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A type-specific blocking ELISA for the detection of infectious bronchitis virus antibody

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A B S T R A C T

Infectious bronchitis virus (IBV) infection has been a major threat to the poultry industry worldwide. Current commercially available ELISA kits detect group-specific antibodies; however, to understand the status of field infection, a monoclonal antibody (mAb) blocking ELISA (b-ELISA) against local IBVs was developed. The selected mAb showed specificity to Taiwan IBV strains but had no cross-reactivity with the vaccine strain H120. Using the hemagglutination inhibition (HI) test as a standard, the cut-off value, sensitivity, and specificity of a b-ELISA using this mAb were evaluated in 390 field samples. The type-specificity of detection was validated using a panel of chicken hyperimmune sera. The results showed that the b-ELISA demonstrated high sensitivity (98.0%) and specificity (97.2%) of detection. The agreement between the results of the b-ELISA and the HI test was statistically significant (Kappa = 0.95), and there was no significant difference between these two methods (McNemar $p = 0.72$). The b-ELISA specifically detected Taiwan IBV serotypes but not three non-Taiwan IBV serotypes nor sera against other avian pathogens. This b-ELISA provides type-specific antibody detection of local IBV strains. It has the potential to serve as a rapid and reliable diagnostic method for IBV clinical infections in the field in Taiwan.

1. Introduction

Infectious bronchitis virus (IBV) belongs to the family *Coronaviridae*, group 3 Coronavirus, and has been a major pathogen affecting the global poultry industry. IBV infects chickens of all ages and causes lesions in respiratory and urogenital organs (Cavanagh, 2007; Cook, 2002). Clinical syndromes include growth retardation in broilers, a drop in egg production in layers, and heavy mortality in poultry (Cavanagh and Naqi, 2003). In addition to hundreds of known serotypes, new viral variants have emerged due to rapid viral evolution and antigenic variation in avian coronaviruses (Cavanagh, 2005; Cavanagh and Naqi, 2003). Despite regular vaccine use, IBV outbreaks occur frequently, and owners of infected flocks suffer from tremendous economic losses.

A number of ELISA tests for IBV antibody detection have been described (Bronzoni et al., 2005; De Wit, 2000; De Wit et al., 1997; Marquardt et al., 1981; Perrotta et al., 1988; Zellen and Thorsen, 1986, 1987). Because it is a simple, rapid, sensitive, and large-scale tool, ELISA has been used widely in IBV serological profiling. The reaction antigen may be either whole virion or recombinant subunits. In previous works, recombinant spike (Wang et al., 2002) and nucleocapsid protein (Chen et al., 2003; Lugovskaya et al., 2006) expressed in bacteria or insect cells have been used as a coating antigen to improve the efficacy of detection. In addition, an ELISA specific to IBV-IgM was described to detect an early infection (De Wit et al., 1998).

The first isolate of IBV in Taiwan was identified in 1958. Taiwan IBVs have been characterised molecularly and grouped into two populations, Taiwan group I (TW-I) and Taiwan group II (TW-II), both of which exist as exclusive virus populations globally (Huang et al., 2004; Wang and Tsai, 1996). Inoculation with Massachusetts (Mass)-type strains such as M41 and H120 has been implemented widely in Taiwan to control IBV for several decades. However, it has been a challenge to perform a sero-surveillance study of IBV in Taiwan due to the lack of serotype-specific diagnostic tools. The current commercially available ELISA kits detect group-specific antibodies (Gelb and Jackwood, 2008) and are not able to identify antibodies specific to local IBV strains responsible for outbreaks.

For the purpose of understanding field infection with IBV, a reliable and type-specific antibody detection method against local IBVs is needed. In the present work, a monoclonal antibody (mAb)-based blocking ELISA (b-ELISA) was developed and validated. In addition, the sensitivity, specificity, precision, repeatability, and linearity of the assay were evaluated with field samples and compared with a standard method of detection.

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2. Materials and methods

2.1. Viruses

IBV isolates 2575/98 (TW-I) (Huang et al., 2004), 2296/95 (TW-II) (Huang et al., 2004), 3263/04 (TW-II) (Chen et al., 2009), and vaccine strain H120 (Abic Biological Laboratories Teva Ltd., Israel) were propagated in 9–11 day-old specific pathogen free (SPF) chicken embryos (Animal Health Research Institute, Council of Agriculture, Tamsui, Taiwan) inoculated via the allantoic route. Briefly, eggs were inoculated with 0.2 ml of virus-infected allantoic fluid (containing approximately $10^{4.4}$ EID_{50}) and incubated at 37 °C for 48 h. Eggs that died within 24 h were discarded. The harvested allantoic fluid (AF) was clarified by centrifuging at 3000 × *g* for 20 min, and the virus was pelleted by centrifuging at 48,000 × *g* for 2 h. The pellet was resuspended in TEN buffer (10 mM Tris-base, 1 mM EDTA, and 100 mM NaCl). The virus solution was layered onto a sucrose gradient solution (20–50% in TEN buffer) and centrifuged at 100,000 × *g* for 2 h. The resulting virus band was pelleted by centrifugation at 100,000 × *g* for 2 h, recovered in TEN buffer and stored at −80 °C. In this study, sucrose gradient-purified viruses were used as the protein antigens in ELISAs and Western blots. The protein concentration of the purified viruses was quantified by the Bradford protein assay (Bio-Rad, Hercules, CA) according to the manufacturer's recommendations.

2.2. Production of monoclonal antibodies

Four 12-week-old female BALB/c mice were immunised intraperitoneally with 1 ml of IBV 2575/98 ($10^{7.4}$ EID_{50}/ml) five times with a two-week interval. Mice received a final booster of 0.1 ml of IBV 2575/98 ($10^{7.4}$ EID_{50}/ml) by intrasplenic injection three days prior to hybridoma fusion. Splenocytes from the mice were fused with Sp2/mIL-6 myeloma cells to produce hybridomas according to the standard protocol (Harlow and Lane, 1988). Only the fused cells grew in hypoxanthine–methotrexate–thymidine selection medium. Positive hybridomas were screened by indirect immunofluorescence assay (IFA) and Western blot (described below). Following three rounds of sub-cloning, selected clones were inoculated intraperitoneally into BALB/c mice primed with incomplete Freund's adjuvant, resulting in the production of ascitic fluid containing mAb. The mAb isotype was analysed with an IsoStrip Isotyping Kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's recommendations. The care and use of mice was approved by the Institutional Animal Care and Use Committee, National Taiwan University to ensure compliance with the local legal and ethical requirements.

2.3. Indirect immunofluorescence assay

Primary chicken embryo kidney (CEK) cells from 19-day-old SPF chicken embryos were prepared according to standard techniques (Schat and Sellers, 2008). For IFA, a CEK cell suspension (100 μl) containing 1×10^4 cells was added to each well of a Corning CellBIND 96-well plate (Corning Inc., Corning, NY). Following overnight incubation, cells were infected with IBV 2575/98 for 24 h. Subsequently, the monolayers were fixed with 80% (v/v) cold acetone for 20 min. Fifty microlitres of hybridoma culture supernatant was added to the monolayers and were incubated at room temperature for 1 h. The cells in each well were then washed with PBS and stained with 50 μl of fluorescein-conjugated goat anti-mouse IgG, IgA, and IgM (ICN Pharmaceuticals, Cleveland, OH) at a 1:1000 dilution at room temperature for 1 h. Results were examined under a fluorescence microscope.

2.4. Indirect ELISA

Flat-bottomed microtitre plates (Nunc, Denmark) were coated with 300 ng of purified IBV 2575/98, 2296/95, H120 viral antigens, and control antigen (SPF-AF) diluted in coating buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6) and incubated at room temperature overnight. The wells were washed three times with PBST (0.1% (v/v) Tween 80 in PBS) and blocked with blocking buffer (5% (w/v) skim milk in PBST) at 37 °C for 1 h. Following three washes, the wells were incubated at room temperature for 1 h with 100 μl of either mAb ascites harvested in this study or a 1:200 dilution of chicken antiserum against IBV 2575/98 in blocking buffer. Following three additional washes, 100 μl of either peroxidase-conjugated affinipure goat anti-mouse IgG (H+L) (Jackson ImmunoResearch, West Grove, PA) or peroxidase-labelled affinity purified antibody to chicken IgY (KPL, Gaithersburg, MD) diluted 1:2500 in blocking buffer was dispensed into each well and incubated at room temperature for 1 h. The wells were washed three times, incubated with 100 μl of SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL), and colour was allowed to develop in the dark at room temperature for 10 min. The reaction was stopped by the addition of 100 μl of TMB stop solution (KPL). The optical density (OD) at 450 nm was read using an automated plate reader (Bio-Tek EL312e reader, Bio-Tek Instruments).

2.5. SDS-PAGE and Western blot

For each sample, 1 μg of purified IBV 2575/98, 2296/95 and H120 viral antigens was mixed with an equal volume of SDS-PAGE sample buffer (100 mM Tris–HCl, 200 mM DTT, 4% SDS, 0.02% bromophenol blue, 20% glycerol, pH 6.8) and boiled for 10 min. The denatured protein solution was separated on an 8% discontinuous SDS-PAGE. Protein gels were either stained with Coomassie brilliant blue R-250 (Amresco, Solon, OH) or transferred onto a 0.45 μm nitrocellulose membrane (Whatman GmBH, Dassel, Germany). The membrane was blocked with blocking buffer at room temperature for 1 h and then soaked with mAbs diluted 1:500 in blocking buffer. After washing three times with wash buffer (0.05% (v/v) Tween 20 in PBS), the membrane was incubated at room temperature for 1 h with peroxidase-conjugated affinipure goat anti-mouse IgG (H+L) (Jackson ImmunoResearch) diluted 1:2500 in blocking buffer. Following an additional three washes, detection was performed using TMB Membrane Peroxidase Substrate (KPL).

2.6. HA antigen preparation, HA test and HI test

In this study, hemagglutination (HA) antigen was prepared using IBV 3263/04 (TW-II) because it is cross-reactive among Taiwan serotypes of IBV. Virus-infected allantoic fluid was collected and clarified by centrifugation at 4 °C at 3000 × *g* for 20 min. Virus was concentrated by pelleting at 48,000 × *g* for 2 h and the pellet was resuspended to 1/100 the original volume with PBS. The virus suspension was treated with neuraminidase (N-2876, Sigma, St. Louis, MO) at a final concentration of 200 mU/ml at 37 °C for 2 h. The resulting HA antigen was aliquoted and stored at −20 °C. The HA test and hemagglutination inhibition (HI) test were performed using the standard method (OIE, 2009). Briefly, the prepared HA antigen was diluted two-fold with PBS and reacted with a 1% suspension of chicken erythrocytes at 4 °C for 40 min in V-bottomed microtitre plates (Nunc). Four HAU of virus antigen was used for the HI test. HI titres were expressed as $\log_2$ of the reciprocal of the highest sample dilution representing complete hemagglutination inhibition. Samples were regarded as positive if they had HI titres of 2^4 or more. For HA and HI tests, all reagents were kept on ice, and procedures were carried out at 4 °C.

2.7. ELISA tracer preparation

IgG in mAb ascites was affinity-purified using Protein A Columns (Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. After quantification, 0.1 μg of purified mAb was labelled with horseradish peroxidase (HRP) using the SureLINK HRP Conjugation Kit (KPL) in preparation for use as the tracer in the b-ELISA described below. Purified mAb was then dialysed against PBS overnight at 4 °C using the Side-A-Lyzer Dialysis Cassette (10K MWCO, Pierce).

2.8. Optimisation of antigen and tracer dilutions using direct ELISA

Varying amounts (100, 300 or 500 ng) of IBV 2575/98 were coated onto flat-bottomed microtitre plates (Nunc) at room temperature overnight. The wells were washed and blocked as stated in the procedure for indirect ELISA. Serially diluted tracer diluted serially (ranging from 1:125 to 1:1000) was added to each well, and the wells were incubated at room temperature for 1 h. After three washes, the wells were filled with 100 μl of SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL), and colour was allowed to develop in the dark at room temperature for 10 min. The reaction was stopped by the addition of 100 μl of TMB stop solution (KPL). The OD at 450 nm was read using an automated plate reader.

2.9. Blocking ELISA

Positive and negative control samples were included on each plate for b-ELISAs. Positive control samples were pooled from six HI-positive field samples and negative control samples were pooled from six HI-negative field samples. The reaction duration, temperature, and dilution fold of the serum were optimised for the b-ELISA conditions reported herein. The final condition as described below was determined from the reaction that produced the highest P/N ratio.

IBV 2575/98 was used as the coating antigen in the b-ELISA. The wells of flat-bottomed microtitre plates (Nunc) were coated with 300 ng of virus diluted in coating buffer and incubated at room temperature overnight (at least 16 h). The plate was washed three times with PBST and then blocked with blocking buffer at 37 °C for 1 h. Following three further washes, 100 μl of each test sample or positive/negative control samples diluted 1:25 in blocking buffer was added to the wells, and the plates were incubated at room temperature for 1 h. Plates were washed in triplicate, 100 μl of the tracer diluted 1:500 in blocking buffer was dispensed into each well, and the plates were incubated at room temperature for 1 h. After washing three times, the wells were filled with 100 μl of SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL), and colour was allowed to develop in the dark at room temperature for 10 min. The reaction was stopped by the addition of 100 μl of TMB stop solution (KPL). The OD at 450 nm was read using an automated plate reader, and the OD values were converted to the percentage of inhibition (PI) according to the following formula: $PI = 100\% \times [1 - (\text{OD of test sample} - \text{OD of positive control})/(\text{OD of negative control} - \text{OD of positive control})]$.

2.10. Determination of cut-off value, sensitivity, and specificity of the blocking ELISA

A total of 390 sera or plasma samples were collected from 33 poultry flocks in central and southern Taiwan in 2009 from breeds of chickens including broiler, broiler breeder, and Taiwan country chicken. Most farms underwent regular vaccination with Mass-type IBV. HI tests against IBV 3263/04 performed as indicated above were used as the standard detection method, and all collected samples were subjected to this test. Fifty-three HI-negative samples from chickens aged 5–16 wk were used for the determination of the cut-off value. The cut-off value was calculated to be two standard deviations above the mean PI. An additional panel of 196 HI-positive samples from chickens aged 5–25 wk was employed in the sensitivity calculation for the b-ELISA and 141 HI-negative samples from chickens aged 3–9 wk were used for the evaluation of specificity. In addition, the correlation coefficient between the antibody levels measured by b-ELISA and by HI test was analysed.

2.11. Validation of the blocking ELISA

A panel of chicken hyperimmune antisera was employed to evaluate the type-specificity of the b-ELISA, including 11 samples of chicken sera against Taiwan IBV strains (Wang and Huang, 2000), 3 samples of commercial chicken antisera of IBV Mass, Conn, JMK (Charles River, Raleigh, NC), 6 samples of commercial chicken antisera against avian influenza virus, Newcastle disease virus, reticuloendotheliosis virus, avian leucosis virus subgroup A, B, J (Charles River), and 3 samples of control sera collected from SPF chickens aged at 4, 9, and 22 wk. All sera were titrated using the ProFlock® IBV ELISA Kit (Synbiotics, San Diego, CA) and subsequently tested using the b-ELISA developed in this study. The precision of the b-ELISA was also evaluated. The intra-assay coefficient of variation was determined by testing three replicates of pooled positive samples. The inter-assay coefficient of variation was determined by performing the assay in triplicate 9 times. In addition, the deviations from the mean calculated from the OD values of the 27 repeats were plotted. The linearity of the assay was also examined using two-fold dilutions of the pooled positive samples. The values of PI were plotted against the dilution factor ($\log_2$).

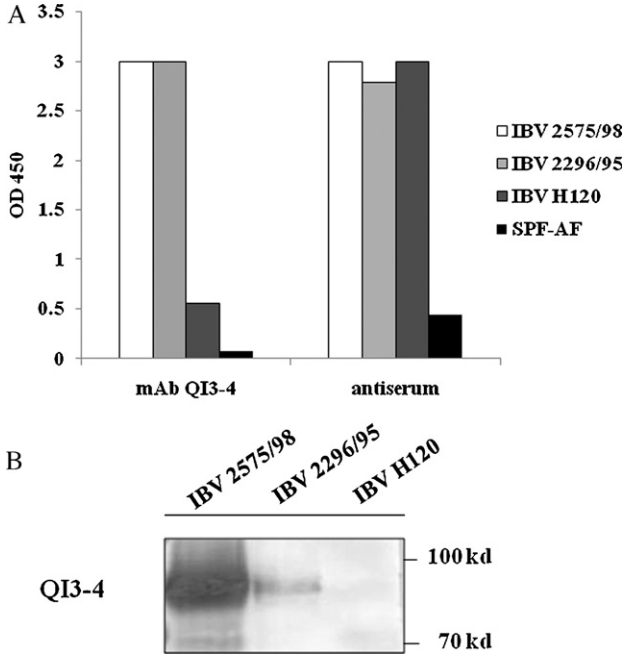

Fig. 1. Cross-reactivity of mAb QI3-4 with different IBV strains was assessed by indirect ELISA (A) and Western blot (B), as described in Section 2. QI3-4 showed high reactivity with Taiwan IBV strains (2575/98, TW-I; 2296/98, TW-II) and relatively low affinity for IBV H120 and uninfected material (SPF-AF). SPF-AF: allantoic fluid from specific pathogen free chicken embryos. Antiserum: chicken hyperimmune serum against IBV 2575/98. * OD value exceeds 3.

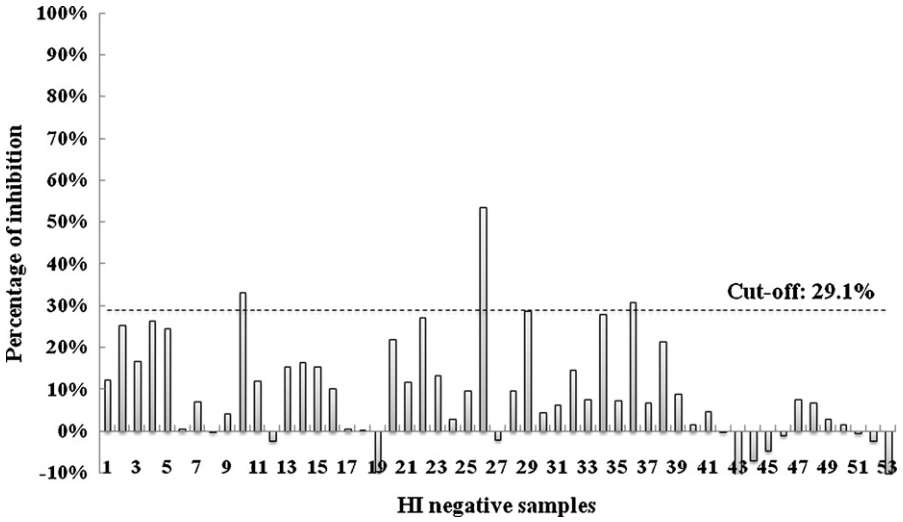

Fig. 2. The cut-off value was 29.1%, calculated as two standard deviations above the mean percentage of inhibition from a total of 53 hemagglutination inhibition (HI) test-negative field samples.

2.12. Statistics

Confidence interval values of sensitivity and specificity were calculated by an efficient-score method with continuity correction (Newcombe, 1998). The Kappa value and the 95% confidence interval values between the HI test and the b-ELISA were calculated using the Kappa statistic with a web-based program (http://faculty.vassar.edu/lowry/kappa.html) (Rosner, 2006). McNemar's test was employed to evaluate the consistency of the two methods.

3. Results

3.1. Monoclonal antibody

mAb QI3-4 was identified by a series of sub-cloning and hybridoma screens (data not shown). The cross-reactivity of mAb QI3-4 against IBV strains was assessed by indirect ELISA and Western blot. As indicated in Fig. 1A, QI3-4 showed strong positive interaction with Taiwan IBV strains (2575/98, TW-I; 2296/98, TW-II) and relatively low affinity with IBV H120 and uninfected material (SPF-AF). In the Western blot (Fig. 1B), QI3-4 recognised a ~95 kDa protein from IBV 2575/95 and IBV 2296/98. No HI activity was observed with this mAb. By isotype characterisation, the QI3-4 heavy chain belonged to IgG_{2b}, and the light chain was a κ chain. HRP-labelled QI3-4 was used as the tracer in b-ELISAs and was designated as QI3-4-HRP.

3.2. Cut-off value, sensitivity, and specificity of the blocking ELISA

The mean and standard deviation of the PI from the 53 HI-negative samples tested were 9.5% and 9.8%, respectively. The cut-off value, calculated to be two standard deviations above the mean, was 29.1% (Fig. 2). Table 1 shows the antibody detection

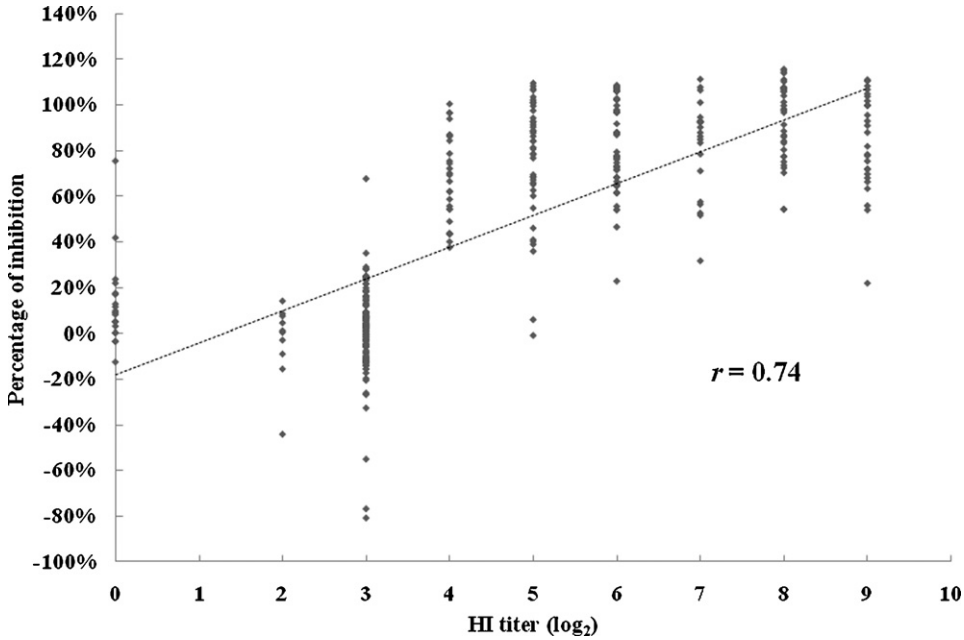

Fig. 3. In a total of 337 field samples examined in this study, the correlation coefficient between the results of the blocking ELISA and hemagglutination inhibition (HI) test was 0.74.

Table 1
The antibody detection results from field samples by blocking ELISA and hemagglutination inhibition (HI) test.

		HI test	
		+	–
Blocking ELISA	+	192	4
	–	4	137

results from the HI test and b-ELISA of collected field samples. The sensitivity of the b-ELISA was 98.0% (95% confidence interval, 96.0–100.0%), evaluated using 196 HI-positive samples. The specificity was 97.2% (95% confidence interval, 94.4–100.0%), calculated using 141 HI-negative samples. The Kappa value was 0.95 (95% confidence interval, 0.92–0.98), indicating high reproducibility between the HI test and b-ELISA. There was no significant difference between the two methods, as analysed by McNemar's test ($p = 0.72$). In addition, the correlation coefficient between antibody levels measured by the HI test and b-ELISA was 0.74 (Fig. 3).

3.3. Validation of the blocking ELISA

The type-specificity of the b-ELISA was validated using a panel of chicken hyperimmune antisera. For all of the IBV antisera, the antibody levels were verified with a commercially available ELISA kit and the obtained titres ranged from 362 to 10,605 (Table 2). When tested using the b-ELISA, all of the Taiwan IBV anti-sera (TW-I and TW-II) were accurately detected as positive (i.e., PI values exceeded 29.1%), whereas sera from three non-Taiwan IBV serotypes, non-IBV avian pathogens and the control sera were detected as negative (i.e., PI values were less than 29.1%).

The intra-assay coefficient of variation was 8.3%. The inter-assay coefficient of variation was 15.4%. In addition, nearly all (26/27) of the OD values from replicates used in the precision assay fell within ±2 standard deviations from the mean (data not shown).

Table 2
Chicken hyperimmune sera used in the validation of the blocking ELISA.

Category	Hyperimmune serum	PI[a] (blocking ELISA)	Titre[b] (commercial ELISA)
IBV TW-I[c]	1171/92	84.4%	2994
	1246/92	91.8%	784
	1449/92	89.4%	4847
	1927/94	83.5%	1697
	1928/94	84.7%	803
	1960/94	106.0%	6071
	2575/98	101.9%	1022
	2651/99	32.8%	9555
IBV TW-II[d]	2012/94	95.0%	1803
	2296/95	98.1%	4497
	2300/95	64.3%	401
Non-Taiwan IBV	Mass	21.9%	10,605
	JMK	23.7%	1176
	Conn	27.9%	362
Non-IBV	AIV	0.7%	1
	NDV	1.4%	36
	ALV-A	−0.1%	10
	ALV-B	1.9%	4
	ALV-J	4.1%	24
	REV	0.3%	0
	Control serum (4 wk)	−4.6%	0
	Control serum (9 wk)	−14.5%	0
	Control serum (22 wk)	−25.3%	0

[a] PI: percentage of inhibition. The cut-off value of the blocking ELISA was 29.1%.
[b] The ProFlock® IBV ELISA Kit was used. A titre of more than 164 was considered positive.
[c] TW-I: Taiwan group I.
[d] TW-II: Taiwan group II.

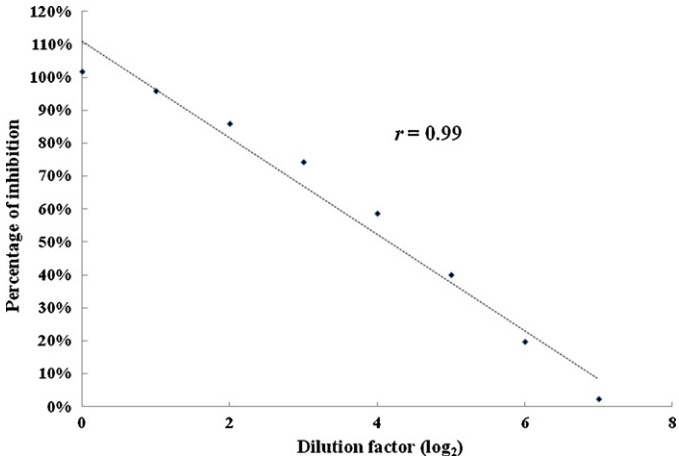

Fig. 4. The linearity assay of the blocking ELISA was determined to be linear within the dynamic range of the test and had a high correlation coefficient ($r = 0.99$).

Finally, the linearity assay of the b-ELISA was determined to be linear within the dynamic range of the test and had a high correlation coefficient ($r = 0.99$) (Fig. 4).

4. Discussion

In the present study, a serotype-specific ELISA was developed to characterise the status of IBV field infection. Unlike other group-specific ELISAs, this ELISA was able to detect antibodies specific to Taiwan IBVs by using a strain-specific mAb as a tracer. Compared with other serotype-specific tests, the virus neutralisation test (VN) and the HI test, which are more laborious and time-consuming, this new ELISA provides a more efficient and economical alternative to serotype-specific antibody detection.

Many aspects were considered in the evaluation of the b-ELISA. First, the cut-off value of the b-ELISA was determined in comparison to the HI test and employed in the examination of a number of clinical samples. This b-ELISA demonstrated high sensitivity and specificity of detection (both higher than 97%). It was found that agreement between these two methods was statistically significant (Kappa = 0.95) and there was no significant difference between them (McNemar $p = 0.72$). Second, a panel of chicken hyperimmune sera against several IBV serotypes and other avian pathogens was used to validate the detection accuracy of the b-ELISA. The precision, repeatability, and linearity of the assay were also shown to fall within the acceptable range and linearity. Furthermore, all of the samples used in this study for determining the cut-off value, sensitivity, and specificity of the b-ELISA were collected from local poultry farms. The ages of sampled chickens ranged from 3 to 25 wk and were distributed evenly in each group of detection. Detection of IBV in the field samples reflects the clinical status of the animals at the time of the sample collection, and the specificity of our assay will aid in the diagnosis and control of this disease in the local chicken populations.

In an attempt to develop a Taiwan IBV serotype specific ELISA, mAbs were chosen based on the specificity limited to Taiwan IBV strains rather than to the vaccine strain H120. In this study, the immunofluorescence technique against IBV-infected primary cells was used to screen mouse hybridomas among a large pool of fused spleen cells. Once IBV-specific hybridomas were identified, indirect ELISA and Western blot were employed to select Taiwan IBV specific hybridomas. The molecular weight of the protein detected using mAb QI3-4 in Western blots corresponds to the spike protein subunit of IBV (S1: 90 kDa and S2: 84 kDa) (Cavanagh, 1983). However, the identification of the exact epitope of the mAb awaits further study.

Two serotype-specific ELISAs for IBV antibody detection were described previously (Karaca and Naqi, 1993). Selected mAbs were employed in ELISAs to detect antibodies specific to IBV serotypes Ark and Mass. However, in the characterisation of these antibodies, neither cut-off value nor further evaluation and application to field samples were described. Additionally, in that work, test sera were not diluted before use. This is a less economical use of sera when compared to the 25-fold dilution of sera used in the present b-ELISA. Furthermore, the direct conjugation of the colour-developing enzyme HRP to the mAb used as a tracer reduces the incubation time by negating the need for a secondary antibody. If the plates are pre-coated and blocked, the whole reaction can be completed within two and a half hours. Given that variations in incubation time and temperature between individual runs of an ELISA affect the results (Perrotta et al., 1988), the reaction condition of this b-ELISA was optimised to achieve a highest P/N ratio.

In Taiwan, IBV infection in the field is fairly common. From a sero-surveillance perspective, the use of b-ELISA has a number of advantages over the HI test. The b-ELISA allows for the large-scale screening of field samples. The interpretation of the results is automated and objective. All of the essential reagents can be prepared in a large volume and stored in aliquots, allowing reaction conditions to be standardised from batch to batch. In addition, only 4 μl of test sample is required for a single detection by b-ELISA, whereas a standard HI test requires 25 μl of test sample to perform the serial dilution. The laborious preparation of chicken erythrocytes for the HI test can be also avoided by using b-ELISA. Unlike the end-point result produced by the HI test, the b-ELISA produces a continuous OD value and a more precise detection of antibody levels, and a recombinant subunit protein may be prepared as a substitute for the coating antigen to reduce the labour required for virus preparation.

It is also worth noting that there was a relatively low correlation coefficient between our b-ELISA and the HI test ($r = 0.74$). This could be explained by differences in the target antibodies detected by the two tests. Specifically, the HI test identifies the HI antibody, whereas the coating antigen (whole virion) of the b-ELISA reacts with a general population of antibodies. In addition, the mAb used in the b-ELISA is not reactive in HI tests, implying that the epitope directing interaction with mAb QI3-4 is distinct from those recognised by the HI antibody.

This new b-ELISA allows type-specific antibody detection of local IBV strains. It has the potential to serve as a rapid and reliable diagnostic method for the characterisation of IBV clinical infections in the field and may greatly benefit the survey of IBV sero-prevalence in Taiwan.

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