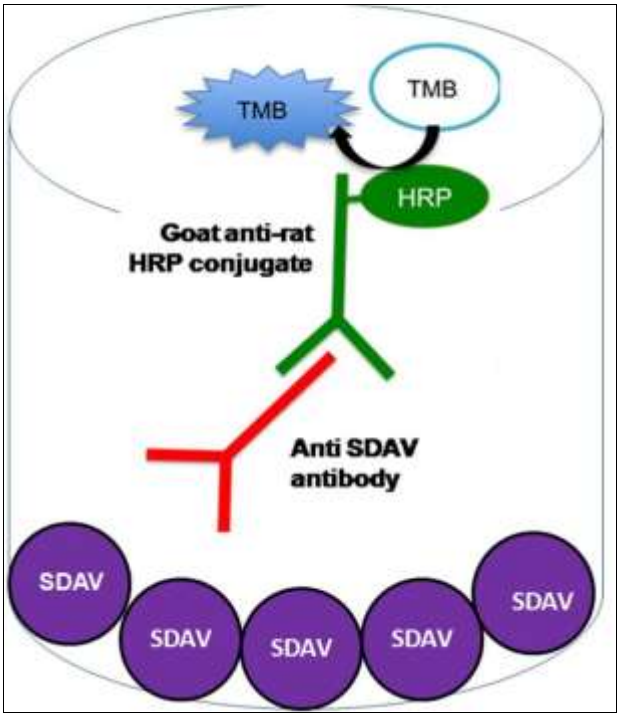

Fig 1: Graphical representation of the indirect ELISA for SDAV antibodies in rat serum

Table 1: Optical density values for the rat serum tested using the indirect ELISA for SDAV antibodies

Negative serum panel				Positive serum panel	
Sample ID	**OD value**	**Sample ID**	**OD value**	**Sample ID**	**OD value**
RCV/IISC/N-1	0.231	RCV/LAM/N-1	0.232	RCV/IISC/P-1	3.185
RCV/IISC/N-2	0.268	RCV/LAM/N-2	0.265	RCV/IISC/P-2	2.971
RCV/IISC/N-3	0.273	RCV/LAM/N-3	0.198	RCV/IISC/P-3	3.267
RCV/IISC/N-4	0.189	RCV/LAM/N-4	0.152	RCV/IISC/P-4	3.007
RCV/IISC/N-5	0.250	RCV/LAM/N-5	0.149	RCV/IISC/P-5	3.057
RCV/IISC/N-6	0.246	RCV/LAM/N-6	0.193	RCV/IISC/P-6	3.038
RCV/IISC/N-7	0.261	RCV/LAM/N-7	0.215	RCV/IISC/P-7	2.381
RCV/IISC/N-8	0.223	RCV/LAM/N-8	0.263	RCV/IISC/P-8	2.726
RCV/IISC/N-9	0.271	RCV/LAM/N-9	0.186	RCV/IISC/P-9	2.105
RCV/IISC/N-10	0.245	RCV/LAM/N-10	0.147	RCV/IISC/P-10	3.184
RCV/IISC/N-11	0.230	RCV/LAM/N-11	0.189	RCV/IISC/P-11	3.049
RCV/IISC/N-12	0.222	RCV/LAM/N-12	0.173	RCV/IISC/P-12	2.867
RCV/IISC/N-13	0.188	RCV/LAM/N-13	0.139	RCV/IISC/P-13	3.219
RCV/IISC/N-14	0.164	RCV/LAM/N-14	0.249	RCV/IISC/P-14	1.998
RCV/IISC/N-15	0.140	RCV/LAM/N-15	0.196	RCV/IISC/P-15	2.365
RCV/IISC/N-16	0.289			RCV/IISC/P-16	2.846
RCV/IISC/N-17	0.233			RCV/IISC/P-17	2.564
RCV/IISC/N-18	0.149			RCV/IISC/P-18	2.173
RCV/IISC/N-19	0.177			RCV/IISC/P-19	1.868
RCV/IISC/N-20	0.123			RCV/IISC/P-20	2.338

Conflict of Interest
Not available

Financial Support
Not available

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