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Binding of avian immunoglobulins to peroxidase-labeled anti-chicken IgY conjugate by double immunodiffusion (Ouchterlony) technique.

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We use this protocol and it's working

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Abstract

In this experiment anti-chicken IgY developed in rabbit cross-reacts with a large panel of avian species including ostrich, duck, pigeon, and quail.

Materials

MATERIALS

 Anti-Chicken IgY, HRP Conjugate, 300ul **Promega Catalog #G1351**

- 1 The binding of avian immunoglobulins to a secondary antibody rabbit anti-chicken IgY antibody was investigated by the Ouchterlony technique.
- 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 rabbit anti-IgY antibody at 1 μ g/ μ l are applied to the centre well.
- 4 The peripheral wells are filled with 25 μ l each of IgY (30 μ g/ μ l) of various avian immunoglobulins Y (IgY) including duck, pigeon, chicken, bantam hen, ostrich, pheasant, guinea hen, goose, quail, cattle egret and other species. This IgY were isolated from the egg by the method of Polson, 1990.
- 5 The gels are incubated at RT for 48–72 hours on a humidity chamber.
- 6 After that the gels are examined for precipitin lines.
- 7 Chicken IgY is included as a positive controls.
- 8 The positive results are taken as the presence of precipitin line/s and negative results, the absence of precipitin lines.