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Immunoblot analyses for investigating SpLA binding to purified mammalian and avian immunoglobulins.

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- 1 Aliquots of 2 µg/µl purified immunoglobulins from birds, laboratory, wild, farm animals and pets are applied to the gels of SDS-PAGE as described elsewhere.
- 2 Gels are transferred to nitrocellulose membranes (Immobilon-Nc, pore size 0.45 µm, Sigma-Aldrich Co, St Louis, Missouri) during 75 minutes at 40 mAmps using a semi-dry electroblotter, HEP-1 Model, Owl Scientific Inc.
- 3 The running buffer contains 25 mM Tris, 192 mM glycine pH 8.3 and 20% methanol.
- 4 The nitrocellulose membranes are blocked overnight in 10% non-fat skim milk in PBS with 0.05% Tween-20 pH 7.4 and then washed 4x, 10 minutes with PBS-Tween 20.
- 5 Recombinant protein LA (Sigma-Aldrich Co, St Louis, Missouri) at a concentration of 5 µg/ml is added to the membranes.

- 6 After that there is an incubation period at 4°C overnight.
- 7 The nitrocellulose membranes were washed as above.
- 8 A secondary antibody (rabbit anti-chicken IgY horseradish peroxidase, Sigma Aldrich) is added at a 1:15 000 dilution.
- 9 It is incubated for one hour at room temperature and washed as above.
- 10 Tetramethyl-benzidine (TMB) solution is added to the nitrocellulose membranes, which are then incubated in the dark for a few minutes. Then, the membranes are shaken gently and rinsed thoroughly in de-ionized water to stop the blotting process and are left to dry.
- 11 Alternatively, Ig samples are transferred to nitrocellulose membranes and directly probed using SpLA-HRP (diluted 1:5000) and TMB (this system was mainly used for detecting avian Igs).