Filter paper sampling of blood for the detection of antibodies to Infectious Bursal Disease virus using a commercial ELISA kit

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Abstract

Aim: The present study was designed to investigate the feasibility of the filter paper sampling of blood for the detection of IBDV antibodies in chicken sera using a commercial ELISA kit.

Materials and Methods: Optimum dilution for filter paper extracts that would give a result equivalent to that of the corresponding serum sample in a commercial ELISA was determined. Correlation between the filter paper extracts based ELISA and conventional serum based ELISA was also examined and the reproducibility of the filter paper extracts based ELISA was tested.

Results: A very good correlation ($r = 0.9$; $n = 15$; $p<0.01$) was observed between the results obtained with filter paper extracts based ELISA and serum based ELISA. The antibody titre determined by two systems were very close and did not exceed more than a two-fold dilution with the exception of samples having very low antibody levels, where relatively higher background reaction was observed with filter paper extracts based ELISA. The filter paper extracts based ELISA appeared to be quite reproducible with a coefficient of variation less than 10%.

Conclusion: Filter paper based ELISA could be a useful alternative to serum dependant ELISA assays for sero-profiling chicken flocks for IBDV antibodies.

Key Words: Filter paper extract based ELISA, IBD, Serum based ELISA, Seroprofiling

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Introduction

Poultry industry is a rising sector in Bangladesh but the development of this sector is often interrupted by frequent outbreaks of a number of emerging and recurrent diseases. One of the most important diseases of commercial poultry is infectious bursal disease (IBD). It is an acute, highly contagious immunosuppressive viral infection of chickens [1,2,3]. Among poultry diseases in Bangladesh, IBD is the number one killer causing up to 80% mortality in field outbreaks [4, 5, 6, 7].

Vaccination is the only means for the prevention of IBD but vaccination failure is quite common due to various factors such maintenance, storage or inadequate immune response following vaccination or neutralization of vaccine virus by the maternal antibodies present in chicks. Therefore, maternal antibody levels in chicks should be determined before vaccination. The enzyme-linked immunosorbent assay (ELISA) is commonly used and considered to be the easiest method for measuring maternal antibody levels against IBDV [8]. The commercial IBDV ELISA kits are available through an ELISA assay for detecting antibodies to IBDV also has recently been developed locally.

Conventionally, serum obtained from blood is used in ELISA, but it is often difficult to collect sufficient blood from very young chicks without killing the birds. As an alternative, a technique has been developed for sampling blood on filter paper, where a small drop of blood is soaked on a filter paper

Table-1. Similarities between absorbance values of sera samples (1: 500 dilutions) and corresponding filter paper extracts (serial two-fold dilutions)

Sample	Serum (1:500)	Filter paper extracts								Corresponding dilution of Filter paper extracts expected to vive absorbance similar to that 1:500 dilution of Serum	
		(1:2)	(1:4)	(1:8)	(1:16)	(1:32)	(1:64)	(1:128)	(1:256)	Dilution (Log_2)	Geometric mean dilution (Log_2)
1	0.167	0.219	0.160	0.141	0.122	0.114	0.089	0.085	0.067	2.3	2.3
2	0.238	0.32	0.214	0.168	0.156	0.137	0.091	0.13	0.075	1.8	
3	0.096	0.146	0.094	0.084	0.078	0.072	0.061	0.059	0.055	1.8	
4	0.262	0.454	0.329	0.282	0.247	0.23	0.139	0.111	0.088	3.9	
5	0.305	0.348	0.275	0.237	0.218	0.198	0.123	0.099	0.082	1.6	

strip, which can be later extracted with PBS and used in ELISA [9]. Filter paper extracts based ELISA has been successfully used to detect antibodies to foot and month disease virus [10], rinderpest virus [11, 12], duck plague virus [13] and *Pasteurella multocida* [14].

However, there is no study about filter paper based ELISA for the detection of antibody to IBDV using commercial kits. Therefore, the present study was designed to investigate the feasibility of the filter paper sampling of blood for the detection of IBDV antibodies in chicken sera using a commercial ELISA kit.

Materials and Methods

Sampling and sera collection: The study was performed during the period of January - June, 2004. Blood samples were obtained from chickens, which were reared in the Department of Pathology, Bangladesh Agricultural University. The chicks were vaccinated against infectious bursal disease virus with a commercial vaccine "Nobilis D-78" (Intervet, the Netherlands) at 14 days of age. Blood samples from 4 birds were obtained at day old, then at 4, 6 and 8 weeks of age, respectively either from the jugular vein during slaughter (day old chicks) or from the wing vein. The 100 µl of blood samples were soaked scientifically and aseptically on commercially available Whatman filter paper no.1(2 cm x 5 cm x 0.3 mm) and allowed to dry at room temperature away from direct sun light after that stored in screw-capped air tight vessels at – 20^0C for subsequent extraction in Dulbecco's phosphate buffer saline and used in filter paper based ELISA.

Blood samples were also collected in vials and allowed to clot at room temperature at slanting position in order to obtain serum. The separated serum was collected in fresh vials and clarified by low speed centrifugation (3000 rpm for 15 minutes) then stored frozen at -20^0C until used. Five discs cut out from each blood soaked filter paper strips with a paper punch were extracted with 300µl PBS for 60 minutes at room temperature.

A commercial ELISA kit (Infectious Bursal Disease Antibody Test Kit-IDEXX Laboratory, Inc., Westbrook, Maine 04092, USA) was used. The test was used and the test performed as described by the manufacturer. The frozen sera samples were thawed. Five hundred fold (1:500) dilution of sera samples were made in sample diluent. The Filter paper extracts were used immediately after extraction. Filter paper extracts were diluted either serially in two-fold dilutions or at an appropriate fixed dilution (1: 5). This was done in the same laboratory, BAU. The optical density (OD) or absorbance values were determined at 650 micron using an ELISA reader (SPECTERA max 340 pc, Molecular Devices Inc., USA) at the Surgery and Obstetrics Department of Bangladesh Agricultural University.

Negative control mean, positive control mean, sample to positive (S/P) ratio and endpoint titres were calculated. Sera samples with S/P ratios of less than or equal to 0.2 were considered negative and S/P ratios greater than 0.2 (titre greater than 396) were considered positive.

Optimum dilution for filter paper extracts: To determine the optimum dilution for filter paper extracts, the ELISA was performed using five selected sera samples at 1: 500 dilution and their corresponding filter paper extracts at two-fold serial dilutions ranging from 1: 2 up to 1: 256.

The absorbance values for the dilution of each filter paper extracts giving an absorbance value close to that of 1: 500 dilution of the corresponding serum sample was determined (Table 1).

Comparison of ELISA results based on sera and filter paper extracts: A total of 15 sera and corresponding filter papers extracts were tested at dilutions of 1:500 and 1:5 accordingly. The antibody titre

Table-2. The ELISA absorbance values and antibody titer of 15 sera samples (1: 500 dilution) and corresponding filter paper extracts (1: 5 dilution)

Sampling occasion	Sample	Mean absorbance		Mean predicted titre	
		Serum 1: 500	Filter paper extract 1: 5	Serum 1: 500	Filter paper extract 1: 5
I	1	0.385	0.357	3889	3525
	2	0.294	0:347	2718	3396
	3	0.543	0.538	5989	5922
II	1	0.072	0.178	107	1289
	2	0.069	0.12	78	617
	3	0.069	0.12	78	617
	4	0.072	0.184	107	1361
III	1	0.479	0.602	5130	6791
	2	0.364	0.472	3616	5036
	3	0.287	0.426	2630	4427
	4	0.234	0.389	1968	3941
IV	1	0.424	0.488	4400	5250
	2	0.549	0.611	6070	6914
	3	0.666	0.657	7670	7546
	4	0.521	0.526	5693	5760

against IBDV in these two systems were calculated and compared by correlation-regression analysis.

Inter-assay variability: For determining the inter-assay variability between ELISA performed using sera and filter paper extracts respectively, replicate filter paper and blood samples were collected from each of the 25 week old birds (n=5) and tested at dilutions explained above. Inter-assay co-efficient of variation (CV) was calculated for each bird.

Results

In this study, filter paper sampling of blood was adopted for detection of IBDV antibodies in chicken sera using a commercial ELISA kit.

Determination of optimum dilution for filter paper extracts: After analysis it was found that, the absorbance value of each serum sample at 1: 500 dilution matched closely to the absorbance value (or Optical Density) of the filter paper extracts at a dilution between 1: 2 and l: 8.

The exact corresponding dilution of the filter paper extracts expected to give an absorbance value equal to that given by the 1: 500 dilution of the serum was also calculated by correlation-regression analysis. The geometric mean of the calculated corresponding filter paper extracts dilutions was found to be Log_2 2.3, which is equal to 4.92. Therefore, a filter paper extract dilution of 1: 5 was considered to be equivalent to 1: 500 dilution of the serum.

Comparison of ELISA results based on sera and filter paper extracts: For comparison of ELISA results based on sera and filter paper extracts, the ELISA was performed using 15 serum samples at 1 : 500 dilution, collected on 4 different occasions and their corresponding filter paper extracts at 1:5 dilution. The absorbance values and calculated titres are presented in Table 2.

The antibody titres determined by two systems were very close and did not exceed more than a two-fold dilution, with the exception of occasion II. The correlation between the ELISA titres determined from the serum and filter paper extracts were analysed by correlation regression analysis. There was a very strong positive correlation ($r = 0.9$; $n = 15$; $p<0.01$). The results are shown in Figure 1.

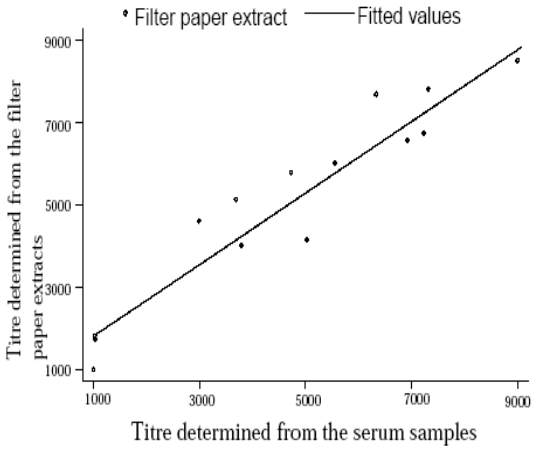

Figure-1. Correlation between ELISA titres determined from the serum and filter paper extract

Inter-assay variability: Sera and filter paper extracts antibody titres, Mean ± SD and % CV are shown in Table 3. The coefficient of variation obtained from this study was less than 10%.

Table-3. Percent coefficient of variation (CV) of ELISA titres determined from 5 replicate samples of each of 5 birds

Sample	Antibody titre							
	Serum (1:500)	Filter paper extracts (1:5)						
		a	b	c	d	e	Mean±SD	%CV
I	12565	18378	16520	16870	15005	15515	16458±1310	7.96
2	13786	15515	15005	15976	15761	16253	15702±475	3.03
3	12093	12837	11274	11056	10916	10203	11257±970	8.62
4	9337	8322	8186	8653	8148	8770	8416±281	3.33
5	13320	13522	13543	14272	14516	14171	14005±449	3.21

Discussion

The whole blood dried on filter paper as an alternative to serum has been used previously in ELISA for detecting antibodies to foot and mouth disease virus [10], Rinderpest virus [11, 12] Duck plague virus [13] anaplasma [14] *Pasteurella multocida* [15] and Schistosome [9]. Although no report is available on the use of filter paper extract to detect antibodies-to IBDV by ELISA [16] used filter paper extract for detecting IBDV by antibodies by agar gel precipitation test. The difference was significant between serum samples and filter-paper eluates in which supports the present findings.

The filter paper extracts based assay used in the present study with a commercial ELISA kit that is routinely used in between poultry industry for measuring maternal antibody levels, in chicks to predict the optimum time for vaccination. The kits are validated for use on sera samples. However, it is often quite difficult to obtain sufficient blood from young chicks due to their small size. The study proposes the filter paper sampling technique and extracts usage as an alternative to bleeding relatively large volumes of blood and sera testing in the commercial assay. The filter paper extracts can be used in the ELISA protocol recommended by the kit manufacturer without any modification except that the extracts should be further diluted 1:5 in the sample diluent instead of the 1:500 recommended for sera samples. This finding is in partial agreement with that of Evengard and Linder [9] who observed that 1:5 dilution of filter paper extracts were equivalent to 1: 400 dilution of corresponding serum sample in bovine of schistosome-specific antibodies. The variation could be due to species differences or differences in the filter paper extraction procedures.

A very good correlation ($r = 0.9$; $n = 15$; $p<0.01$) was observed between the results obtained with the filter paper extracts and serum based ELISA in the present study respectively. The antibody titre determined by two systems were very close and did not exceed more than a two-fold dilution, with the exception of one particular sampling occasion (Occasion II). In fact, at that particular occasion the birds had very low levels of antibodies (having the titre between 78 to 107 with serum based ELISA). However, with filter paper based ELISA the antibodies were between 617 and 1361. This discrepancy would suggest that at very low antibody levels, the filter paper extracts based ELISA might give relatively a high background reaction. Further studies are required in this regard. One possible option is to extract the blood soaked filter paper in sample diluent instead of PBS to reduce nonspecific binding.

Besides the drawback mentioned above, the filter paper based ELISA appeared to be quite reproducible with a coefficient of variation less than 10%. Heller [11] also suggested that the accuracy and precision of filter paper extract based ELISA were excellent with a sensitivity of 100% and specificity of 98.26%.

The major limitation of the present study was the small sample size for determination of the optimum dilution of filter paper extracts and for comparison of the filter paper extracts based ELISA and serum based ELISA. This was due to the resource constraint as only one kit (5 antigen-coated plates) was available for the study.

Conclusion

Filter paper based ELISA could be a useful alternative to serum dependant ELISA assays for sero-profiling chicken flocks for IBDV antibodies.

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Competing interests

The authors declare that they have no competing interests.

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