

AN ELISA FOR THE DETECTION OF ANTIBODIES AGAINST NEWCASTLE DISEASE VIRUS IN AFRICAN VILLAGE POULTRY

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Abstract

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A standardised and robust ELISA was developed for detecting antibodies against Newcastle disease in poultry. Results from 28 field samples were compared with the results from a haemagglutination test. It will be necessary to validate the ELISA in the field by assessing the results of serum samples collected from village chickens following vaccination or field infection.

1. INTRODUCTION

Newcastle disease is a major constraint to the production of village poultry in Africa. A serological test to detect antibodies against the causal virus is necessary for two reasons: to evaluate the prevalence of the disease in areas where vaccination does not take place, and to verify the antigenic response to vaccination. The haemagglutination inhibition (HI) test is simple to perform, but difficult to standardise amongst laboratories [1]. Kits for detection of antibodies against Newcastle disease virus (NDV) using enzyme-linked immunosorbent assays (ELISAs) are widely available commercially, but the cost is too high for them to be useful in the village poultry sector. The Animal Production Unit of the FAO/IAEA Biotechnology Laboratory has considerable experience in the production of ELISA kits for the detection of antibodies against other animal pathogens and their distribution to African countries [2]. These kits have been specifically designed to be robust enough to withstand the fluctuations in temperature and transit delays experienced in distribution to tropical countries, and the protocols are designed to give uniform results when carried out in laboratories with widely varying ambient temperatures. This article describes a kit produced through the adaption of one of these existing kits, for the detection of bovine antibodies against *Brucella*, to detect chicken antibodies against NDV. It is based on a prototype kit first described in World Animal Review [3].

2. MATERIALS AND METHODS

The antigen was produced in embryonated eggs [4]. Batches of 150 specific pathogen-free (SPF) eggs (Lohmann, Germany) were inoculated with the Lasota vaccinal strain of NDV (Intervet, Holland). The vaccine was reconstituted with 5ml of sterile distilled water and 5000 units/ml of penicillin and 5000µg/ml of streptomycin was added to prevent possible bacterial contamination. The eggs were candled prior to inoculation to eliminate unfertile eggs and each fertile egg was inoculated with 0.1ml of the reconstituted vaccine. Three days later the eggs were candled to eliminate contaminated eggs prior to chilling at 4°C for 2 hours. Then the allantoic fluid was harvested from the eggs and pooled. The presence of NDV was verified using the rapid slide agglutination test with a 10% suspension of chicken red blood cells in phosphate buffered saline (PBS).

The antigen was purified according to the method of Snyder et al. [5]. The allantoic fluid was clarified by centrifugation at 7000g for 30 minutes at 4°C. Then the virus was pelleted by centrifugation at 59 000 g for 90 minutes. It was re-suspended at 10 x the original concentration in tris-maleate buffer at pH 6.0. Then it was layered over a discontinuous sucrose gradient comprising 2ml of 55% (weight/volume) sucrose and 5ml of 30% sucrose per tube. These were centrifuged for 4h at 90 000 g and the band containing the virus between the two sucrose layers was collected and run on an identical gradient. The virus fraction was collected again and pelleted at 59 000 g for 2 hours. The virus was re-suspended at 100 x its original concentration in 0.15M NaCl.

A pool of positive chicken sera with a high level of antibodies to NDV, obtained from the poultry department of the Veterinary School of the University of Vienna, was used as a positive control. SPF chicken serum, supplied by Lohmann, Germany, was used as a negative control.

The conjugate used was a rabbit anti-chicken whole molecule IgG conjugated to horse radish peroxidase. All other reagents were identical to those described for the Brucellosis ELISA kit

produced by the Animal Production Unit, FAO/IAEA Biotechnology Laboratory (version BRA 1.3, December 1994). 0.05M carbonate/bicarbonate buffer, pH 9.6 (± 0.05) is used as coating buffer; the washing buffer and sera and conjugate diluting buffer are respectively 0.02M and 0.1M PBS + 0.05% Tween 20 (Sigma USA), pH 7.4 (± 0.2). The chromogen and the substrate are ABTS and hydrogen peroxide (perhydrit) tablets. They are used dissolved in the substrate/chromogen buffer (0.05M phosphate/citrate buffer, pH 5.0 (± 0.5)) at final concentrations of respectively 3.6 mM and 4.4 mM. The reaction is stopped with 4% SDS solution.

A simple indirect ELISA was used. Plates were coated overnight at 4°C using 100 μ l of antigen, then washed 3 times in PBS + Tween 20. They were incubated with 100 μ l of serum sample for 30 min. at 37°C with shaking, washed 3 times in PBS + Tween 20, then incubated again for 30 min. at 37°C with shaking after the addition of 100 μ l of conjugate. Then they were washed again in the same way before addition of the substrate and chromogen. Following incubation for 15 min with shaking at 37°C, the reaction was stopped without washing by the addition of 100 μ l of stopper. Absorbance was then measured in an ELISA reader at 405 nm.

3. RESULTS

In order to transport the antigen to different laboratories around Africa, it would be preferable to supply it in a freeze-dried form. To evaluate the effect of freeze-drying on the activity of the antigen in the assay, an aliquot of 1ml of antigen at a 1/10 dilution was freeze-dried, and titrated in comparison with liquid antigen using positive and negative serum, fixing the serum dilution at 1/1600 and the conjugate dilution at 1/6400 (Fig.1). Since the effect of freeze-drying was only equivalent to a two-fold dilution of the antigen, freeze-dried antigen was adopted for use in the kit. Antigen was freeze-dried after a 1/10 dilution in saline.

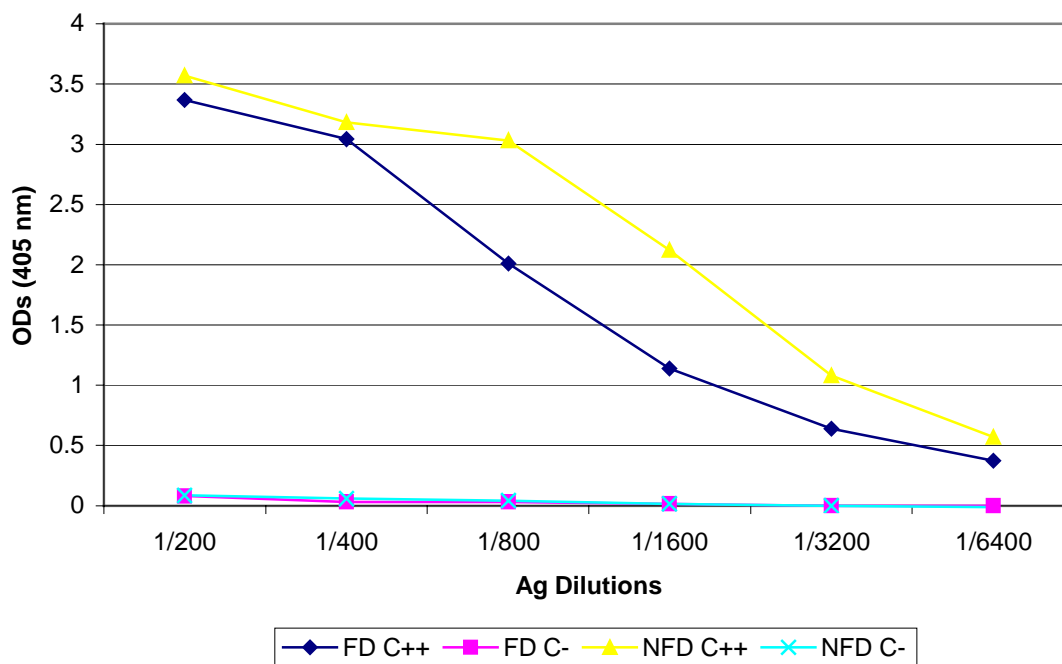


FIG. 1. NDV iELISA: Antigen titration before and after freeze-drying

Different dilutions of antigen, conjugate and serum were titrated in combination using the positive and negative control sera and the results evaluated for the maximum separation between absorbances for the negative and positive sera. An example of one of these titrations is shown (Fig. 2). On the basis of the results of these titrations, the following dilutions were finally selected: freeze-dried antigen 1/160, conjugate 1/3000, serum 1/2000.

Twenty-eight field samples were titrated at fixed conjugate and antigen dilutions (Fig. 3).

In order to compare the ELISA with the haemagglutination inhibition test over a complete range of titres, 40 different serum samples of varying positivity were made by mixing 4 strongly positive samples with SPF serum at 10 different dilutions, and tested with both tests. The optical density (OD)

of the ELISA results have been plotted against the titres in the HI test (Fig. 4). The coefficient of correlation was 0.914.

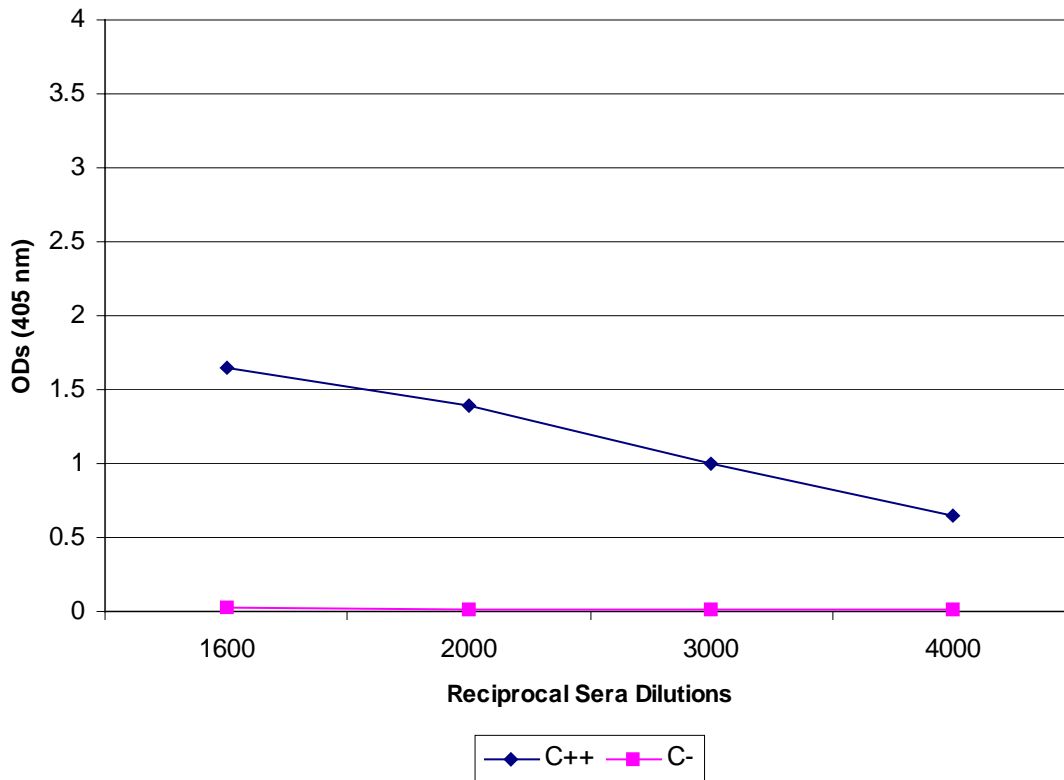


FIG. 2. NDV iELISA: Sera Titration, conjugate dilution: 1/3000, antigen (FD 1/10) dilution: 1/160

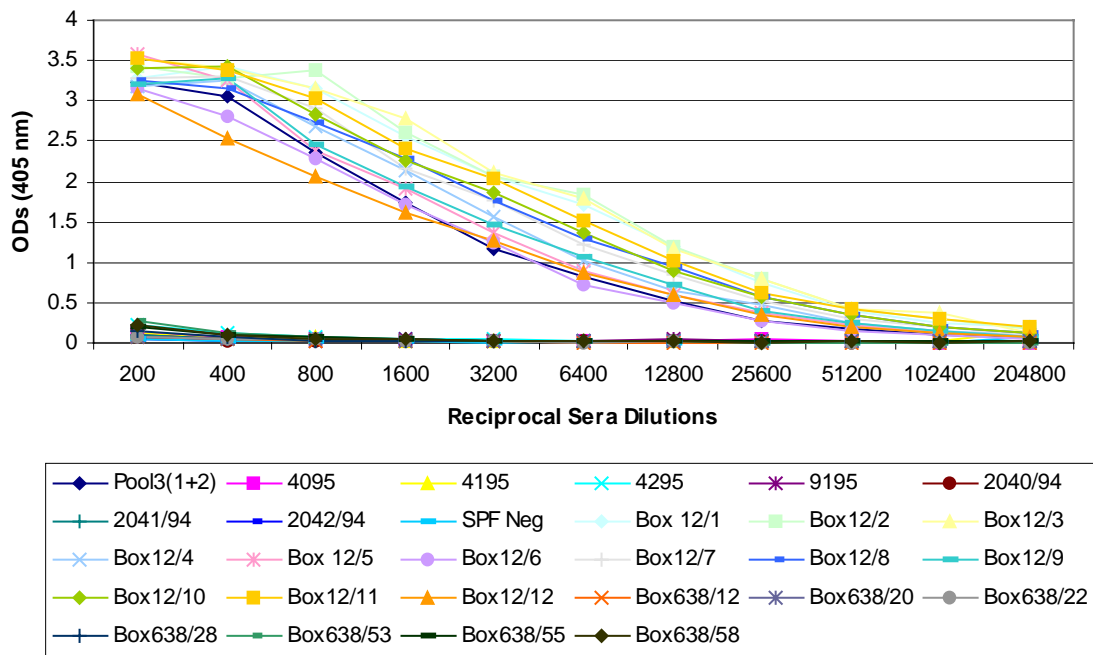


FIG. 3. NDV iELISA: sera titration, conjugate dilution: 1/1600, antigen (FD 1/10) dilution: 1/160

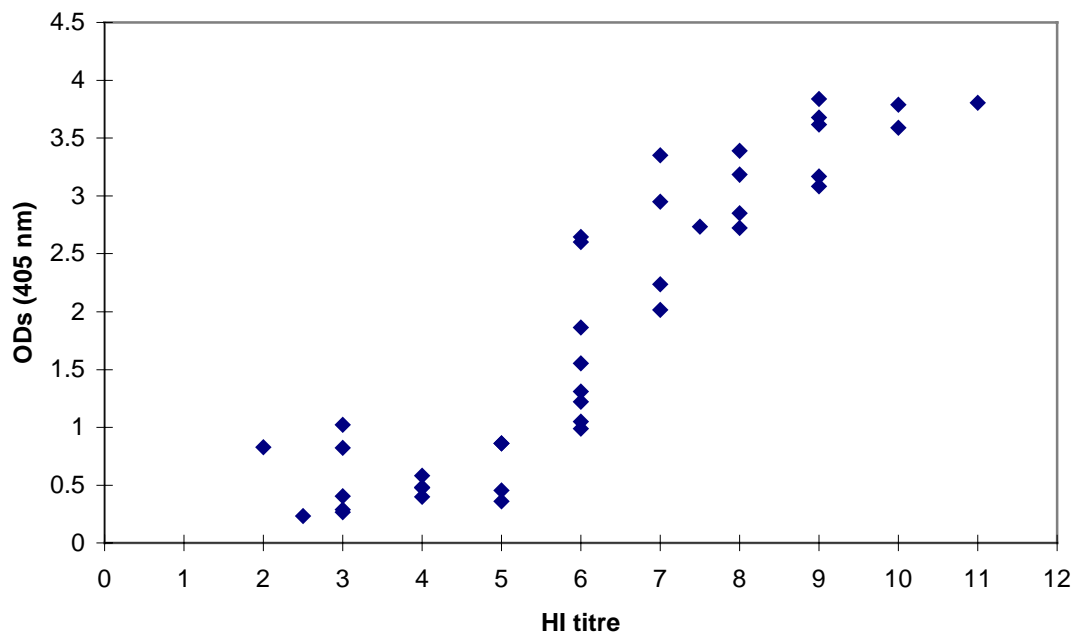


FIG. 4. Correlation between ELISA ODs and haemagglutination inhibition (HI) titres (antigen 1/160, conjugate 1/6000, serum 1/1000)

4. DISCUSSION

Results are expressed as percent positive by calculating the absorbance value of the test sample as a percentage of the value given by the positive control. An HI titre of 3 is considered the minimum indicative of protection against the virulent virus, and an HI titre of 6 is indicative of good protection, and in the absence of vaccination, a recent case of the disease. Given this, by referring to the correlation with ELISA absorbances given in Fig. 4, and the absorbance obtained with positive control serum, it was calculated that, very approximately, a percent positive value of 30% is indicative of a minimum level of protection, and a value of 100% or higher indicates excellent protection. It will now be necessary to validate the assay in the field, by studying the results of the assay of serum samples taken from village chickens following vaccination and cases of the disease.

This assay has the advantage that the same basic test could also be used for detecting antibodies against other avian pathogens using the same reagents, with the exception of the antigen only. It could also be adapted to differentiate between antibodies against wild type virus and vaccinal virus in the case where a vaccine with only one of the viral antigens is used.

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