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Protein-A and Protein-LG Sandwich ELISA

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ABSTRACT

This ELISA was used to study the interactions between Staphylococcal protein-A (SpA) and protein-LG (SpLG) with different immunoglobulin preparations from mammalian and avian species.

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We use this protocol and it's working

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- 1 This ELISA was used to study the interactions between Staphylococcal protein-A (SpA) and protein-LG (SpLG) with different immunoglobulin preparations from mammalian and avian species. The 96 well microtiter plate was coated overnight at 4°C with 2 µg/µl per well of SpA in carbonate-bicarbonate buffer pH 9.6.

- 2** The plate was then treated with bovine serum albumin solution and washed 4X with PBS-Tween. 50 µl of immunoglobulins (1 mg/ml) is added and incubated for 1h at room temperature, and the microplate is rewashed 4X with PBS-Tween.
- 3** Then, 50 µL of peroxidase-labeled SpLG conjugate diluted 1:5000 in PBS-non-fat milk was added to each well and incubated for 1h at RT. The plate was washed 4X with PBS-Tween. Then, 50 µL of o-phenylenediamine solution (4 mg/mL) was added, and the plate was incubated for 15 min at RT in the dark.
- 4** The reaction was stopped with 50 µL of a 3M H₂SO₄ solution. The plate was visually assessed for color development and read on a microplate reader at 492 nm. A cut-off point was calculated as the mean of the optical density of the negative controls multiplied by two. The cut-off point was set to 0.32.