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Protein A and Protein AG Sandwich ELISA

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Carbon

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ABSTRACT

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

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- 1 This ELISA was used to study the interactions between Staphylococcal protein-A (SpA) and protein-AG (SpAG) 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 SpAG 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.
- 4 Then, 50 µL of 4 mg/mL o-phenylenediamine solution was added, and the plate was incubated for 15 min at RT in the dark. The reaction was stopped with 50 µL of a 3M H2SO4 solution.

- 5 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 value was 0.34.