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Single Radial Hemolysis Technique in Comparison with Indirect Hemagglutination and Agar Gel Precipitation tests for Assaying Antibodies against Infectious Bursal Disease Virus

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Abstract

The interaction between antibodies and the surface antigens of infectious bursal disease virus particles was studied in the agarose gel by using single radial hemolysis (SRH) test. Sonicated antigen was used to sensitize the Human "O" erythrocytes. Hemolytic zones, using the filter paper discs were observed after overnight incubation in humid chamber at 37^0C. Indirect Hemagglutination (IHA) and agar gel precipitation tests (AGPT) were also performed and compared with SRH. Out of 50 serum samples, the percentage of positive samples using SRH, IHA and AGP tests were 70, 58 and 38 respectively, which showed the sensitivity in decreasing order. Results revealed that SRH is simple, sensitive, quick and particularly suitable for the routine screening of serum samples and assessing the immune status of birds against infectious bursal disease virus.

Keywords: Single radial hemolysis test, Serodiagnosis, Infectious bursal disease

Introduction

Infectious bursal disease (IBD) is an immunosuppressive and economically important disease of poultry causing considerable losses (Anjum *et al*., 1993). Immunosupression results from the depletion of B-lymphocytes and secondary infections associated with Infectious bursal disease (Lukert and Saif, 1991). Chickens of 3-6 weeks of age are more susceptible to clinical infection (Ley *et al*., 1983). Very virulent infectious bursal disease virus can cause even 60-100% mortality (Cao *et al*., 1995).

A number of serodiagnostic tests are available to diagnose the clinical cases of infectious bursal disease including indirect hemagglutination test (Aliev *et al*., 1990), agar gel precipitation test (Castello *et al*., 1987), Enzyme linked immunosorbent assay (Cao *et a*l., 1995) and counter immuno-electrophoresis (Hussain *et al*., 2002).

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Single radial hemolysis test has generally been reported to be a simple, sensitive and reliable test that now is being frequently used as a highly specific and reproducible test for detecting Influenza virus (Gibson *et al*.,1985), Rubella virus (De Ory *et al*.,1985), Parvovirus (Fastier, 1981), Arbovirus (Mel Nikova, 1980) Vaccinia virus (Prakash *et al*.,1977), Adenovirus (Grandien and Norrby, 1975), Measles virus (Fenwich, 1986) and Foot-and-mouth disease virus (Rweyemamu *et al*.,1980) , Rinderpest virus (Bansal *et al*.,1986), Reovirus (D'Ambrosio, 1981), NewCastle disease virus (Shiv Cheran *et al*.,1980), Rabies virus (Ferguson and Schild, 1982) and Poliovirus (Schild *et al*.,1980).

No work has been reported to detect the antibodies against IBD using SRH. The present paper reports the standardize and application of SRH test for the detection of IBD antibodies and its comparison with with IHA and AGP tests.

Materials and Methods

Preparation of antigen

Antigen was prepared following the method adapted by Hussain *et al*., (2002). Briefly, after collection of IBDV affected bursa, they were chopped, mixed with PBS (pH 7.2) to prepare 10% (w/v) suspension, homogenized and finally were subjected to ultra-sonification in a jacketed vessel using rapidis 600 at an intensity of 75 watts/cm^2 with titanium probe (15cm dia) for 5 minutes. The temperature was kept under 20°C. The sonicated antigen was centrifuged at 5000 rmp for 15 minutes; supernatant was collected in a sterile stoppered glass tube as sonicated antigen.

Hyper immune serum

The hyper-immune serum was raised against D78 (commercial vaccine) in rabbits according to Barnes *et al.,* (1982). IBDV (field isolate) was confirmed using the modified counter immuno-electrophoresis (Hussain *et al*., 2002) fand agar gel precipitation (Castello *et al*., 1987) tests.

Washed human group “O” and sheep erythrocytes were used in the present study. The erythrocytes were sensitized according the procedure adapted by Rehman *et al.* (1990).

Serum Samples

Fifty serum samples of broiler birds were collected from different commercial broiler farms in Faisalabad. After heat inactivation (56°C for 25-30 minutes) in water bath they were subjected to agar gel precipitation, indirect hemagglutination and single radial hemolysis tests.

Single radial hemolysis test

Single radial hemolysis test was performed as described by Rehman et al., (1990). One percent Noble agar was prepared in physiological normal saline, autoclaved and kept at 46°C. One ml of sensitized RBCs suspension was added to 5 ml molten Noble agar (1 %) with 0.05 ml of fresh guinea pig complement (serum). The material was layered (2-3 mm thickness) on microscopic glass slides and was shifted to refrigerator at 4^0C for 15 minutes for proper solidification. Filter paper discs (6mm diameter) soaked in inactivated serum samples (absorbed 35 µl amounts each), were placed on the agar slides 2 cm apart from each other. These slides were kept at 37 °C in a humid chamber and at 4^0C for overnight incubation. Sensitized sheep erythrocytes were treated similarly. Zones of hemolysis were observed and measured.

Agar gel precipitation test (AGPT)

Agar gel precipitation test was carried out as described by Sulochana and Lalithakanjamma, (1991).

Indirect hemagglutination test (IHA)

Indirect hemagglutination test was performed according to the method of Aliev *et al*., (1990). Briefly, after making two-fold serial dilution of the test serum, equal quantity of sensitized human "O" RBCs (1%) were added to each well of one plate and in the other, sensitized sheep RBCs were used. The plates were gently tapped to ensure even dispersion of erythrocytes and then kept at 37^0C for 30 minutes of incubation.

Results and Discussion

Out of 50 serum samples, the percentage of positive results by using SRH, IHA and AGPT were 70, 58 and 38 respectively, which showed the sensitivity in decreasing order. Indirect hemagglutination antibody titre ranged from 1 :4 to 1 :128 both by using the sheep and human 'O' erythrocytes (Table1 and 2). It was observed that human 'O' erythrocytes gave comparatively bigger and easily demarcated zone of hemolysis (Table 2) and the colour of human 'O' erythrocytes remained unaltered while sheep erythrocytes turned light brown after sensitization. Similar results were reported by Rehman *et al*., (1990). Human 'O' erythrocytes and chromium chloride ($CrCl_3$) treated sheep erythrocytes were used for sensitization of sonicated IBDV antigen and it was seen that the quality of hemolysis produced by either of the RBCs found quite comparable but on account of ease and time saving human 'O' erythrocytes may be preffered for use in SRH and IHA tests. It was also observed that hemolytic zones obtained using sheep erythrocytes appeared somewhat hazzy and poorly demarcated. Rehman *et al*., (1990) used the veronal buffer, which is commonly used in complement fixation test, and is a suitable medium as it helps to adsorb the complement on to the surface of agar gel (Cruickshank, 1975) but in the present study physiological saline was used which gave equally good and comparable results.

No non-specific zone of hemolysis was observed in heat inactivated serum samples indicating the inactivation of avian complement by heating. The most suitable temperature for getting maximum zone of hemolysis was 37 ^{0}C in a humid chamber for overnight incubation. No zone of hemolysis was observed at 4^0C incubated for overnight. Fastier (1981) and Rehman *et al*., (1990) reported similar temperature conditions for SRH test.

Table 1: Average hemolytic zone diameter with respect to IHA titer and AGPT.

IHA positive Samples	IHA Titer	SRH (Diameter mm)	Mean ± SD	AGPT Positive samples
9 (31.03%)	1:4	6-8	6.86 ± 0.90	2 (10.52%)
6 (20.69%)	1:8	7-9	8.20 ± 1.30	3 (15.79%)
3 (10.34%)	1:16	9-10	9.67 ± 1.52	3 (15.79%)
4 (13.80%)	1:32	10-11	10.75 ± 1.25	4 (21.05%)
5 (17.24%)	1:64	11-14	12.80 ± 0.89	5 (28.31%)
2 (6.90%)	1:128	14-16	15.50 ± 0.70	2 (10.52%)

Table 2: Average zone diameter with respect to temperature using Sheep erythrocytes and Human "O" erythrocytes.

IHA Titer	SRH (Zone Diameter)			
	Sheep RBCs		Human "O" RBCs	
	(37 ^{0}C)	(4 ^{0}C)	(37^{0}C)	(4^{0}C)
1:4	6.57 ± 0.78	-	6.86 ± 0.90	-
1:8	7.40 ± 0.89	-	8.2 ± 1.30	-
1:16	8.66 ± 00.57	-	9.67 ±1.52	-
1:32	9.25 ± 0.95	-	10.75 ± 1.25	-
1:64	10.60 ± 01.34	-	12.80 ± 0.89	-
1:128	12.00 ± 01.41	-	15.50 ± 0.70	-

SRH and IHA tests were compared statistically to find out the correlation. Calculated correlation co-efficient (r) between SRH and IHA was 0.837, which was highly significant (P>0.01). The regression equation to the above data was SRH = 6.05 + 0.07 (IHA). χ^2 (Chi-square) values for SRH vs. AGPT and SRH vs. IHA comparisons were found to be 9.623 and 23.31 respectively, both of which were found significant at 0.01 probability level.

The present study revealed that SRH test is simple, quick, inexpensive and gave comparable results with IHA and AGPT for measuring antibody titer in birds against IBDV.

References

Aliev, A.S., Dzhavadov, E.D. and Leont'eva, M.M. Indirect Hemagglutination test for infectious bursal disease of fowls. Vet. Bull., 1990, 60: 47-67.

Anjum, A.D., Hussain, S. and Arbi, G.S. Infectious bursal disease in chickens in Pakistan. Pakistan Vet. J., 1993, 3 (2): 54-58.

Bansal, R.P., Josin, R.C., Sharma, B., Bandyopadhyay, S.K. and Chandra, U. Single radial hemolysis test for detection of rinderpest antibody. Trop. Anim. Hlth. Prod., 1986, 18: 133-136.

Barnes, H.J., Wheeler, J. and Reed, D. Serological evidence of infectious bursal disease virus infection in Iowa turkeys. Avian Dis., 1982, 26: 560-565.

Cao, Y.C., YingZuo, B.I. and JiMei, Zhu, Application of enzyme-linked immuno-sorbent assay for evaluation of immunological efficency of chicks against IBD. Chinese J. Vet. Med., 1995, 21(11): 9-10.

Castello, L., Martinez, B.L. and Heronodez, K. Comparison of the agar gel precipitation, virus neutralization and enzyme-linked immunosorbent assay in the determination of antibodies to infectious bursal disease virus. Veterinaria-Mexico, 1987, 18(4): 317-323.

Cruickshank, R., Medical Microbiology, 11th ed. The English Language Book Society and E&S Livingstone (Ltd) London, 1975, pp: 812-843.

D' Ambrosio, E., Battaglia, M.F. and Midulla, M. Single radial hemolysis test for the determination of antiboody to reoviruses. Arch. Virol., 1981, 68(3/4): 309-312.

De'Ory, F., Sainz, M.C., Echevarria, J.M., Galindez, C.L. and Najera, R. Evaluation of the technique of single radial hemolysis for the determination of rubella antibodies. Infectologika, 1985, 6(1): 41-46.

Fastier, L.B.. A Single radial hemolysis test for measuring canine parvovirus antibody. Vet. Rec., 1981, 108: 299-301.

Fenwich, M. Single radial hemolysis for demonstrating measles virus antibody. Med. Lab. Sci., 1986, 43(1): 34-37.

Ferguson, M., and Schild, G.C. A single radial immunodiffusion technique for the assay of rabies glycoprotein antigen: Application for potency tests of vaccines against rabies. J. Gen. Virol., 1982, 59: 197-201.

Gibson, C.A., Wood, J.M., Mumford, J., Schild, G.C. and Bevan, A.M. A single radial hemolysis technique for measurement of antibody to influenza virus neuraminidase in equine sera. J. Virol. Meth., 1985, 11(4): 299-308.

Grandien, M. and Norrby, E., Characterization of adenovirus antibodies by single radial diffusion in agarose gels containing immobilized intact virus particles. J. Gen. Virol., 1975, 27(3): 343-353.

Hussain, I., Nisar, A., Rahman, S.U., Mehmood, S., Zahoor, A., Ashfaque, M., and Akhtar, M. Counter-immunoelectrophoresis-A rapid test for the detection and quatification of infectious bursal disease virus. Proc. Regional Seminar on Prevalent and Newly emerging Poultry diseases held on April 15-16, 2002, University of Agriculture, Faisalabad.

Ley, D.H., Yamamorto, R. and Bickfor, A.A. The pathogenesis of infectious bursal disease virus: serologic, histopathological and clinical observation. Avian Dis., 1983, 27: 1060-1085.

Lukert, P.D. and Saif, Y.M. Infectious bursal disease. In: Diseases of Poultry, 9th Ed. B. W. Calnek., Iowa State University Press., Ames, Iowa, 1991, pp: 648-663.

Mel'Nikova, E.E. and Gaidamovich, S.Ya., Methodical basis for the radial hemolysis reaction by arboviruses. Vopr. Virusol., 1980. 0: 492-494,

Prakash, V.J., Norrby, E. and Payne, L. Single radial immunodiffusion test for detecting antibodies against surface antigens of intracellular and extracellular vaccinia virus. J. Gen. Virol., 1977, 35(3): 465-472.

Rehman, S.U., Ashfaque, M., Ajmal, M., Khushi, M. and Rehman, Z.U. Single radial hemolysis test for measuring antibodies against Robert type I strain of *Pasteurella multocida*. Pakistan Vet. J., 1990, 10(3): 119-124.

Rweyemamu, M.M., Parry, N.R. and Joan, S. The application of single radial hemolysis technique to foot-and-mouth disease virus antibody Study. Arch. Virol., 1980, 64(1): 47-56.

Schild, G.C., Wood, J.M. Minor, P.D. Dandawate, C.N. and Magrath, D.I. Immunoassay of Poliovirus antigens by Single-radial diffusion: development and characterization of a sensitive autoradiographic zone size enhancement(ZE) technique. J. Gen. Virol., 1980, 51: 157-170.

Shiv'Cheran, A.R., Mahajan, V.M. and Agarwal, L.P. Modified single radial hemolysis technique for measurement of antibodies to new-castle disease virus. J. Immun. Method., 1980, 40: 239-242.

Sulochana, S. and Lalithakanjamma, C.R. Infectious bursal disease virus in Kerala. Cheiron, 1991, 0: 180-183.