A monoclonal antibody capture enzyme-linked immunosorbent assay for detecting antibodies to infectious bursal disease virus

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(Accepted 2 September 1991)

Summary

A monoclonal antibody capture enzyme-linked immunosorbent assay (mAb-ELISA) for antibodies to infectious bursal disease virus (IBDV) in chicken sera was developed and compared with conventional ELISA. When sera from farm chickens were tested by the two ELISAs and serum neutralization (SN), the correlation rate between SN and mAb-ELISA was 100% (49/49), and that between SN and conventional ELISA was 81.6% (40/49). In mAb-ELISA, all of the sera that were antibody-negative by SN had low absorbance values (below 0.05), and the absorbance values correlated closely with the SN titers. In the conventional ELISA, however, the sera antibody-negative by SN had various absorbance values ranging from 0.06 to 0.32. mAb-ELISA had much lower non-specific reactions than the conventional ELISA against sera from IBD-negative chickens.

Infectious bursal disease; mAb-ELISA; Antibody; Detection

Introduction

Infectious bursal disease virus (IBDV) is the etiologic agent of a highly contagious disease of young chickens characterized mainly by severe damage in

the bursa of Fabricius followed by immunosuppression (Allan, 1972; Fadly et al., 1976; Rosenberger and Gelb, Jr. 1978). The humoral immune response of chickens to IBDV has been studied extensively. In addition to providing an important tool for the diagnosis of infectious bursal disease (IBD), the determination of IBD antibodies would also permit the assessment of maternal antibodies in the progeny at hatch for preventing subclinical IBDV infections and would predict more accurately the efficacy of a progeny vaccination program for preventing clinical IBDV infections (Lucio and Hitchner, 1979; Giambrone et al., 1982; Nagi et al., 1983; Solano et al., 1986). Antibody responses to IBDV were measured initially by the agar-gel precipitation test (AGP) (Cursiefen et al., 1979; Lukert et al., 1975) and later by the serum-neutralization test (SN) (Skeel et al., 1979; Giambrone, 1980; Snyder et al., 1984). Recently, enzyme-linked immunosorbent assay (ELISA) emerged as a sensitive, quantitative, and efficient diagnostic tool for detecting IBDV antibodies in chickens (Marquardt et al., 1980; Howie and Thorsen, 1981; Snyder et al., 1983; Solano et al., 1986). However, non-specific reactions were noted when the serum dilution was less than 1:50 (Marquardt et al., 1980). Antigens initially captured by antibody coated on the ELISA plate have been used for ELISA to detect antibodies to some viruses (Edman et al., 1984; Zhang et al., 1989). The results showed that non-specific reactions could be reduced. This paper describes the application of a monoclonal antibody (mAb) capture ELISA (mAb-ELISA) for detecting antibody against IBDV in chicken serum. The mAb-ELISA greatly reduced non-specific reactions and the SN-positive and -negative sera had a better correlation with mAb-ELISA than with conventional ELISA.

Materials and Methods

Virus propagation and antigen preparation

The IBDV strain P3009 used in this study was a local isolate and was identified as serotype 1 virus of IBDV based on the virus neutralization with antiserum to IBDV LU strain (obtained from Dr. P.D. Lukert, Department of Medical Microbiology, University of Georgia, Athens, GA, U.S.A.) as described by Lee et al. (1988). The virus was propagated in chicken embryo fibroblast (CEF) by inoculating 0.05 ml of 10^6 $TCID_{50}$/per flask (175 cm^2, Nunc, Roskilde, Denmark) at 37°C for 48 h. The culture was treated by freezing and thawing three times, and the culture fluid was clarified by centrifugation at $10\,000 \times g$ for 15 min. The supernatant obtained was treated with 1% Triton-X100 in TNE buffer (Tris-HCl, 0.01 M; NaCl, 0.1 M; EDTA, 0.001 M; PH 7.6) for 1 h at 37°C and was used as crude antigen preparation and stored at -70°C. To prepare purified antigens, virus-containing supernatant was concentrated 100-fold using polyethylene glycol 16000 (Merck, Darmstadt, F.R.G.). Virus was partially purified by the extraction of virus with

fluorocarbon (Merck, Darmstadt, F.R.G.) and highly purified virus was obtained essentially as described by Dobos et al. (1979). The virus band formed in density gradient was collected and pelleted through a 35% sucrose cushion. The pellet was resuspended in TNE buffer.

Monoclonal antibody

The production and characterization of monoclonal antibodies (mAb) to IBDV P3009 were described elsewhere (Lee et al., 1989) In brief, spleen cells from BALB/c mice immunized with P3009 of IBDV were fused with NS-1 mouse myeloma cells. Hybridomas secreting antibody to IBDV were selected by ELISA test and confirmed by the indirect fluorescent antibody staining on virus-infected CEF cells. The mAb used in this study was 2G10 which immunoprecipitated one of two major capsid proteins of IBDV P3009 with molecular weights of 32 kDa. mAb 2G10 was purified from ascites fluids using an affinity column of protein-A Sepharose CL-4B (Pharmacia, Uppsala, Sweden) as described by Ey et al. (1978).

Chicken sera

IBD-hyperimmune sera were obtained from specific pathogen-free (SPF) chickens that had been vaccinated twice at 4 weeks and 6 weeks of age, intramuscularly, with killed P3009 viruses emulsified with complete Freund's adjuvant. The sera were collected at 9 weeks of age. SN-positive and -negative sera were collected from 30-week-old chickens at two commercial poultry farms.

Standardization of the mAb-ELISA procedure

To determine the optimum dilution of IBDV antigens in mAb-ELISA, checkerboard titrations were carried out between IBDV antigen and positive serum. mAb 2G10 was diluted at 1:300 in 0.05 M carbonate buffer (pH 9.6) and 50 μl of mAb was coated on the well of an ELISA plate (Nunc, Roskilde, Denmark). Crude or purified antigen of several dilutions was captured on the well surface and tested with twofold serially diluted positive serum. Horse-radish peroxidase (HRP)-labeled goat anti-chicken IgG (L and H) conjugate (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, U.S.A.) was used at a dilution of 1:2000.

To determine the optimum dilution of conjugate, a checkerboard titration was performed between the HRP-labeled goat anti-chicken IgG (L and H) conjugate and the positive and negative sera. The plate coated with mAb 2G10 at the concentration of a 1:300 was used to capture the crude antigen that had been diluted at 1:80 followed by adding twofold-diluted positive or negative serum. HRP-labeled goat anti-chicken IgG (L and H) conjugate was then added at various dilutions.

To determine the optimum dilution of mAb, a checkerboard titration was performed between the mAb and the negative sera. A serial twofold dilution of mAb was coated on the well and tested with several dilutions of negative serum. Crude antigen and HRP-labeled goat anti-chicken IgG (L and H) conjugate were respectively used at the dilutions of 1:80 and 1:4000.

mAb-ELISA procedure

mAb 2G10 was diluted at the optimum concentration in 0.05 M carbonate buffer (pH 9.6), and 50 μl of mAb was added in each well of the ELISA plate. The mAb was coated in the well by incubation at 37°C for 2 h. The wells were washed three times with washing buffer PBST (phosphate-buffered saline, 0.05% Tween 20, pH 7.4). The crude antigen was diluted in a solution of phosphate-buffered saline (PBS, 1:80) and 50 μl of antigen was added to each well. The plate was incubated at 37°C for 1 h and washed with washing buffer three times. The chicken serum was serially diluted twice in a solution of PBS containing 0.1% Tween 20, and 50 μl of serum was added to each well. The plate was incubated at 37°C for 1 h and washed three times with washing buffer. HRP-labeled goat anti-chicken IgG (L and H) was diluted in PBST to the optimum concentration, and 50 μl of the solution was added to each well. The plate was incubated at 37°C for 1 h and washed three times with washing buffer. A volume of 100 μl substrate solution containing 0.04% orthophenylenediamine (Sigma, MO, U.S.A.) in 0.05 M phosphate-citrate buffer, pH 5.0 with 0.04% H_2O_2 was added. The reaction was carried out for 30 min at room temperature and stopped by adding 50 μl of 2.5 M H_2SO_4 to each well. Absorbance readings were made at 409 nm with a Dynatech ELISA reader (Dynatech, Alexandria, VI, U.S.A.).

Conventional ELISA

The procedure followed was according to the method described by Howie and Thorsen (1981). Antigen was directly coated on the well of the ELISA plate. The binding of IBD antibody to antigen plate and the reaction with the substrate were essentially the same as for mAb-ELISA.

Serum neutralization test

The serum neutralization (SN) test was conducted in 96-well microtiter plates as described by Giambrone (1980). Titers (log_2 scale) for mAb-ELISA and SN tests from non-immunized birds of 3.9 or less were considered negative. Titers 4.0 or greater were considered positive.
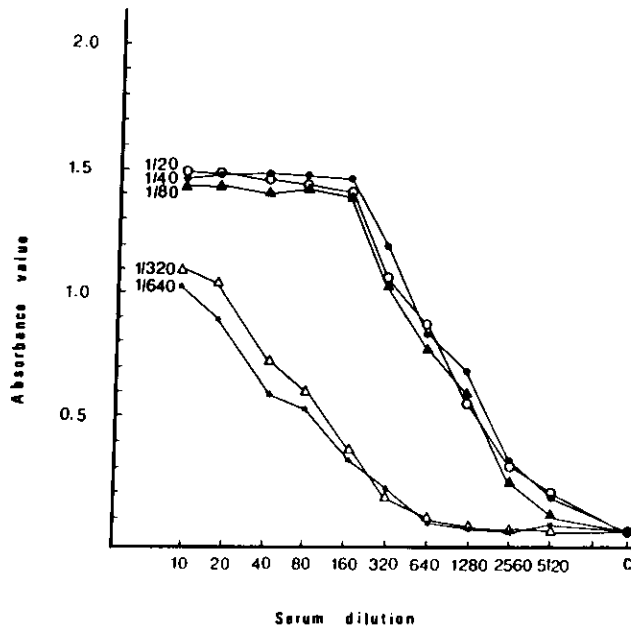
Fig. 1. Checkerboard titration between IBDV antigen and positive serum in mAb-ELISA. Reaction curves were expressed with antigen dilution of 1:20 (○), 1:40 (●) 1:80 (▲), 1:320 (△), and 1:640 (★). The control reaction without serum (C) is also indicated.
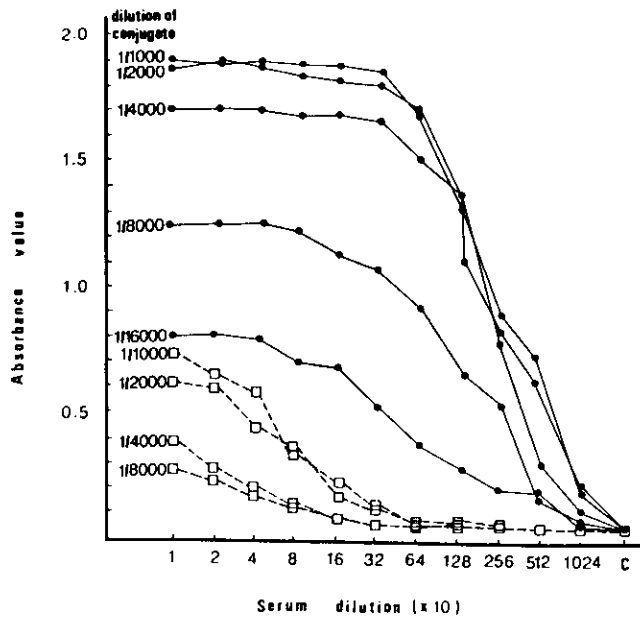

Fig. 2. Checkerboard titration between HRP-labeled goat anti-chicken IgG (L + H) conjugate and positive and negative sera. Various dilutions of HRP-labeled goat anti-chicken IgG conjugate were tested against the positive (●) and the negative (□) reference sera diluted twofold serially using the condition as described in text. The conjugate was diluted 1:1000, 1:2000, 1:4000, 1:8000 and 1:16000, and the reaction curve for each dilution is shown. The control reaction without serum (C) is also shown.

Results

Standardization of the mAb-ELISA procedure

As indicated in Fig. 1, the reaction curve of the crude antigen concentration at dilutions of 1:20, 1:40, and 1:80 showed a plateau between serum dilutions of 1:10 and 1:160 and declined with further dilutions of the serum. The absorbance values of the three antigen dilutions of 1:20, 1:40, and 1:80 did not differ markedly. However, the reaction curves of the antigens diluted 1:320 and 1:640 did not exhibit the plateau, and the absorbance value was less than those at 1:20, 1:40, and 1:80. Therefore, the antigen dilution chosen for further use was 1:80. Similar results were obtained when several different dilutions of IBD purified virus antigens were used in the checkerboard titration (data not shown).

Fig. 2 shows that the absorbance values decreased in accord with the dilution of serum. The reaction curves were almost the same when dilutions of conjugate were 1:4000 or less and were at a plateau when serum dilutions were between 1:10 and 1:320. The absorbance values of the conjugate at 1:8000 or greater dilutions were far lower than those at dilutions of 1:4000 dilution ·or less. The 1:4000 dilution of the conjugate was chosen as the optimum dilution, because it gave a high absorbance value in positive serum but a very low non-specific background (less than 0.3) in negative serum.
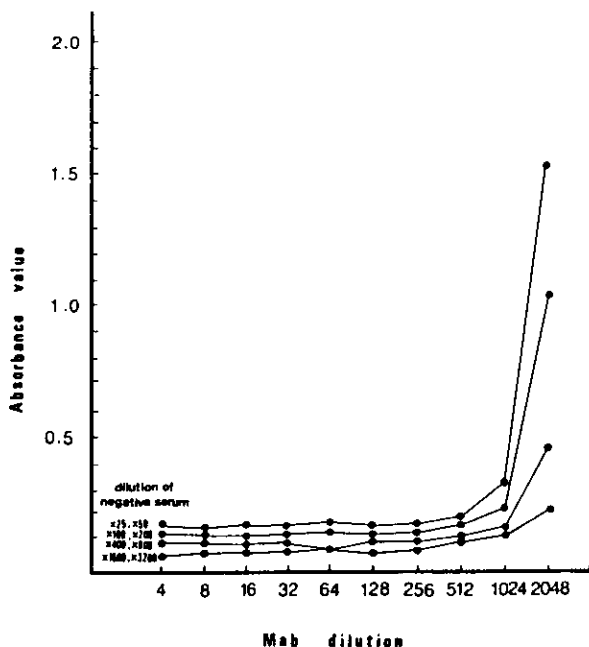

Fig. 3. Checkerboard titration between monoclonal antibody and the negative sera. A serial twofold dilution of mAb coated on the plate wells was tested against the negative reference serum diluted twofold serially using the conditions as described in text.
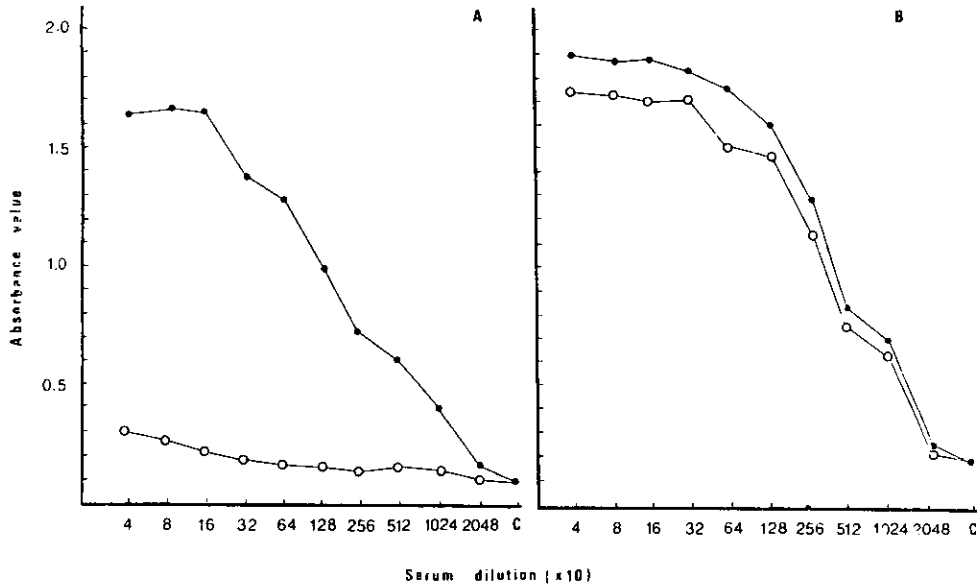
Fig. 4. Comparison of mAb-ELISA (A) and conventional ELISA (B) absorbance values in IBD-positive and -negative sera. Symbols show the absorbance values of positive sera (●) with an SN titer of $2^{10\cdot6}$ and negative sera (○) with an SN titer of less than $2^{3\cdot9}$, C represents control reaction without serum.

As shown in Fig. 3, the reaction curves were almost the same when several dilutions of negative serum were used. Non-specific background remained very low (less than 0.2) until the 1:512 dilution of mAb where slightly increased non-
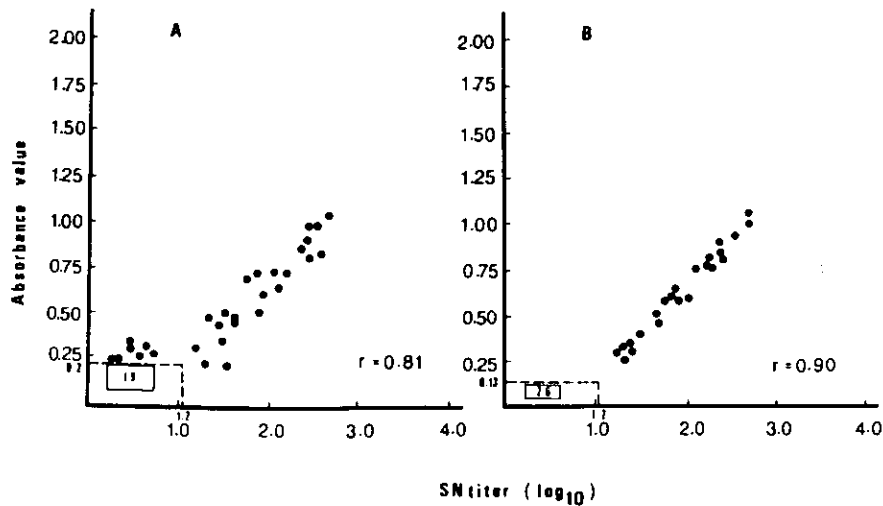

Fig. 5. Comparison of conventional ELISA (A) and mAb-ELISA (B) absorbance values and the SN titers ($\log_2$) of 49 serum samples from commercial chicken farms. The number in the square in B is the total number of negative sera by both test methods. The total number of negative sera in A includes the number in the square (19 samples) as well as 7 samples (●) above the square which are positive in the ELISA test. The SN titer of 2^4 was judged to be the cut-off value between positive and negative. An ELISA absorbance value of 0.2 and mAb-ELISA absorbance value of 0.13 were judged to be cut-off values for positive and negative. r represents coefficient of correlation.

TABLE 1

Correlation of conventional ELISA and mAb-ELISA absorbance values and the SN titers for 49 serum samples from commercial poultry farm chickens

SN	Conventional ELISA		mAb-ELISA	
	Pos.	Neg.	Pos.	Neg.
Pos.	21	2	23	0
Neg.	7	19	0	26
Correlation rate (%)	81.6		100	

specific background (less than 0.2) could be observed. The absorbance values rapidly increased when mAb was diluted at 1:1024 or greater. Therefore, the mAb dilution chosen for further use was 1:400.

Absorbance values of IBD-positive and -negative sera

To determine the ability of conventional ELISA and mAb-ELISA to detect IBD antibody in commercially raised aged chickens, sera were collected from an IBD-antibody-negative chicken with an SN titer less than $2^{3.9}$ and a positive chicken with an SN titer of $2^{10.6}$, both 30-weeks old from the farm flock. In conventional ELISA, there was no clear difference in absorbance values between IBD-positive and -negative sera, even at a low serum dilution, and the difference was not so great as in mAb-ELISA (Fig. 4). On the other hand, mAb-ELISA gave a clear difference in absorbance values between negative and positive sera until the serum dilution reached $1:2^{13.3}$.

Correlation of SN titers and absorbance values in sera from chickens

To determine the correlation between the SN titers and the absorbance values with conventional ELISA and mAb-ELISA, SN-positive sera ($n = 23$) and -negative sera ($n = 26$) from 30-week-old chickens were examined in the two kinds of ELISA. With the conventional ELISA, the SN-positive sera gave the absorbance values ranging from 0.19 to 1.03, which were correlated with SN titers: coefficient of correlation (r) = 0.81. The r value was calculated between SN titers and absorbance values of conventional ELISA (data not shown). However, the SN-negative sera (titers less than $2^{3.9}$) showed various absorbance values ranging from 0.06 to 0.32. With mAb-ELISA, the SN-positive sera had absorbance values ranging from 0.25 to 1.06, compared with those in conventional ELISA, but all SN-negative sera had much lower absorbance values (below 0.05). With mAb-ELISA, there was a good correlation between the SN titers and the absorbance values ($r = 0.90$). Because an SN titer higher than 2^4 was considered to indicate IBD-positive antibody, the absorbance values set as the cut-off values were 0.2 for conventional ELISA and 0.13 for mAb-ELISA (data not shown). The correlation rates between the SN titers and both ELISAs were calculated for

both positive and negative antibodies. The results are shown in Table 1. The correlation rates for conventional ELISA and mAb-ELISA were 81.6% and 100%, respectively.

Discussion

The results demonstrate that mAb-ELISA was developed successfully for the detection of antibody to IBD virus in chicken sera. This test has advantages over the conventional ELISA. mAb-ELISA could distinguish the SN-positive and -negative sera in 30-week-old chickens (Fig. 4). The conventional ELISA could not differentiate between positive and negative sera at various dilutions, but mAb-ELISA showed clear differences between positive and negative sera, even at higher serum dilutions. Furthermore, the SN-positive and -negative sera had a higher correlation with mAb-ELISA than with conventional ELISA (Table 1). Therefore, mAb-ELISA shown in this report seems to be accompanied by fewer non-specific reactions than conventional ELISA.

Many reports have demonstrated the possible causes of the non-specific reactions in several ELISA tests using avian sera. York et al. (1982), Meulemens and Halen (1982), and Ohkubo et al. (1984) suggested an age-dependent increase in non-specific reaction in chicken sera. The chicken immunoglobulins (Igs) tend to absorb to polystyrene plates in a non-specific manner (Slaught et al., 1979). The nature of the antigen in the well and the quantity of IgM in the serum were also reported to affect non-specific reactions (Meulemens and Halen, 1982). Marquardt et al. (1980) detected antibody to IBDV using ELISA. A non-specific reaction would be obtained when the serum dilution was less than 1:50. The results reported by Meulemens and Halen (1982) showed that non-specific binding of avian Igs to the infectious laryngotracheitis virus antigens is largely reduced by the use of carbonate-bicarbonate buffer for antigen coating instead of PBS buffer. But negative sera gave some absorption of the antigen. Additionally, York et al. (1982) did not reduce non-specific reactions following the pretreatment of chicken sera with kaolin and N-acetylcysteine. Ohkubo et al. (1984) used the avidin-biotin enzyme system in their ELISA. This system reduced markedly the non-specific binding reactions, compared to the conventional HRP-labeled enzyme system. They pointed out that the avidin-biotin interaction was so strong, making it possible to use biotin-labeled anti-chicken IgG at a high dilution (1:4000).

In the mAb-ELISA, non-specific binding reactions could be overcome without pretreatment of the sera, even by using crude antigen preparations. It is possible that the mAb coated on the well of the ELISA plate captures specifically IBDV antigens and the factors which contribute to the non-specific reactions are subsequently eliminated after washing procedures. In conclusion, mAb-ELISA has the advantages of less non-specific reaction, rapidity, and stable sensitivity and may be used to determine the immune status of a chicken flock or to detect chickens infected with IBD.

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References

Allan, W.H., Faragher, J.T. and Cullen, C.A. (1972) Immunosuppression by the infectious bursal agent in chickens immunized against Newcastle disease. Vet. Rec. 93, 511–512.

Cursiefen, D., Vielitz, E., Landgraf, H. and Becht, H. (1979) Evaluation of a vaccine against infectious bursal disease in field trials. Avian Pathol. 8, 341–351.

Dobos, P., Hill, B.J., Hallet, R., Kells, D.T.C., Becht, H. and Teninges, D. (1979) Biophysical and biochemical characterization of five animal viruses with bisegmented double-stranded RNA genome. J. Virol. 32, 593–605.

Edman, D.D., Gray, G.W. and Anderson, L.J. (1984) Development and evaluation of an IgM capture enzyme immunoassay for diagnosis of recent Norwalk virus infection. J. Virol. Methods 24, 57–66.

Ey, P.L., Prowse, S.J. and Jenkin, C.R. (1978) Isolation of pure IgG1, IgG2A and IgG2B immunoglobulins from mouse serum using protein A-Sepharose. Immuno-chem. 15, 429–436.

Fadly, A.M., Winterfield, R.W. and Olander, H.J. (1976) Role of the bursal of Fabricius in the pathogenicity of inclusion body hepatitis and infectious bursal disease virus. Avian Dis. 20, 467–477.

Giambrone, J.J. (1980) Microculture neutralization test for serodiagnosis of three avian viral infections. Avian Dis. 24, 284–287.

Giambrone, J.J., Yu, M. and Eckman, M.K. (1982) Field trials with an oil emulsion infectious bursal disease vaccine in broiler breeder pullets. Poult. Sci. 61, 1823–1827.

Howie, R. and Thorsen, J. (1981) An enzyme-linked immunosorbent assay for infectious bursal disease virus. J. Comp. Med. 45, 51–55.

Lee, L.H., Liu, H.J. and Lin, Y.P. (1989) Characterization of antigenic region sites on 32 kDa capsid protein of infectious bursal disease virus using monoclonal antibody. J. Chinese Soc. Vet. Sci. 15, 317–325.

Lee, L.H., Lu, J.S. and Lii, N.J. (1988) Characterization of infectious bursal disease virus isolated in Taiwan. J. Chinese Soc. Vet. Sci. 14, 89–100.

Lucio, B. and Hitchner, S.B. (1979) Infectious bursal disease emulsified vaccine: effect upon neutralizing-antibody levels in the dam and subsequent protection of the progeny. Avian Dis. 23, 446–478.

Lukert, P.D., Leonard, J. and Davis, R.B. (1975) Infectious bursal disease-antigen production and immunity. Am. J. Vet. Res. 36, 539–540.

Marquardt, W.W., Johnson, R.B., Odenwald, W.F. and Scholttoler, B.A. (1980) An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. Avian Dis. 24, 375–385.

Meulemens, G. and Halen, P. (1982) Enzyme-linked immunosorbent assay for detecting infectious laryngotracheitis virus antibodies in chicken serum. Avian Path. 11, 361–368.

Naqi, S.A., Marquez, B. and Sahin, N. (1983) Maternal antibody and its effect on infectious bursal disease immunization. Avian Dis. 27, 623–631.

Ohkubo, Y., Takashima, I., Hashimoto, N. and Fujita, I. (1984) Enzyme-linked immunosorbent assay for detecting IgM and IgG antibodies to Japanese encephalitis virus in sera from experimentally infected swine. Jpn. Vet. Sci. 46, 57–64.

Rosenberger, J.K. and Gelb, J., Jr. (1978) Response to several avian respiratory viruses as affected by infectious bursal disease virus. Avian Dis. 22, 95–105.

Skeels, J.K., Lukert, P.D., De Buyssches, E.V., Flitcher, P.J. and Brown, J. (1979) Infectious bursal disease viral infection. I. Complement and virus-neutralizing antibody responses following infection of susceptible chickens. Avian Dis. 23, 95–106.

Slaught, S.S., Yang, T.J. and van der Heide, L. (1979) Adaptation of enzyme-linked immunosorbent assay to the avian system. J. Clin. Microbiol. 10, 698–700.

Snyder, D.B., Marquardt, W.W. and Russek, E. (1983) Rapid serological profiling by enzyme-linked immunosorbent assay. II. Comparison of computational methods for measuring antibody titer in a single serum dilution. Avian Dis. 27, 474–484.

Solano, W., Giambrone, J.J. and Panangala, V.S. (1986) Comparison of a kinetic-based enzyme-linked immunosorbent assay (KELISA) and virus-neutralization test for infectious bursal disease virus. II. Decay of maternal antibody in progeny from white leghorns receiving various vaccination regimens. Avian Dis. 30, 126–131.

York, J.J., Fahey, K.J. and Bagust, T.J. (1982) Development and evaluation of an ELISA for the detection of antibody to infectious laryngotracheitis virus in chickens. Avian Dis. 27, 409–421.

Zhang, A.Q., Smith, J.R. and Burgess, G.W. (1989) A capture antibody enzyme-linked immunosorbent assay for detection of antibodies against bovine entero-virus. J. Virol. Methods 24, 223–226.

以單源抗體捕抓酵素結合免疫吸附試驗
檢測傳染性華氏囊病抗體

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　　以單源抗體捕抓酵素結合免疫吸附試驗（ mAb‐ELISA ）來檢測鷄血清中傳染性華氏囊病毒（IBDV）抗體已開發成功，並與傳統ELISA比較。由養鷄場採取之鷄血清以上述二種ELISA法及血清中和試驗（SN）比較，mAb‐ELISA與SN之相符率為100％（$^{49}/_{49}$），而SN與傳統ELISA之相符率則為81.6％（$^{40}/_{49}$）。所有SN試驗陰性之血清，於mAb‐ELISA之吸收值均甚低（小於0.05），且其吸收值與SN力價也甚相近。然而，SN陰性之血清，於傳統ELISA之吸收值範圍則在0.06至0.32之間。於鷄IBD陰性血清上，mAb‐ELISA較傳統之ELISA其非特異性反應較低。