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DETECTION OF RINDERPEST ANTIGEN BY LATEX AGGLUTINATION TEST

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Summary. — A slightly modified latex agglutination test was applied for detection of rinderpest antigen. The antigen was added to sensitized latex particles in the presence of hyperimmune antiserum to facilitate agglutination. Out of 129 samples tested by latex agglutination (LA), solid phase aggregation of coated erythrocytes (SPACE), reverse phase passive haemagglutination (RPHA) and counter immunoelectrophoresis (CIE) test, 86.0, 86.8, 84.4 and 79.8 per cent, respectively, were found positive.

Key words: rinderpest antigen; rinderpest antibodies; latex agglutination; CIE; reverse phase passive haemagglutination (RPHA); solid phase aggregation of coated erythrocytes (SPACE)

Latex agglutination test has been used for the detection of a number of viral antigens (Seidl, 1978; Sanekata *et al.*, 1981; Shimizu *et al.*, 1983; Agius *et al.*, 1984; Sanders *et al.*, 1986). This test has been shown simple, reliable and rapid. Recently, a number of tests has been used for the detection of rinderpest antigen, such as agar gel precipitation (AGPT), counter immuno--electrophoresis (CIE), immunoperoxidase test (IPT), enzyme-linked immunosorbent assay (ELISA), fluorescent antibody technique (FAT), SPACE and RPHA. Here we describe the application of latex agglutination test for detection of rinderpest antigen.

Hyperimmune serum (HIS) against rinderpest antigen was raised in calf as described by Scott and Brown (1961). Immunoglobulins (Ig) were precipitated with 33 per cent ammonium sulphate solution. Visceral organs such as spleen, lymph nodes, pancreas, caecum, rectum, abomasum, adrenal glands, haemolymph glands and tonsils were collected from 15 dead or sacrificed cattle experimentally infected with the virulent Bangalore isolate of rinderpest virus. Clinical samples like gum and tongue scrappings were also collected. Thirty three per cent (w/v) suspension of different tissues was prepared in chilled physiological saline or in distilled water. Lymph nodes collected from healthy calves were used to prepare the control antigen.

Counter immuno-electrophoresis test was carried out as described by Bansal *et al.* (1981). Reverse phase passive haemagglutination test was performed according to Bansal *et al.* (1987*a*). Solid phase aggregation of coated erythrocytes (SPACE) test was made as described (Bansal *et al.*, 1987). For sensitization of latex particles 0.6 ml of 1 per cent latex particles (0.79 μm dia, Sigma) in carbonate-bicarbonate buffer (pH 9.6) and 1.0 ml of Ig (1 mg/ml) in carbonate-bicarbonate buffer (pH 9.6) were mixed thoroughly and kept overnight at 4 °C. The excess of Ig was

Table 1. Comparative sensitivity of LA, SPACE, RPHA and CIE tests for detection of rinderpest antigens in tissues

Description	LA	SPACE	RPHA	CIE
Number positive/ Number of samples	111/129	112—129	109/129	103/129
Per cent positive	86.0	86.8	84.4	79.8
Range of titre	1 : 8—1 : 1024	1 : 8—1 : 256	1 : 8—1 : 128	—
Mean titre	1 : 198.86	1 : 35.39	1 : 26.47	—

washed off three times; after the last washing the latex particles were resuspended in carbonate-bicarbonate buffer (pH 9.6) at 0.35 w/v per cent concentration.

Twofold serial dilution of 33 per cent suspension of test antigen was prepared in carbonate--bicarbonate buffer (pH 9.6) or in sterile distilled water. One drop (approximately 0.1 ml) of latex particles (sensitized with Ig) was dropped on the microscopic slide followed by one drop (approximately 0.1 ml) of antiserum. Then one drop of antigen was added to the mixture and carefully mixed. The slides were left at room temperature for 15 to 20 min and thereafter examined by naked eye under bright light for agglutination. The known positive and negative controls were kept for each test.

The latex agglutination depends on the agglutination of latex particles sensitized with antibody in the presence of specific antigen. In a typical latex agglutination test the sensitized latex particles are mixed with the test antigen until agglutination occurs. In such latex agglutination test for the detection of rinderpest antigen, we had found very difficult to get agglutination of sensitized latex particles in the presence of rinderpest antigen. In SPACE test, sensitized RBC are mixed with test antigen on microtitration plates and sensitized with hyperimmune serum to get heamagglutination. Similarly, hyperimmune serum was added to the mixture of sensitized latex particles and antigen to see whether agglutination of latex particles was facilitated. The addition of HIS to the mixture of sensitized latex particles and antigen enhanced agglutination of latex particles (Fig. 1).

Table 2. Comparative sensitivity of LA, SPACE, RPHA and CIE test for detection of rinderpest antigen from clinical samples

Number of materials	LA	SPACE	RPHA	CIE
Gum scrapping	*12/15	13/15	12/15	11/15
Tongue scrapping	*12/15	12/15	11/15	10/15
Total	24/30	25/30	23/30	21/30
Per cent positive	86.66	83.33	76.66	70.00

* No. positive/No. tested

The results of latex agglutination, SPACE, RPHA and CIE in detecting rinderpest antigen are presented in Table 1. It is obvious that all these tests give somewhat similar results without significant difference. The LA seems to be more sensitive as the antigen can be detected in much higher dilutions; as it is evident from range of titres and mean titres. Table 2 shows the result of these tests in detecting rinderpest antigen from clinical samples. It is clear from this Table that there is no significant difference in the sensitivity of these tests. Latex aglgutination test is very simple to perform, requires no equipment, the results can be read in half an hour. The test is as sensitive as other routine tests used for the diagnosis of rinderpest. We think that it can be adopted for field use in the diagnosis of rinderpest.

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Legend to Figure (Plate XXX):

Fig. 1. An example of latex agglutination test
1,2 — positive antigen control;
3 — tested antigen from the lymph node;
4 — tested antigen from the tongue scrapping;
5 — tested antigen from gum scrapping
6 — tested antigen from abomasum;
7,8 — negative antigen control