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Usefulness of dot-ELISA in detection of canine distemper virus antigen

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Canine distemper virus is a more commonly prevalent disease in dogs. A rapid and simple test is needed to control the disease effectively. Dot-enzyme linked immunosorbent assay is one of solid phase immunoassay developed by Hawkes *et al.* (1982) to detect either antigen or antibody. The test has got several advantages over other immuno assays in that it is cheap, simple and applicable to screen large number of samples without any sophisticated equipment.

Nasal and occular swabs were collected in 100 ml sterile phosphate buffer saline (PBS) from clinically suspected dogs. These samples were subjected to brief centrifugation to sediment the debris. Then 1 ml of sample was spotted onto nitrocellulose (NC) membrane sheet. After drying at room temperature, the antigen was fixed with 0.25% glutaraldehyde at 4°C for 15 min. The NC membrane was then washed with PBS, blocked with 1% gelatin at 4°C for 15 min and incubated with 1: 50 dilution of hyper immune serum raised in rabbits as per the method of Vandevelde *et al.* (1978). After incubation at 4°C for 30 min washed with PBST (PBS containing 0.05% tween 20), the bound antibody was fixed with 0.25% glutaraldehyde at 4°C for 15 min. Finally, the NC membrane was washed once with PBS. The NC membrane was incubated with 1: 1000 dilution of antirabbit IgG-HRP conjugate (sigma) at 40°C for 30 min. Finally, substrate was added (5mg of diaminobenzidine in 10ml of PBS with 10 µl of 30% hydrogen penoxide) for 10-15 min at room temperature. The enzymatic reaction was stopped by washing the membrane with tap water. One ml of CDV vaccine strain as a positive control and 1 ml of canine parovirus as a negative control were employed in this assay.

The antigen used in dot-ELISA was solubilized during processing thus resulting in lower sensitivity. To avoid such problems, glutaraldehyde was used as a fixative to fix the antigen as well as antigen-antibody complex. The use of fixatives so as to increase the sensitivity of the assay has been reported by Natsukari *et al.* (1989) and Maiti *et al.* (1993).

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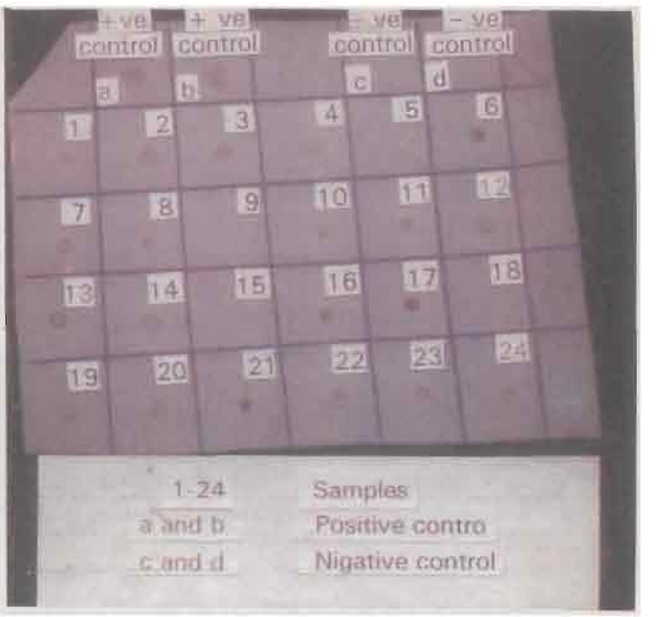

Fig. 1. Dot- ELISA for canine distemper virus.

Initially, skim milk power was used as a blocking agent. Milk powder seemed to lower the antigen-antibody reaction due to milk protein binding with the antigen and mask the epitope to react with antibody as reported by Mohammad *et al.* (1989). Gelatin was used as a alternative blocking agent which increased the intensity of the reaction. In conclusion, dot-ELISA is a cheap, simple, fast and also reliable qualitative immunoassay to detect the presence of the antigen in suspected samples.

In dot-ELISA, the positive samples were easily distinguished from the negative samples by appearance of colour development after the addition of substrate. There was no reaction in negative control which clearly indicated that this test was effectively used to screen specific antigens (Fig.1). Out of 120 samples tested, 68% were positive in both nasal and occular secretions of animals and hence the dot immunobinding assay eliminates the need of isolation or purification of antigen.

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