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Binding properties of immunoglobulin-binding protein by double immunodiffusion (Ouchterlony) technique.

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We use this protocol and it's working

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- 1 The binding of SpLA, SpL and SpG with animal sera, avian IgY, avian egg whites and purified IgG are investigated by double immunodiffusion.

- 2 Briefly, 1% agarose gels are prepared and wells cut into the gel using a template.
- 3 Initially, aliquots of 25 μ l each of SpLA, SpL or SpG at 1 μ g/ μ l were applied to the centre well.
- 4 The peripheral wells are filled with 25 μ l each of IgY (30 μ g/ μ l), avian egg white diluted 1:2 in PBS pH 7.4, or animal serum.
- 5 The gels are incubated at RT for 48–72 hours.
- 6 After that the gels are examined for precipitin lines.
- 7 Human serum and human IgG are included as positive controls.
- 8 The positive results are taken as the presence of precipitin line/s and negative results, the absence of precipitin lines.
- 9 The experiments were repeated using concentrations of each bacterial Ig receptor and animal serum or purified immunoglobulin ranging from 1–51 μ g/ μ l.