J.Vet. Malaysia (2002)14 (1 &2): 19-24

DEVELOPMENT OF INDIRECT AND DOUBLE ANTIBODY SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY FOR INFECTIOUS BURSAL DISEASE

S. F. Phong, M. Hair-Bejo, A. R. Omar and I. Aini

Faculty of Veterinary Medicine,
Universiti Putra Malaysia, 43400 UPM,
Serdang, Selangor, Malaysia

SUMMARY

Indirect and double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) was successfully developed using the P97/302 field local isolate of very virulent (vv) infectious bursal disease virus (IBDV) to detect both the antibody and antigen against IBDV. The regression line equation of the indirect ELISA was $Log_{10}Titre = -1.1495(1/SP) + 4.5349$ with $R^2 = 0.975$ and DAS ELISA was Log_{10} Titre $= 2.6527Log_{10}$ (SP) + 3.0853 with $R^2 = 0.93$ which was calculated from the standard curves. Serum samples were then obtained from the commercial and specific-pathogen-free (SPF) chickens for commercial IDEXX, developed indirect and DAS ELISA evaluations, and the results showed significant correlation ($p<0.01$) between IDEXX and indirect with $R = 0.88$ and between IDEXX and DAS with $R= 0.93$. The developed DAS ELISA for antigen detection is also highly specific. It can detect various IBDV field isolates from the bursa tissue and various IBDV vaccine strains.

Keywords: Indirect and DAS ELISA, infectious bursal disease, IBDV

INTRODUCTION

The use of enzyme-linked immunosorbent assay (ELISA) has increased rapidly since the introduction of the technique in 1972 (Carlson *et al.,* 1972). Initially, the most common methods used to detect flock exposure to infectious bursal disease virus (IBDV) are the agar gel precipitin (AGP) and virus neutralization (VN) test (Weisman and Hitchner, 1978). The AGP test is economical and simple to perform, but precipitins are sometimes not detectable although chickens are challenged with the virus. The VN test appears to be a better indicator of flock immunity, but it is more expensive, cumbersomes and time-consuming (Weisman and Hitchner, 1978). The limitation of both tests has lead to the development of ELISA. The ELISA has delivered a much more cost effective, scientifically credible method for doing the volume of testing the poultry industry needs, over other methodologies as such VN or AGP test (Odor, 1995). The ELISA offers the best sensitivity, specificity and practicality for large scales use (Marquardt *et al.,* 1980). The ELISA has been developed into an assay which can be used as a routine diagnosis for serosurveys of chicken flock and examination of vaccination efficiency (Snyder *et al.*, 1986; Solano, *et al.*, 1985). The use of double-antibody sandwich (DAS) ELISA for IBD has been reported (Cardoso *et al.*, 1998; Kumar and Rao, 1991; Mohamed *et al*, 1996). The DAS ELISA has more advantages over indirect ELISA, as it does not need purify antigen and it can be used both for virus detection and for measuring specific antibody using the same basic reagent (Cardosa, *et al.,* 1998). Therefore the objectives of the studies were to develop indirect ELISA for IBD antibody detection and development of DAS ELISA for IBD antibody and antigen detection using P97/302 IBDV local isolate.

MATERIALS AND METHODS

IBD antigen

Field isolate of P97/302, which is identified as very virulent (vv) IBDV strain was obtained from an IBD outbreak of the IBD vaccinated commercial layer farm. This IBDV isolate was propagated in specific-pathogen-free (SPF) embryonated eggs. The IBDV infected chorioallantoic membrane (CAM) was collected and homogenated with mortar and pestle, using sterile sand in 1:4 (w:v) dilution of phosphate buffer saline (PBS). It was then centrifuged at 3000 rpm for 15 minutes at 4°C (Sigma 4kD, B. Braun). The supernatant was collected, filtered through a 0.45um syringe filters, treated with antibiotic-antimycotic (GIBCO Lab., USA) in 1:10 (v:v) and incubated at 4°C for 1 hour. For the indirect ELISA coating, the supernatant was layered onto the 40% sucrose cushion, centrifuged at 115 200 g for 2.5 hours. The virus pellet was then suspended, treated with 1% Triton-X 100 in PBS at 37°C for 1 hour and centrifuged at 20 000 g for 20 minutes at 10°C. The treated IBDV antigen was then measured for protein concentration using the protein assay dye (Bio-Rad, USA).

Indirect ELISA for antibody detection

The microtitre plate of Immulon® 2 High Binding Strips, flat bottom (Dynex Technologies, USA) was used for antigen coating of the indirect ELISA. The optimum dilutions of all reagents were determined by checkerboard titration. The wells were coated with 1:100 dilution of the

prepared antigen and blocked with 5% bovine serum albumin (BSA). After three washes in PBS with 0.1% Tween 20, 100ul of chicken serum samples (test, positive and negative control samples) in the dilution of 1:500 was added into each well, incubated for 1 hour in a 37 °C orbital shaker at 150 rpm. The positive control serum samples were prepared in SPF chickens using P97/302 IBDV isolate. After three washes, the goat anti-chicken IgG (KPL, USA) which was diluted into 1:10 000 was then added into the wells and incubated at 37 °C in an 150 rpm orbital shaker for 1 hour. After three washes, 100ul of the tetramethybenzidine (TMB) substrate was added into each well and incubated for 25 minutes in a 37 °C orbital shaker. The plate was then read by the ELISA reader (Dynatech, MR 7000, USA) at 650nm.

DAS ELISA for antibody detection

The purified antibody was prepared in New Zealand White rabbits by intravascular injection of purified P97/302 IBDV and subcutaneous injection of this purified IBDV emulsified in Freund complete and incomplete adjuvant (Sigma, USA) at week intervals. The amount of the purified virus was 100ug ($1 \times 10^{5.5}$ EID_{50} / 0.1ml) per injection. Econo-Pac serum IgG purification kit (Bio-Rad, USA) was used in the purification of the rabbit hyperimmune serum (HIS). The optimum dilutions of all reagents were determined by checkerboard titration. The plate (Immulon® 2 High Binding Strips) was coated with 1:100 of the purified rabbit HIS, blocked (5% BSA) and 1:100 dilution of the unpurified IBD antigen was added into each well. The chicken serum (test, negative and positive control samples) were diluted into 1:500 and added (100ul) into each wells and incubated for 1 hour in a 37 °C orbital shaker at 150rpm. After the incubation, the wells were washed three times as above. The goat anti-chicken IgG (1:10 000) was then added and incubated at 37 °C, in an 150rpm orbital shaker for 1 hour. After three washes, 100ul of the TMB substrate was added and incubated for 25 minutes in a 37°C orbital shaker. The plate was read at 650nm using ELISA reader.

Standard curve of indirect and DAS ELISA

Three chickens HIS were used as reference serum in serial dilutions to obtain the standard curve. The chicken HIS were prepared in SPF chickens by intraocular administration of purified P97/302 IBDV and intramuscular injection of this purified IBDV emulsified in Freund complete and incomplete adjuvant at week intervals. The amount of the purified virus was 100ug ($1 \times 10^{5.5}$ EID_{50} / 0.1ml) per injection. The chicken HIS was then collected and tested with commercial IDEXX ELISA kits (IDEXX Lab., Inc., USA). The IBD antibody titres of the three reference sera used were 2355, 11352 and 21613, respectively. Serial dilutions of these reference sera were made (1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12800, 1:25600, 1:51200, 1:102400, 1:204800, 1: 409600, 1:819200, 1:1638400 and 1: 3 276800). The reference and negative control sera were diluted 1:500 in PBS with 0.1% Tween 20. The chickens HIS with antibody titre of 1548 were diluted at 1:500 and used as positive control. Two wells per plate were used for each of the positive and negative controls. When the test assays were run, the absorbance value for the serially diluted individual reference serums were obtained and then converted to sample-to-positive (SP) ratio values, where

$$SP = \frac{\text{Mean absorbance (samples} \quad \text{negative control)}}{\text{Mean absorbance (positive control} \quad \text{negative control)}}$$

The titres of the reference serum samples were transformed to Log_{10}. The standard curve relating Log_{10} titre to SP was obtained. The curve estimation regression in the software of Statistical Programme for Social Science (SPSS 1999, version 10.0) was used to generate the regression equation line from the indirect and DAS ELISA standard curve.

Positive-negative threshold (PNT) for indirect and DAS ELISA

Total numbers of 24 negative sera were collected from the SPF chickens. The mean OD was calculated and the standard deviation (SD) was determined. The positive-negative threshold (PNT) in OD value was determined when the mean OD plus three times the SD (assuming a 1% probability of misclassification). The PNT in OD value was then converted to SP value. The PNT in SP value was converted to antibody titre value using the equation regression line generated from the standard curve of the indirect and DAS ELISA.

Evaluation of indirect and DAS ELISA for antibody detection

Four groups of serum samples were obtained from a broiler Farm A with a chicken population of sixty thousands. Each group had thirty serum samples with total amount of one hundred and twenty. The breed of the chicken was Abor Acre. The chickens were given IB/ND live vaccine at 7-day-old, IBD live vaccine at 14-day-old and second booster with IB/ND live vaccine at 18-day-old. Group one blood samples was obtained from the chicken at 21-day-old (one week after the IBD vaccination). The following groups of blood samples were obtained from the subsequent weeks (at 4-, 5-, and 6-week-old).

Another group of thirty serum samples was obtained from a broiler Farm B with a chicken population of sixty thousand. The breed of the chicken in this farm was Avian. The chickens were vaccinated with IB/ND live vaccine at 1-day-old, IBD live vaccine at 7- and 14-day-old and second booster with IB/ND at 18-day-old. The blood samples were obtained from the chickens at 40-day-old.

Four groups of serum samples were also obtained from SPF chicken. Each group had thirty serum samples taken from the chickens at days 28, 29, 30 and 31 of age. The serum samples obtained from Farms A, B and the SPF chicken were used to evaluate the developed indirect and DAS ELISA by comparing with the IDEXX commercial

ELISA.

The mean antibody titres of IDEXX, indirect and DAS ELISA were entered into SPSS program (version 10.0), and bivariate correlations were performed. The Pearson's correlation coefficient (R) was computed to determine the correlation of the IDEXX ELISA data with that of indirect and DAS ELISA.

DAS ELISA for antigen detection

The antibody coated (1:100) and blocked (5% BSA) wells were prepared as above. The tests antigen (1:100) were added into each well and allowed to incubate for 1 hour in a 37°C orbital shaker at 150 rpm. Eight negative control antigen samples were used to calculate the cut-off value by assuming a 1 % probability of misclassification. After three washes, the chicken HIS (1:100) was added into each wells and incubated for 1 hour at 37°C in the orbital shaker. The wells were then washed three times, drained dried and 100ul of the goat anti-chicken IgG (1: 10 000) was then added into each well and incubated for 1 hour. After three washes, 100ul of the TMB substrate was then added into each well and incubated for 25 minutes in a 37°C orbital shaker. The plate was then read using the ELISA reader at 650nm.

Evaluation of DAS ELISA for antigen detection

Five IBDV isolates, P97/302, P97/61, P94/283, P94/273, P99/551 from the unpurified virus of bursa samples and three vaccine strains, CU-1M, LC75 and D78 were diluted at 1:100 and were used for evaluation of the developed DAS ELISA for IBDV detection. Samples of Newcastle disease virus (NDV) from SPF allantoid fluid and chicken anaemia virus (CAV) from tissue culture were also used to determine the specificity of the developed DAS ELISA.

RESULTS

Standard curve

In indirect ELISA, the formula and R^2 of the standard curve were obtained using the inverse model in the curve estimation regression of the SPSS software. The curve formula was Log_{10}titre = -1.1495(1/SP) + 4.5349 with the $R^2 = 0.975$ and was used in the range of SP ratio between 0.3 to 7.

In the DAS ELISA, the formula and R^2 of the standard curve were obtained using the logarithmic model in the curve estimation regression of the SPSS software. The curve was 93% ($R^2 = 0.93$) fit with logarithm curve. The logarithm formula was Log_{10} titre = $2.6527Log_{10}$ (SP) + 3.0853 which can be used in SP ratio in the range between 0.3 to 5.

Positive-negative threshold (PNT)

In indirect ELISA, the mean OD value was 0.26 with the SD value of 0.04. Therefore, the PNT was 0.377 (mean OD plus three times SD). The PNT in antibody titre value was 1081 which was calculated based on the standard curve formula of Log_{10} titre = -1.1495(1/SP) + 4.5349.

In DAS ELISA, the mean OD value was 0.30 with the SD value of 0.09. The PNT was 0.573 (mean OD plus three time SD) and therefore the PNT in antibody titre value was 1978 calculated, based on the standard curve formula of Log_{10} titre = $2.6527Log_{10}$ (SP) + 3.0853.

Evaluation of indirect and DAS ELISA

The mean antibody titres of broiler chickens from farm A (Table 1) were 444±339 (21-day-old), 111±47 (28-day-old), 2519±617 (35-day-old) and 1985±741 (42-day-old) measured by IDEXX commercial ELISA kit. In the developed indirect ELISA, the mean antibody titres from farm A were 1980± 2570 (21-day-old), 2449±3365 (28-day-old)), 6033±3888 (35-day-old) and 4409± 3125 (42-day-old). In DAS ELISA, the mean antibody titres from farm A were 70±60 (21-day-old), 1179±2430 (28-day-old), 20391±16738 (35-day-old) and 11275±9395 (42-day-old).

The mean antibody titre from 40-day-old broiler chickens from farm B (Table 1) was 1454±1060 when measured with IDEXX commercial ELISA kit. In indirect ELISA, the mean antibody titre was 6806± 3517 whereas in DAS ELISA, the antibody titre was 3947±1901.

In the SPF chickens (Table 1), the mean antibody titres were 52±10 (28-day-old), 63 ±8 (29-day-old), 48±12 (30-day-old) and 95±44 (31-day-old) which were measured using IDEXX commercial ELISA. These SPF chickens were negative for IBD antibody. In indirect ELISA, the mean antibody titres of the SPF chickens were 58±143 (28-day-old), 17±51 (29-day-old), 73±83 (30-day-old) and 78±272 (31-day-old) whereas DAS ELISA demonstrated 270±311 (28-day-old), 220±327 (30-day-old), 275±554 (31-day-old) and 224±305 (31-day-old). The coefficient of correlation for the indirect and IDEXX ELISA (Table 2) was $R = 0.88$ ($p < 0.01$); DAS and IDEXX ELISA (Table 2) was $R = 0.93$ ($p < 0.01$).

DAS ELISA for antigen detection

The uninfected SPF bursa of Fabricius as negative control with the mean absorbance of eight samples was found to be 0.359. The SD was 0.016. Therefore, the cut-off level between ELISA negative and positive absorbance values was found to be 0.408. The absorbance values of the five unpurified IBDV isolates obtained from the bursa were 2.337(P97/302), 2.387(P97/61), 2.381 (P94/284), 1.878 (P94/273), 1.417 (P99/551) and from three vaccine strains were 1.387 (Cu-1M), 2.616(LC 75) and 1.738(D78) as shown in Fig.1. All the OD values of the IBDV isolates were above the cut-off level which revealed the presence of IBDV antigen. The absorbance value of the NDV and CAV samples were found to be 0.348 and 0.233, respectively, which were below the cut-off level, indicating the absence of IBDV antigen.

Table 1. The mean IBD antibody titres of the chickens from Farm A, B and SPF chickens which evaluated by commercial IDEXX, indirect and DAS ELISA.

Chickens	Age(day-old)	IDEXX	ELISA Indirect	DAS
Farm A	21	444± 339	1980±2570	70±60
	28	111± 47	2449±3365	1179±2430
	35	2519±617	6033±3888	20391±16738
	42	1985±741	4409±3125	11275±9395
Farm B	40	1454±1060	6806±3517	3947±1901
SPF	28	52±10	58±143	270±311
	29	63±8	17±51	220±327
	30	48±12	73±83	275±554
	31	95±44	78±272	224±305

Table 2. The coefficient of correlation for the indirect and DAS ELISA compared to commercial IDEXX ELISA

	Indirect (R)	DAS (R)
IDEXX	0.88	0.93

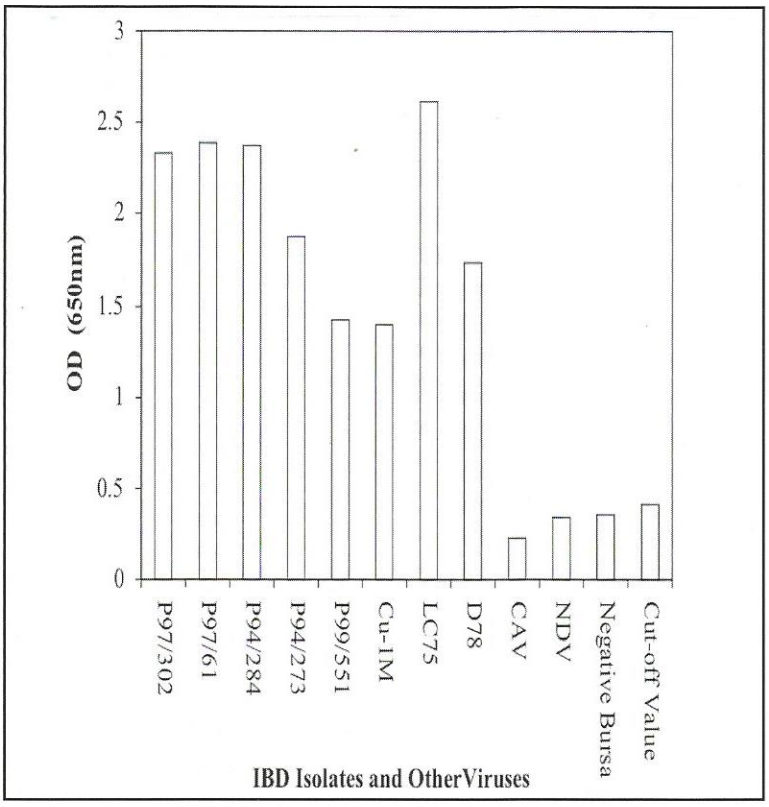

Fig. 1. The absorbance values of five field isolates (P97/302, P97/61, P94/273, and P99/551) three vaccines strains (Cu- 1M, LC75, D78), two other avian viruses (CAV, NDV), negative control bursa and cut-off value by DAS ELISA.

DISCUSSION

The standard curves of indirect and DAS ELISA were successfully determined from the optimised indirect and DAS ELISA. From the standard curves, the equation regression lines were generated using the SPSS software and can be used to measure the IBD antibody titre. The development of ELISA using various methods for measuring antibody titre to IBDV has been reported (Marquardt *et al.*, 1980; Snyder *et al.*, 1984).

The indirect and DAS ELISA using local P97/302 IBDV isolate in this study were successfully optimised and developed for measuring IBD antibody titre. These ELISAs used regression equation line generated from the standard curves to predict IBD ELISA antibody titre. The ELISAs were highly significantly correlated ($p<0.01$) when compared to IDEXX commercial ELISA. The DAS ELISA has better correlation with IDEXX ELISA compared to indirect ELISA, indicating that DAS ELISA was more sensitive than indirect ELISA. The DAS ELISA was more sensitive probably because of it being coated with the purified and high titre of the specific antibody. Furthermore, this specific antibody which is coated to the solid phase was capable of capturing and concentrating the antigen. Thus, the results generally indicated that the indirect and DAS ELISA have the potential to be used for detection and measurement of antibodies to IBDV in chicken. Therefore, it should be of value for assessing flock immunity after vaccination or natural exposure to the disease.

The estimated reagent cost per well for both ELISAs, including the antigen and antibody preparations is cheaper than the current commercially available ELISA. Furthermore, the conversion to change absorbance to SP value and using the regression equation line to change the SP values to final ELISA titre make these ELISAs highly reproducible as described by Barbour *et al.* (1991).

The ELISAs were developed using the local vv IBDV isolate for ELISA coating. The use of local strain in developing ELISA has advantages over the commercially available ELISA. According to Jackwood *et al.* (1999), the antigen used in the ELISA can affect the titre to different antigenic types of IBDV.

The ability of the commercial ELISA to predict protection to IBD may not be as accurate today because of the emergence of antigenic variants (Jackwood, *et al.*, 1999) and vv strain of IBDV (Chettle, *et al.*, 1989: Stuart, 1989; Van Den Berg, *et al.*, 1991). The use of a local strain in developing ELISA may enhance the accuracy to predict IBD protection .

The ELISA for antibody detection has already been proven as a useful tool for monitoring poultry health. However, interpretation of results is often still a great challenge (Snyder and Marquardt, 1989). This is because flock antibody levels can be influenced by many related factors, such as natural infection, vaccination and age of chickens. In addition, antibody titres as measured by ELISA are often more indicative of overall exposure to a particular agent than they are an estimate of absolute virulent challenge against it.

The diagnostic tool of DAS ELISA for antigen detection was successfully optimized and developed. This DAS was able to detect various IBDV field isolates from the bursa tissue and various IBDV vaccine strains. The absorbance values of below cut-off level were obtained in the NDV and CAV tissues, indicating the IBDV was undetected and the DAS ELISA was highly specific. Therefore, it has the potential to be used as routine diagnostic test to detect IBDV. The DAS ELISA has advantages over the indirect ELISA because the purified antigen for coating is unnecessary and it can be used both for antigen and antibody detections. Moreover, the correlation coefficient between DAS and IDEXX commercial ELISA in this study showed higher correlation when compared to indirect ELISA. Therefore, DAS ELISA is a better choice as diagnostic tool for screening large number of antibody and antigen samples for epidemiology studies.

In conclusion, the developed indirect and DAS ELISA for antibody detection were highly significant correlated ($p<0.01$) when compared to commercial IDEXX ELISA. Meanwhile, the DAS ELISA can also be used for antigen detection and it was highly specific. Therefore, both the indirect and DAS ELISA are valuable as diagnostic tools to detect antigen and measuring flock antibody to IBD.

REFERENCES

Barbour, E. K., Brinton, M. K., Torkelson, S. D., Johnson, J. B. and Poss, P. E. (1991). An enzyme-linked immunosorbent assay for detection of *Bordetella avium* infection in turkey flocks: sensitivity, specificity and reproducibility. *Avian Dis.* **35**:308-314.

Cardosa, T. C., Sousa, R. L. M., Alessi, A. C., Montassier, H. J. and Pinto, A. A. (1998). A double antibody sandwich ELISA for rapid diagnosis of virus infection and to measure the humoral response against infectious bursal disease on clinical material. *Avian Pathol.* **27**: 450-454.

Carlson, H. E., Lindberg, A. A. and Hammarstorm, A. (1972). Titration of antibodies to Salmonella on antigen by enzyme-linked immunosorbent assay. *Infect. Immun.* **6**:703-708.

Chettle, N., Stuart, J. C. and Wyeth, P. J. (1989). Outbreak of virulent infectious bursal disease in East Anglia. *Vet. Rec.* **125**:271-272.

Jackwood, D. J., Sommer, S. E. and Odor, E. (1999). Correlation of enzyme-linked immunosorbent assay titers with protection against infectious bursal disease virus. *Avian Dis.* **43**:189-197.

Kumar, A. and Rao, A. T. (1991). Double-antibody sandwich ELISA for detection of Infectious bursal disease virus. *Bri. Vet. J.* **147**: 251-255.

Marquardt, W.W., Johnson, R. B., Odenwald, W. F. and Schlotthober, 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.

Mohamed, K. Hassan, Saif, Y. M. and Shawky, S. (1996). Comparison between antigen capture ELISA and conventional methods used for titration of infectious bursal disease virus. *Avian Dis.* **40**:562-566.

Odor, E. M. (1995). ELISA Serology, Application to Infectious bursal Disease in Broiler production. In : Proc. of International Poultry Symposium Summit on Infectious Bursal Disease, University of Georgia, April 3-4, pp 104- 105.

Snyder, D. B., Marquardt, W. W., Mallinson, E. T , Savage, P. K. and Allen, D. C. (1984). Rapid serological profiling by enzyme-linked imunosorbent assay. III. Simultaneous measurements of antibody titer to infectious bronchitis, infectious bursal disease and Newcastle disease viruses in a single serum dilution. *Avian Dis.* **28**:12-24.

Snyder, D. B., Marquardt, W. W., Mallison, E. T., Russek-Cohan, E., Savage, P. K. and Allen, D. C. (1986). Rapid serologic profiling by enzyme linked immunosorbent assay. IV. Association of infectious bursal disease serology with broiler flock performance. *Avian Dis.* **30:**139-148.

Snyder, D. B. and Marquardt, W. W. (1989). Enzyme immunoassay for poultry disease monitoring. In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens. (3rd Edition). American Association of Avian Pathologists, pp 201-207.

Solano, W., Giambrone, J. J. and Panangala, V. S. (1985). Comparison of kinetic based enzyme-linked immunosorbent assay (KELISA) and virus-neutralization test for infectious bursal disease virus. I. Quantitation of antibody in white leghorn hens. *Avian Dis.* **29**:662-671.

Stuart, D. E . (1989). Acute infectious Bursal disease in poultry. *Vet. Rec.* 125:281.

Van Den Berg, T. P., Gonze, M. and Meulemans, G. (1991). Acute infectious bursal disease in poultry: isolation and characterization of highly virulent strains. *Avian Pathol.* **20**:133-143.

Weisman, J. and Hitchner, S. B. (1978). Virus-neutralization versus agar-gel precipitation tests for detecting serologic response to infectious bursal disease virus. *Avian Dis.* **22:**598-603.

RINGKASAN

DEVELOPMENT OF INDIRECT AND DOUBLE ANTIBODY SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY FOR INFECTIOUS BURSAL DISEASE

Indirect dan double antibodi sandwich (DAS) asai immunoerap terangkai enzim (ELISA) telah berjaya dicipta berdasarkan penggunaan isolat tempatan P97/302 penyakit bursa berjangkit yang amat virulen (vvIBDV), yang bertujuan untuk mengesan antibodi dan antigen terhadap IBD. Formula regresi untuk indirect ELISA ialah Log_{10} Titre = -1.1495(1/SP) + 4.5349 dengan R^2=0.975. Manakala DAS ELISA pula ialah Log_{10} Titre= 2.6527Log10(SP) + 3.0853 dengan R^2=0.93. Sampel sera yang diperolehi dari ladang ayam komersial dan ayam bebas-patogen khusus (SPF) telah digunakan untuk penilaian IDEXX komersial, indirect dan DAS ELISA tersebut. Keputusan kajian menunjukkan kolerasi ($p<0.01$) yang sangat ketara di antara IDEXX dan indirect ELISA, iaitu R =0.88, manakala IDEXX dan DAS ialah R=0.93. DAS ELISA yang dibentuk untuk mengesan antigen IBD juga didapati sangat khusus. Ia boleh mengesan berbagai jenis IBDV isolat tempatan sama ada dari tisu bursa ataupun vaksin strain.