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LATEX IMMUNOASSAY FOR RAPID DETECTION OF NEWCASTLE DISEASE VIRUS

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SUMMARY

A rapid test has been developed based on the technique of latex immunoassay for the detection of Newcastle disease virus from suspected tissue suspensions. The latex particles were sensitised with globulins and were used for antigen detection. Of the 258 samples tested, 165 samples were positive by this kit which was compared for its efficacy with the standard OIE approved haemagglutination (HA) and haemagglutination inhibition (HI) tests. No significant difference ($P > 0{\cdot}05$) was observed between the tests. The sensitivity and specificity of the developed test was 94·19% and 87·63% respectively.

INTRODUCTION

Newcastle disease (ND) is a highly contagious and infectious disease affecting poultry and has caused considerable loss to the poultry industry from mortality and loss in egg production. Despite various control measures including slaughter and compensation, quarantine measures, and regular and systematic vaccination, ND continues to plague the poultry industry. Since laboratory services for NDV detection are not always available in rural areas, a sensitive, simple, inexpensive and specific field test for rapid and accurate diagnosis is necessary for immediate control measures to combat the disease and to avoid further dissemination. This study highlights the usefulness of the latex agglutination test (LAT) in the detection of NDV antigen in suspected biological materials collected from ailing or dead birds.

MATERIALS AND METHODS

Tissue suspensions

Suspected tissue samples (brain, trachea, spleen, proventriculus, ileocaecal tonsil and intestinal contents) were collected in 50% glycerol saline from field outbreaks and transported to the laboratory. The samples were rinsed and ground in PBS (pH 7·2) and the tissue suspensions were used in the study. Positive diagnosis of the samples was first based on the agglutination of chicken erythrocytes and inhibition of the agglutination by specific antisera. The tissue samples were then coded before testing by LAT and assayed at least twice.

Preparation of antiserum and globulins

Antisera and globulins were prepared in chickens as per the methods described earlier (Hudson and Hay, 1980). The globulins were sequentially precipitated by a 45% saturated ammonium sulphate solution and the final precipitate was dissolved in a minimum quantity of PBS (pH 7·2) and dialysed extensively against PBS at 4°C.

Sensitisation of latex particles

The latex particles were sensitised as per the method of Bansal *et al.* (1988) with minor modifications. A 10% suspension (100 μl) of latex beads (0·8 μ, Sigma, USA) was mixed with 900 μl of carbonate-bicarbonate buffer to give a 1% suspension. This was mixed with an equal volume of globulin suspension in carbonate-bicarbonate buffer (protein content of the suspension adjusted to 2 mg/ml) and kept at 4°C overnight. The coated beads were centrifuged and the non-specific sites on the beads were blocked with BSA in PBS (pH 7·2) at 37°C for 2 hours. The beads were washed in carbonate-bicarbonate buffer and finally made as a 0·6% suspension.

Latex test

One drop of 0·6% of the coated beads was mixed with one drop of clarified supernatant of the suspected material on a glass slide. Positive and negative controls were included in the test. Agglutination of the beads indicated the positivity of the sample.

Haemagglutination (HA) and haemagglutination inhibition (HI) tests

HA and HI tests were conducted as per the standard procedure prescribed by OIE (1992).

RESULTS

The samples were first screened by HA and HI test prior to screening by the LAT. Of the 258 samples tested by LAT, 165 (63·95%) were positive and 93 were negative (Fig. 1 and Table I). No significant difference ($P > 0·05$) was observed between the LAT and combined HA and HI tests. The LAT was found to be 97·3% and 87·64% sensitive and specific respectively for the detection of NDV antigen (Table I). A highly significant concordance (94·2%) ($P \leq 0·01$) was observed between latex agglutination test and HA and HI tests (Table I).

DISCUSSION

The LAT is easy to carry out and the results are available within a few minutes. This test has been successfully applied for the detection of other antigens, e.g. rotavirus (Hughes *et al.*, 1984), rinderpest virus (Bansal *et al.*, 1988) and infectious bursal disease virus (Nakamura *et al.*, 1993) but there is no known report regarding the applicability of LAT to NDV diagnosis.

The developed test has the advantage that it requires only a drop of the suspected material and also it does not require expensive equipment. Contents of the kit can be kept in a refrigerator and are stable for a period of 6 months (maximum period tested). The addition of hyperimmune serum to the mixture of sensitised latex particles and antigen enhanced the agglutination of latex particles and the results were obtained within

TABLE I

Results of the samples tested by LAT and HA and HI

S. no.	Tests	Total no. of samples tested	No. of samples positive	No. of samples negative	% of positivity
1	HA and HI	258	180	78	69·76
2	LAT	258	165	93	63·95

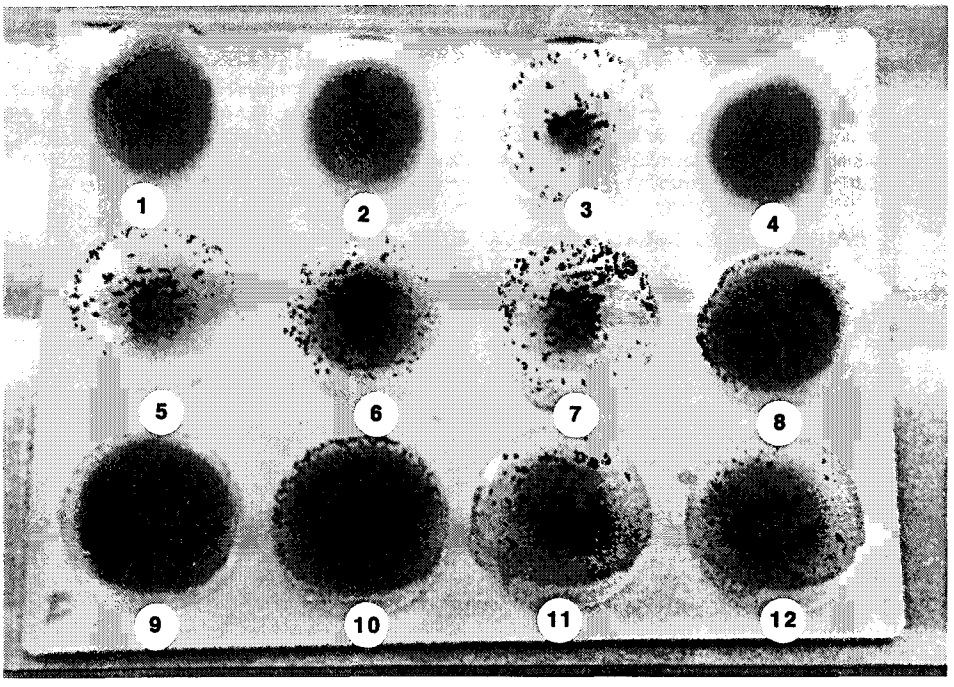

FIG. 1. Latex agglutination test for the detection of Newcastle disease virus. 1,2—negative control; 11,12—positive control.

one to 2 minutes. The sensitivity and specificity of the LAT correlated well with the standard test, and its ease of use and rapidity indicate that this test could be used as a good field diagnosis test kit.

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TEST IMMUNOLOGIQUE AU LATEX POUR LA DETECTION RAPIDE DU VIRUS DE LA MALADIE DE NEWCASTLE

Résumé—Un test rapide a été développé grâce à une technique immunologique au latex pour la détection du virus de la maladie de Newcastle chez des suspensions de tissus pouvant être infectés. Les particules de latex, sensibilisées avec des globulines furent utilisées pour la détection de l'antigène. Sur les 258 échantillons testés, 165 furent positifs avec ce type de kit dont l'efficacité fut comparée avec les méthodes standards de l'OIE: l'hémaglutination et le test d'inhibition de l'hémaglutination. Aucune différence ne fut significative ($P > 0,05$) entre les différents tests. La sensibilité et la spécificité furent respectivement pour ce test de 94,19% et 87,63%.

INMUNOENSAYO EN LATEX PARA LA DETECCION RAPIDA DEL VIRUS DE LA ENFERMEDAD DE NEWCASTLE

Resumen—Se desarrolló un test rápido basado en la técnica del inmunoensayo en látex para la detección del virus de la enfermedad de Newcastle en suspensiones tisulares. Las partículas de latex se sensibilizaron con globulinas y se utilizaron para la detección de antígenos. De un total de 258 muestras analizadas, 165 fueron positivas de acuerdo con este test. La eficacia del test se comparó con los tests estándar de hemoaglutinación (HA) e inhibición de la hemoaglutinación (HI). No se encontraron diferencias entre tests. La sensibilidad y especificidad del test desarrollado fue del 94·19% y 87·63% respectivamente.