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

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1 Works for me [dx.doi.org/10.17504/protocols.io.bjsrknd6](https://dx.doi.org/10.17504/protocols.io.bjsrknd6)

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## ABSTRACT

Keyhole limpet hemocyanin (KLH) is a cooper-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

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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

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## MATERIALS

NAME	CATALOG #	VENDOR
10mg KLH (Keyhole Limpet Hemocyanin) (Immunological Grade)	786-088	G-Biosciences

- 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 µl 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 µl 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.