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## Detection of anti- SpA antibodies in egg white tested by double immunodiffusion (Ouchterlony) technique.

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

Keyhole limpet hemocyanin (KLH) is a copper-containing protein comprising of subunits with MW of 400 kDa. This protein is found in the hemolymph of the sea mollusk *Megathura crenulata*. It has the ability to enhance the host's immune response by interacting with monocytes, T cells and macrophages. KLH has been used primarily as a carrier for vaccines and antigens [1]. It was found that chicken immunized with KLH bound peptide raised an anti-KLH immunoresponse [2]. This can be tested by a single method such as the Ouchterlony technique.

## Reference

1. Aarntzen EH, de Vries IJ, Göertz JH, et al. Humoral anti-KLH responses in cancer patients treated with dendritic cell-based immunotherapy are dictated by different vaccination parameters. *Cancer Immunol Immunother*. 2012;61(11):2003-2011. doi:10.1007/s00262-012-1263-z
2. Justiz Vaillant AA, Anderson MF, Smikle M, Wisdom B, Mohammed W, et al. (2013) Development of Anti HIV Gp120 and HIV Gp41 Peptide Vaccines. *J Vaccines Vaccin* 4: 206. doi: 10.4172/2157-7560.1000206

## Materials

### MATERIALS

 10mg KLH (Keyhole Limpet Hemocyanin) (Immunological Grade) **G-Biosciences Catalog #786-088**

- 1 Detection of anti-SpA antibody in the egg white of eggs from chicken vaccinated with SpA is carried out.
- 2 Briefly, 1% agarose gels are prepared and wells cut into the gel using a template.
- 3 Initially, aliquots of 25  $\mu$ l each of protein-A (SpA) in concentration of 1 mg/ml are applied to the centre well.
- 4 The peripheral wells are filled with 25  $\mu$ l of 1:4 dilutions of egg whites from eggs of chicken immunized with SpA.
- 5 The gels are incubated at RT for 48–72 hours.
- 6 After that the gels are examined for precipitin lines.
- 7 An anti-SpA antibody developed in rat is included as positive control and turtle serum as a negative control.
- 8 The positive results are taken as the presence of precipitin line/s and negative results, the absence of precipitin lines.