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

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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 µl each of SpLA, SpL or SpG at 1 µg/µl were applied to the centre well.

4 The peripheral wells are filled with 25 µl each of IgY (30 µg/µl), 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 µg/µl.

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