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# Enzyme linked immunosorbent assay for investigating the immunoglobulin-binding bacterial protein (IBBP) to avian egg yolk antibodies.

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- 1 This ELISA is used to study the interaction of proteins A, L and LA with different avian IgY preparations.
- 2 The 96 well microtitre plate is coated overnight at 4°C with 1 µl/mg per well of unlabelled SpL, SpA or SpLA in carbonate-bicarbonate buffer pH 9.6.
- 3 Then plate is treated with bovine serum albumin solution and washed 4X with PBS-Tween.
- 4 50 µl of different IgY preparations (1 mg/ml) are added and incubated for 1 h at room temperature and washed.

- 5 Then 50 µl of rabbit anti-chicken IgG-HRP conjugate diluted 1:15000 in PBS-non-fat milk is added to each well and incubated for 1h at RT. The plate is washed 4X with PBS-Tween.
- 6 50 µl of 4 mg/ml o-phenylenediamine solution (OPD) is added and the plates are incubated 15 minutes at RT.
- 7 The reaction is stopped with 50 µl of 3M H<sub>2</sub>SO<sub>4</sub> solution.
- 8 The plate is visually assessed for the development of colour.
- 9 The plate is read in a microplate reader at 492 nm.
- 10 A cut-off point is calculated.