

## IVD.II - Usefulness of IgY technology for hepatitis B virus diagnosis

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Introdução:

Hepatitis B virus (HBV) infection has worldwide distribution and causes 887,000 deaths per year, mainly due to complications (including cirrhosis and hepatocellular carcinoma). Diagnosis assays employ mammalian immunoglobulin G (IgG) that present high costs due to low quantity of protein and sophisticated procedure. Chicken IgY antibody, the major serum immunoglobulin in birds, has functions like mammalian IgG and present some advantages since it is a stable immunoglobulin that can be simply obtained from egg yolk with high quantity with no necessity to bleed the laboratory animal. IgY technology is convenient and cost-effective that could be useful for hepatitis B diagnosis.

**Objective:** The main objective of this study is to immunize chickens with hepatitis B vaccine in order to produce highly effective and pure antibodies (IgY), as well as extract, characterize, quantify, and evaluate the usefulness of these antibodies to develop an enzyme-linked immunosorbent assay (ELISA) for diagnosis of hepatitis B.

**Methodology:** Three groups of birds were immunized with hepatitis B vaccine with and without adjuvant CPG-ODN and a negative control group were also included. Eggs were collected from 21 weeks and IgY was extracted using polyethylene glycol precipitation and purified using affinity chromatography. IgY anti-HBs characterization was performed using polyacrylamide gel electrophoresis (SDS-PAGE), dot blot and enzyme-linked immunosorbent assay (ELISA). Total protein concentrations were measured using spectrophotometer. Samples presenting higher total protein concentration were evaluated as capture protein for in house ELISA to detect HBsAg.

**Results:** The characterization of IgY revealed bands of stained peptides with molecular weight between 75 and 50kDa and 37 and 25kDa using SDS Page. In the dot blot test was observed that there was antigen-antibody reaction throughout the sample period. Mean  $\pm$  standard deviation (SD) of concentration of total protein obtained after purification by affinity chromatography was 0.386 mg / mL  $\pm$  0.440 mg/mL.

After evaluation of different dilutions of IgY as capture protein for ELISA, it was possible to distinguish positive from negative controls that presented mean  $\pm$ SD of optical density (OD) values of 1.99  $\pm$  0.18 and 0.45  $\pm$  0.06, respectively.

**Conclusion:** The results indicated that HBV is able to generate a high production of specific immunoglobulins in chickens and these proteins could be used as capture protein for ELISA. Further studies will be conducting to develop in house ELISA for hepatitis B diagnosis.

**Keywords:** hepatitis B; diagnosis; IgY