ELISA is a cheap, fast and simple method for the detection of food allergens. Use of chicken antibodies in the analysis of food allergens and specifically the effect of the immunisation frequency on antibody generation is not well documented. This study aimed at comparing the activity of the immunoglobulins generated using a three-week and a six-week immunisation protocol. Isa brown chicken were immunised with 500 mg of roasted peanut protein extract using varying booster injection intervals. After induction, antibody production was sustained during the immunisation period in the six-week protocol with a marked drop in the three-week regime. The activity of antibodies was higher in the six-week protocol than the three-week protocol. An assay was optimised in the detection range of 1 ppm. Upon heating, the peanut protein binding by IgYs decreased with subsequent fluctuations. This was associated with denaturation of proteins and unmasking of new epitopes and stable fractions persisting.