한국가금학회지 제 37권 제 2호, 167~172 (2010)
Korean J. Poult. Sci. Vol. 37, No.2, 167~172 (2010)

167

Rapid Detection of Infectious Bursal Disease Virus (IBDV) in Chickens by an Immunochromatographic Assay Kit

Kang-Seuk Choi[1,†], Jin Sik Oh[2], Woo Jin Jeon[1], Keon Sok Na[2], Eun Kyoung Lee[1], Youn Jeong Lee[1], Hwan Woo Sung[3], Gun Woo Ha[2] and Jun Hun Kwon[1]

[1]Avian Diseases Division, National Veterinary Research and Quarantine Service, Anyang 430-757, Korea
[2]BioNote, Inc., Hwasung 445-170, Korea
[3]Kangwon National University, Chuncheon 200-701, Korea

ABSTRACT An immunochromatograhy (IC) based infectious bursal disease virus (IBDV) detection kit, which employed two anti-IBDV VP2 monoclonal antibodies, was evaluated for rapid diagnosis of infectious bursal disease virus (IBD). The detection limit of the IC kit for IBDV was $10^{3.1}$ to $10^{3.9}$ EID_{50}/mL, indicating that the IC kit detected IBDV sensitively as same as double antigen capture ELISA but less than a RT-PCR assay. The IC kit did not detect other viral pathogens such as Newcastle disease virus, infectious bronchitis, avian influenza virus, and infectious larynotracheitis virus. When applied to tissue samples of experimental chickens died 3 or 4 days post infection after very virulent IBDV (strain Kr/D62) infection, the IC kit detected IBDV in all samples of the bursa of Fabricius, spleen, kidney, cecal tonsil and in 87.5%, 37.5% and 0% of liver, thymus and proventriculus samples. In particular, BF tissue samples showed stronger signal bands than other tissues. Positive signal was observed. All except for one thymus sample of samples having negative results by the IC kit showed the same result with DAS-ELISA but RT-PCR assay detected IBDV in some of IC kit negative samples of thymus and proventriculus. When swab samples from the bursa of Fabricius of dead chickens (n=231) on field farms were tested, the sensitivity and specificity of the IC assay relative to RT-PCR was 100% (109/109) and 97.5% (119/122), respectively and kappa value between both assay was 0.97. The kit can provide a useful aid for rapid detection of IBDV in chickens under field circumstances.

(Key words : infectious bursal disease virus, immunochromatography, rapid detection)

INTRODUCTION

Infectious bursal disease (IBD), caused by infectious bursal disease virus (IBDV) is an acute and highly contagious viral disease in chickens and causes major economic losses in the poultry industry worldwide. The principle target organ for IBDV replication is the bursa of Fabricius (Lukert and Saif, 1997). IBDV infects the precusors of antibody-producing B cells in the bursa of Fabricius (BF), which causes severe immunosuppression and mortality for young chickens.

IBDV is a member of the *Avibirnavirus* genus in the family *Birnaviridae* (Lukert and Saif, 1997). The IBDV causes severe immunosuppression and mortality for chickens by destruction of immature B cells in the BF. There are two serotypes of IBDV (serotypes 1 and 2). Strains of serotype 1 IBDV causes clinical disease in chickens whereas serotype 2 viruses are avirulent for chickens (Ismail et al., 1988).

Very virulent strains of IBDV (vvIBDV) with high mortality have been the source of great economic losses in the poultry industry in Asia, Africa, and South America (Chettle et al., 1989, Di Fabio et al., 1999, Jackwood and Sommer-Wagner, 2007) whereas antigenic variant continues to be a problem in the poultry industry in North America (Jackwood and Sommer-Wagner, 2005).

Several immunoassays have been developed for the detection of IBDV in clinical samples from chicken flocks suspected of IBD. These assays include agar gel immunodiffusion (AGID), antigen-capture enzyme-linked immunosorbent assay (AC-ELISA), reverse transcription-polymerase chain reaction (RT-PCR) (Jackwood, 2004; Wu et al., 2007). However, those methods have limited application in clinical diagnosis because they require a laboratory equipped with special instruments and professional technicians. Development of an immunochromatography (IC) based assay for rapid detection of IBDV was reported (Zhang

---

et al., 2005). Recently a commercial IC kit (Anigen Rapid IBDV Ag Test Kit) is now commercially available but are not evaluated for applicability in diagnosing IBD in field yet.

In this study, we evaluated the commercial IC kit for rapid detection of IBDV whether the kit has diagnostic utility in clinical samples from chickens suspected of IBD.

MATERIALS AND METHODS

1. Viruses

Two strains (Winterfield2512 and Kr/D62) of IBDV were used in this study; the Winterfield2512 strain is commonly used as vaccine strain and the Kr/D62 strain is a field strain belonging to vvIBDV. Newcastle disease virus (B1 strain), H9N2 avian influenza virus (MS96 strain), infectious bronchitis virus (H120 strain), and infectious laryngotracheitis virus (Sterwin strain) were also used for specificity test. All viruses were supplied from the National Veterinary Research & Quarantine Service (NVRQS) in Korea. These viruses were propagated and titrated using embryonating chicken eggs from specific pathogen free (SPF) chickens before use. Infective titers of IBDV were calculated by the reciprocal of virus dilution that can infect 50% SPF chicken embryos.

2. Tissue Samples from Experimentally IBDV-infected Birds

Eight SPF chickens died within 4 days after experimental challenge with very virulent IBDV strain Kr/D62 ($10^{5.1}$ EID_{50} per bird) were used. All of dead birds showed IBDV characteristic lesions such as atrophied bursa, and hemorrhage on small intestine, at the juncture of the proventriculus and gizzard, muscle, slightly enlarged spleen with necrotic foci on the membrane. Three SPF chickens of the same age were also used as control. Seven tissues including BF, cecal tonsil, spleen, kidney, proventriculus, liver and thymus were collected for all of infected and control birds. A 10% (spleen, kidney, proventriculus, liver and thymus) or 2% (BF and cecal tonsil) suspension of these tissue samples were applied for the IC kit (BioNote Inc., Korea) and double antigen sandwich (DAS)-ELISA (Jeon et al., 2008).

3. Sampling from Dead Birds on Farm

To further evaluate the applicability of the kit for the diag-

nosis of IBD under field conditions, a total of 231 dead birds between 3 and 6 weeks old were examined. Of them, 109 birds from two broiler farms A (n=47) and B (n=65) were confirmed as very virulent IBDV infection while the other birds from one broiler farm (farm C) (n=122) were diagnosed as no IBDV infection. Swab samples were collected from the BF of dead birds under field conditions and then resuspended in 1 mL 0.01 M PBS before use. The swab samples were tested for IBDV.

4. Immunochromatographic Kit

An immunochromatographic (IC) kit for detection of IBDV was supplied from BioNote, Inc., Korea (Fig. 1). The IC test kit was designed to detect IBDV in clinical samples based on the use of two anti-IBDV monoclonal antibodies (mAbs) 1 and 2. The IC test strip was assembled with sample pad, conjugate pad, nitrocellulose membrane, and adsorption pad. The antibodies are immobilized to three different regions; colloidal gold-IBDV mAb1 conjugate on the sample pad (S) zone, IBDV mAb2 on the test (T) line, and the rabbit anti-mouse IgG on the control (C) line. The IC test was performed by adding four drops (approximately $100 \sim 150\,\mu$L) of test sample solution to the S zone.
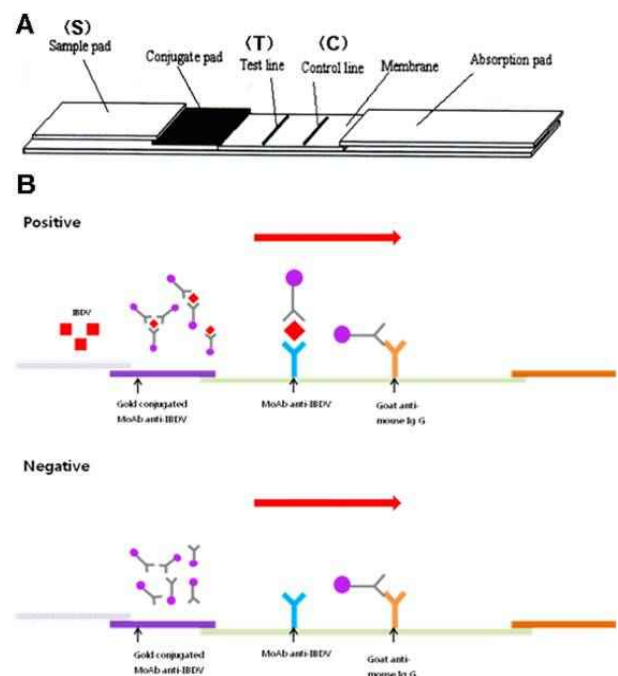
Fig. 1. Diagrams of the IBD diagnostic strip structure and principle. (A) Structure of the IBD diagnostic strip. (B) Principle for the IBD diagnostic strip detection.

The membrane was observed visually after a 10-min incubation period at room temperature. Two bands of T and C lines indicate the presence of the IBDV antigen while single band in the C line indicates the absence of IBDV antigen.

5. Double Antigen Sandwich ELISA

A double antigen sandwich ELISA for detection of IBDV (Jeon et al., 2008) was performed as follows; MaxiSorp™ ELISA plates (Nalge Nunc International, Roskilde, Denmark) were coated with 50 μL of IBDV-specific monoclonal antibody R63 at the concentration of 4 μg/mL in 0.01 M phosphate buffered saline (PBS), pH 7.4 at 37℃ for 1 h with constant shaking. Plates were washed three times with PBST (0.002 M PBS containing 0.05% tween 20) and then incubated with 50 μL of tissue homogenates diluted 1:5 in blocking buffer (0.01 M PBS, 0.02% tween 20, 3% skimmed milk) for 1 h at 37℃. BF homogenate from experimentally IBDV-infected chicken (NVRQS, Anyang, Korea) and BF homogenate from mock-infected SPF chicken were used as positive and negative controls, respectively. Following washing step, the plates were incubated at 37℃ for 1 h with hyperimmune anti-IBDV chicken serum diluted 1:1,000 in the blocking buffer. Following another washing step, the plates were incubated at 37℃ for 1 h with horseradish peroxidase (HRP)-labelled anti-chicken immunoglobulins antibody (100 ng/ mL) (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). Following another washing step, the plates were incubated for 10 min with the substrate TMB solution (Sigma, USA) and then colorimetric reaction was stopped by adding a 50 μL volume of 1.0 M HCl solution to all wells. Optical density (OD) readings were measured at the 450 nm wavelength using ELISA reader (TECAN, Research Triangle Park, NC, USA). Sample having OD value of 0.3 or greater was considered positive.

6. RT-PCR Assay

Total RNA from tissue or swab samples was extracted with RNeasy mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Approximately 1 μg of extracted RNA was used for reverse transcription-polymerase chain reaction (RT-PCR). Oligonucleotides for RT-PCR (forward primer 5'-ACAGGCCCAGAGTCTACACCATAA-3', reverse primer 5'-ATCCTGTTGCCACTCTTTCGTAGG-3') were designed for a 479 bp-amplification (position: nt 733~1212) of VP2 gene (Bar-

lic-Maganja et al, 2002). The RT-PCR was carried out with one-step RT-PCR kit (Qiagen) in the PCR machine (PTC-220, MJ-research, Waltham, MA, USA) under the following conditions: 45 min at 48℃ for reverse transcription and 2 min at 94℃ for AMV reverse transcriptase inactivation and RNA/ cDNA/primer denaturation and 40 cycles of 30 sec at 94℃, 1 min at 60℃, and 2 min at 68℃ for PCR, and a final extension step of 7 min at 68℃. The reaction products were analyzed by gel electrophoresis on 1% agarose gel stained with ethidium bromide.

RESULTS

1. Sensitivity and Specificity

The sensitivity and specificity of the kit was examined with 2 strains of IBDV and 4 strains of non-IBDV. Serial two fold dilutions of allantoic fluids of these viruses were applied to the S zone. When classical IBDV strains 228E and Winterfield 2512/90 were tested, the detection limit of the kit was $10^{3.1}$ EID_{50} per mL and $10^{3.9}$ EID_{50} per mL, respectively. The IC kit did not react with any of the other viruses including Newcastle disease virus (B1 strain), H9N2 avian influenza virus (MS96 strain), infectious bronchitis virus (H120 strain), and infectious laryngotracheitis virus (Sterwin strain), which use for local production of poultry vaccines in Korea.

2. Detection of IBDV from Experimentally Infected Chickens

To evaluate the applicability of the IC kit for the diagnosis of IBD, eight SPF chickens died within 4 days after very virulent IBDV (strain Kr/D62) infection were tested. Seven tissues including BF, cecal tonsil, spleen, kidney, proventriculus, liver and thymus were included for the testing. These tissue samples from IBDV infected birds were applied for the IC kit. All samples of BF, spleen, kidney, cecal tonsil were positive for IBDV. In particular, all of BF tissue samples showed stronger positive signal than other tissues. Positive signal was observed in 87.5%, 37.5% and 0% of liver, thymus and proventriculus samples, respectively. The result by IC kit was compared with result by DAS-ELISA and RT-PCR assay for the same birds (Table 1). All of positive samples by the IC kit gave also positive results

Table 1. A comparison of the results of the immunochromatographic kit and antigen capture ELISA in experimentally very virulent infectious bursal disease virus infected chickens

	Tissue tested						
	Bursa of the fabricius	Cecal tonsil	Spleen	Liver	Kidney	Thymus	Proventriculus
Infected birds[1]							
IC-kit	8/82	8/8	8/8	7/8	8/8	3/8	0/8
DAS-ELISA	8/8	8/8	8/8	7/8	8/8	4/8	0/8
RT-PCR	8/8	8/8	8/8	7/8	8/8	8/8	4/8
Control birds[3]							
IC-kit	0/3	0/3	0/3	0/3	0/3	0/3	0/3
DAS-ELISA	0/3	0/3	0/3	0/3	0/3	0/3	0/3
RT-PCR	0/3	0/3	0/3	0/3	0/3	0/3	0/3

[1]Eight SPF chickens died within 4 days after experimental challenge with very virulent IBDV strain Kr/D62 ($10^{5.1}$ EID_{50} per bird).
[2]No. Positive/No. tested.
[3]Three SPF chickens without vvIBDV challenge.

by DAS-ELISAs and RT-PCR assays. All except for one thymus sample of samples having negative results by the IC kit showed the same result with DAS-ELISA but RT-PCR assay detected IBDV in some of IC kit negative samples (thymus and proventriculus). All samples collected from control birds were negative for IBDV by IC kit, DAS-ELISA and RT-PCR assay.

3. Detection of IBDV from Dead Birds in Field

To further evaluate the applicability of the kit for the diagnosis of IBD under field conditions, a total of 231 dead birds between 3 and 6 weeks old were examined (Table 2). Of them,

Table 2. A comparison of the results of the immunochromatographic kit and RT-PCR assay based on bursal swab samples from dead chickens (n=231) on commercial broiler farms

RT-PCR assay	Immunochromatographic kit		
	Positive	Negative	Total
Pos	109	0	109
Neg	3	119	122
Total	112	119	231

Relative sensitivity=100% (109/109), relative specificity = 97.5% (119/122), kappa value between two tests=0.97.

109 birds from two broiler farms A (n=47) and B (n=65) were confirmed very virulent IBDV infection while the other birds from one broiler farm (n=122) were diagnosed as no IBDV infection. Each BF sample was tested using the IC kit and a RT-PCR assay (Barlic-Maganja et al, 2002). The IC kit results were compared with RT-PCR assay method As shown in Table 1, all of IBDV positive samples with positive RT-PCR results (n=109) were also positive with the IC kit. When applied to 122 IBDV negative samples, the IC kit gave negative results for IBDV in 119 birds while the RT-PCR assay were negative for IBDV in all of birds tested. By the kappa quotient value (Martin et al., 1988) the agreement between two tests was 0.97. When inoculated to 10 day-old SPF chicken embryonated eggs, no IBDV were isolated from three BF samples exhibiting contradictory results between the IC kit and the RT-PCR assay, indicating that the three samples may be false positive for IBDV in the IC kit.

DISCUSSION

The use of the IC kit has several advantages against RT-PCR assay. First, the IC kit can be undertaken at the veterinary clinics or on farm, unlike the RT-PCR assay only undertaken in

the laboratory by a laboratory technician. The procedure is very simple, rapid and importantly comparable with RT-PCR assay for detecting IBDV when applied to dead birds suspected of IBD. Sample preparation (e.g., swab sample) and test performance (one-step test by sample dropping onto the device) require no extra equipment unlike RT-PCR assay. In this study, we used swab sampling as a procedure for BF sample preparation since a 10% (w/v) homogenates of BF tissue is not suitable for a field veterinarian or farm owner to test easily dead birds under field conditions. Collectively, these observations indicated that the IC kit might be a rapid and simple immunoassay useful for detecting IBDV. Second, the IC kit allows it to be implemented rapidly an appropriate preventive control measures such as movement control, vaccination, other bio-securities. This is very important since IBD is highly contagious to susceptible chickens. Third, the IC kit can be stored at room temperature for long time (more than 15 months). Several reagents for RT-PCR assay require storage at $-20℃$ or lower. This storage condition makes it an appropriate test method for ambulatory practice at veterinary clinics, on farm or at diagnostic laboratory.

Of tissue samples from dead birds with vvIBDV infection, BF showed the strongest positive band signal in IC kit, indicating principle target organ for detecting IBDV by the IC kit. This is consistent with the fact that BF has predilection to IBDV replication (Käufer and Weiss, 1980; Winterfield et al., 1972). When applied to BF swab samples of dead birds on IBD outbreak farm, IC test results were highly correlated with those of RT-PCR assay (kappa value of 0.92), indicating that the kit can still provide a useful aid for rapid diagnosis of IBD in chickens under field circumstances. Thus, test samples for detecting IBDV are recommended to be taken from primary target organ (e.g., BF) of dead birds rather recovered or subclinical birds. In addition, it is notable that IBDV was detected IBDV in all of the BF, cecal tonsil, spleen, and kidney samples of dead birds by both IC kit and AC-ELISA. This indicates that if BF sample is not available, samples of other lymphoid organs (cecal tonsil, spleen) and kidney may be alternative for diagnosis of IBD. Contradictory results between the IC kit and a RT-PCR assay were observed in some of thymus and proventriculus samples of dead birds after vvIBV infection. In this study, the detection limit of the IC kit was $10^{3.1}$ EID$_{50}$ per mL to $10^{3.9}$ EID$_{50}$ per mL. The detection limit of the IC kit was the same as double antigen

capture ELISA ($10^{3.8}$ EID$_{50}$/mL) but lower than RT-PCR ($10^{2.5}$ EID$_{50}$/mL) according to the results by Jeon et al.(2008). Thus, the negative result of some of RT-PCR positive samples by the IC kit appears to be associated with the kit's sensitivity due to low viral load in the samples.

Interpretation of the IC kit result can also be confused by vaccine strain of IBDV since live attenuated IBDV vaccines are commonly used in young chickens on farm. Thus, the clinical picture and pathological changes especially BF in chicken flocks should be considered and IC kit positive samples, at occasions, require the pathotyping of IBDV by either sequence analysis or *in vivo* pathogenicity test at a diagnostic laboratory. Despite some limitations, the kit can still provide a useful aid for rapid diagnosis of IBD in chickens under field circumstances.

적 요

닭 전염성 F낭병 (IBD)은 닭에서 전염성이 강하고 발병으로 인하여 양계 산업에 막대한 경제적 피해를 입히는 닭의 바이러스성 전염병이다. 이 연구에서는 수 분 이내에 검사 시료로부터 닭 전염성 F낭병 바이러스를 검출할 수 있는 시판용 면역크로마토그래피법 검사 킷트를 이용하여 IBD 진단에 있어서의 유용성을 조사하였다. 사용한 면역크로마토그래피법 검사 킷트는 닭 전염성 F낭병 바이러스 VP2에 특이적인 단클론 항체를 이용하여 닭 전염성 F낭병 바이러스를 검출하도록 고안되었다. 바이러스 감염 역가를 알고 있는 IBDV를 사용하여 조사한 결과, IC 검사 킷트의 검출 한계는 $10^{3.1}$ 내지 $10^{3.9}$ EID$_{50}$/mL이었다. 이 검사 킷트는 닭의 다른 전염성 바이러스인 뉴캣슬병 바이러스, 닭 전염성 기관지염 바이러스, 조류인플루엔자 바이러스 및 전염성 후두기관염 바이러스에 대하여 비특이 반응을 나타내지 않았다. 고병원성의 IBDV를 실험적으로 감염시킨 후 3일 내지 4일에 폐사한 닭의 장기별로 조사한 결과, 모든 폐사 닭의 F낭, 장편도, 비장, 신장 시료들은 면역크로마토그래피법 검사 킷트에서 강한 양성반응을 나타내었다. 검사 시료 중 F낭 시료가 IC 검사 킷트에서 가장 강한 양성반응을 나타내었다. 폐사 닭의 간, 흉선, 선위의 경우, 각각 검사시료의 87.5%, 37.5% and 0%가 양성 반응을 나타내었다. 면역크로마토그래피법 검사 킷트에서 음성이었던 시료 중 흉선 시료 한 점을 제외한 모든 시료는 DAS-ELISA와 동일한 검사 결과를 나타내었으나, 검사 시료 중 흉선과 선위 일부에서 RT-PCR 검사에서 양성

반응을 나타내었다. 야외 IBD 발생 농장과 비발생 농장에서 수거한 폐사닭 231수의 조직을 면봉으로 도말하여 채취한 시료를 조사하여 RT-PCR법과 비교한 결과, 상대적 민감도와 특이도는 각각 100% (109/109) 및 97.5% (119/122)를 나타났으며, 두 검사 방법간 kappa value는 0.97이었다. 우리의 연구 결과는 IC 검사 킷트는 야외 양계 농장에서 폐사 닭을 대상으로 IBD를 진단하는 데 적용하기에 매우 유용하다는 것을 말해준다.

(색인어: 전염성 F낭병 바이러스, 면역크로마토그래피법, 신속검출, 진단)

ACKNOWLEDGEMENTS

LITERATURES CITED

Barlic-Maganja D, Zorman-Rojs O, Grom J 2002 Detection of infectious bursal disease virus in different lymphoid organs by single-step reverse transcription polymerase chain reaction and microplate hybridization assay. J Vet Diagn Invest 14:243-246.

Chettle N, Stuart JC, Wyeth PJ 1989 Outbreak of virulent infectious bursal disease in East Anglia. Vet Rec 125:271-272.

Di Fabio J, Rossini LI, Eterradossi N, Toquin MD, Gardin Y 1999 European-like pathogenic infectious bursal disease viruses in Brazil. Vet Rec 145:203-204.

Ismail NM, Saif YM, Moorhead PD 1988 Lack of pathogenicity of five serotype 2 infectious bursal disease viruses in chickens. Avian Dis 32:757-759.

Jackwood DJ 2004 Recent trends in the molecular diagnosis of infectious bursal disease viruses. Anim Health Res Rev 5:313-316.

Jackwood DJ, Sommer-Wagner SE 2005 Molecular epidemiology of infectious bursal disease viruses: Distribution and genetic analysis of newly emerging viruses in the United States. Avian Dis 49:220-226.

Jackwood DJ, Sommer-Wagner S 2007 Genetic characteristics of infectious bursal disease viruses from four continents. Virology 365:369-375.

Jeon WJ, Chang BS, Park MJ, Lee EK, Joo HD, Kwon JH, Choi KS 2008 Detection of infectious bursal disease virus by double antibody sandwich ELISA. J Bacteriol Virol 48:139-147.

Käufer I, Weiss E 1980 Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens. Infect Immun 27:364-367.

Lukert PD, Saif YM 2001 Infectious bursal disease. *In:* Disease of Poultry, ed. Calnek BW, 10th ed., pp 721-738, Iowa State University Press, Ames, IA.

Martin SW, Meek AH, Willegerg P 1988 Measurement of disease frequency and production. *In*: Veterinary Epidemiology. Principles and Methods, pp 48-76, Iowa State University Press, IA.

Winterfield RW, Fadly AM, Bickford A 1972 Infectivity and distribution of infectious bursal disease virus in the chicken. Persistence of the virus and lesions. Avian Dis 16:622-632.

Wu CC, Rubinelli P, Lin TL 2007 Molecular detection and differentiation of infectious bursal disease virus. Avian Dis 51:515-526.

Zhang GP, Li QM, Yang YY, Guo JQ, Li XW, Deng RG, Xiao ZJ, Xing GX, Yang JF, Zhao D, Cai SJ, Zang WM 2005 Development of a one-step strip test for the diagnosis of chicken infectious bursal disease. Avian Dis 49:177-181.