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Competitive enzyme-linked immunosorbent assay for investigating SpL binding to mammalian and avian immunoglobulins

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ABSTRACT

This ELISA was based on the theory that antibodies present in different samples would compete with human IgG for binding to SpL, resulting in inhibition of human IgG-SpL interactions [1].

Reference:

1. Justiz-Vaillant AA, Akpaka PE, McFarlane-Anderson N, Smikle MF. Comparison of techniques of detecting immunoglobulin-binding protein reactivity to immunoglobulin produced by different avian and mammalian species. *West Indian Med J.* 2013;62(1):12-20.

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1 This ELISA is based on the theory that antibodies present in different samples would compete with human IgG for binding to SpL, resulting in inhibition of human IgG-SpL interactions.

2 The samples tested are commercially prepared pooled sera from skunk, coyote, raccoon, duck, and also commercially prepared purified immunoglobulins from cat and chicken (SigmaAldrich Co, St Louis, Missouri).

3 The microplate is coated with 50 µl of commercial human IgG (1 µg/well overnight at 4°C).

4 Serial doubling dilutions (1:4 to 1:1024) of 30 µl of each sample are made in a separate microplate to which 30 µl of the conjugate SpL-HRP diluted 1:1000 in non-fat milk is added.

5 The microplate is incubated for one hour at RT and then 50 µl of each sample is transferred to the human IgG coated microplate and incubated for one hour.

6 The microplate is then washed four times with PBS-Tween 20 buffer (SigmaAldrich Co, St Louis, Missouri), and 50 µl of the substrate OPD (3 mg/ml) is added to each well and incubated at RT for 15 minutes.

7 The reaction is stopped with 3M H_2SO_4 and the microplate is visually assessed and read at 492 nm.

8 The percentage of the binding inhibition (I%) of the SpL-human IgG interactions by different samples was calculated.

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