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FIXED CHICKEN ERYTHROCYTES IN HAEMAGGLUTINATION TEST FOR DIAGNOSIS OF NEWCASTLE DISEASE *

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Haemagglutination (HA) of Newcastle disease virus (NDV)was first described by Burnet (1942) who found that this action to be inhibited by specific antiserum. HA of chicken erythrocytes (CRBC) and haemagglutination inhibition (HI) using specific antiserum is an accepted test for diagnosis of Newscastle disease (ND), since the test is sensitive, easy to perform and cost effective. HA and HI could also be used for field level diagnosis but preservation of CRBC is a main constraint. The present study was undertaken to increase the shelf life of CRBC by fixation using aldehydes for successful use of CRBC in HA and HI test in diagnosis of ND.

Materials and Methods

Preparation of formaldehyde fixed CRBC: The method of Hirata and Brandriss (1968) was followed with some modification. One volume of 3 percent formaldehyde solution in 0.1 M PBS (pH7.2) was added to one volume of 8 per cent CRBC suspension in PBS and mixed thoroughly. The mixture was left at room temperature (22°C-28°C) for 17 hours, washed five times in PBS and filtered through gauze cloth. To the suspended 8 per cent CRBC, sodium borohydride (3.8 mg /100 ml.) was added and incubated at 37°C for 30 minutes. Cells were again washed with PBS for another two times. Finally CRBC were resuspended in PBS to give a 10 percent suspension of cells and stored at 4°C in the presence of 0.2 per cent BSA and 0.02 per cent sodium azide. For conducting HA test one percent suspension of CRBC in PBS was used.

Preparation of Glutaraldehyde fixed CRBC: The method of Scott (1986) was followed with some modification. One volume of 2.5 per cent glutaraldehyde in 0.1 M PBS (pH 7.2) was added to 8 volumes of 2.5 per cent CRBC. The mixure was agitated by a magnetic stirrer for one hour at 37°C. The cells were washed five times in PBS and filtered through a gauze cloth. To the resuspended CRBC (8 percent), sodium borohydride (3.8 mg/100 ml.) was added and incubated at 37°C for 30 minutes. Cells were washed for another two times in PBS. Finally CRBC were resuspended in PBS to give a 2.5 per cent suspension of cells and stored at 4°C in presence of 0.2 per cent BSA and 0.02 per cent sodium azide. For conducting HA test

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one per cent suspension of CRBC in PBS was used.

HA test: HA test was done as described by Alexandar (1988) using fixed CRBC and simultaneously with fresh CRBC as indicator system and known NDV as antigen.

HI test: Serum showing high HI titre was diluted in PBS and the diluted serum showing HI titre of 1:64 was used for inhibiting the HA activity of the virus.

Results and Discussion

Formaldehyde or glutaraldehyde was used for fixing CRBC. Glutaraldehyde fixed cells were found stable at room temperature for five days whereas the formaldehyde fixed cells deteriorated even before 2 days. However, both the cells were stable at 4°C for an observation period of 60 days. At weekly intervels the fixed CRBC were used for HA test using 10 NDV samples selected at random along with fresh CRBC as controls. The HA titre with fixed CRBC compared favourably with fresh CRBC. The results are presented in the table.

It was found that the fixed CRBC was as good as fresh CRBC for HA and HI tests. This result correlates well with the earlier reports (Zheng *et al.*, 1989). When both the fixed CRBC were compared with fresh CRBC, no significant difference ($P>0.05$) in HA activity was noticed. After fixation of CRBC with aldehydes, sodium borohydride was used to remove unsaturated free aldehydes (Roy and McEwen, 1977).

Glutaraldehyde fixed CRBC was more stable at room temperature compared to formaldehyde fixed CRBC. This is possibly because of structural differences of the two different aldehydes used. Glutaraldehyde has more free aldehyde groups compared to formaldehyde which binds with thiol groups (-SH) present on the surface of

TABLE
HAEMAGGLUTINATION (HA) TEST USING FIXED CHICKEN ERYTHROCYTES
GEOMETRIC MEAN HA TITRE**

Test After weeks	Formaldehyde fixed CRBC	Glutaraldehyde fixed CRBC	Fresh CRBC
1	6.2 ± 0.36	6.2 ± 0.36	6.4 ± 0.32
2	6.2 ± 0.36	6.5 ± 0.36	6.8 ± 0.36
3	6.6 ± 0.36	6.6 ± 0.29	6.3 ± 0.24
4	6.2 ± 0.34	6.0 ± 0.24	6.0 ± 0.24
5	6.3 ± 0.31	6.7 ± 0.34	6.4 ± 0.35
6	6.2 ± 0.31	6.2 ± 0.31	6.2 ± 0.31
7	6.4 ± 0.29	6.2 ± 0.27	6.2 ± 0.27
8	6.2 ± 0.23	6.4 ± 0.29	6.2 ± 0.23

** Values are expressed in log 2 base.

CRBC and re-Orientation of electrons are important for strong binding. If binding is weak, bonds will be broken at room temperature and ultimately erythrocytes will be lysed. Further formaldehyde is volatile, whereas glutaraldehyde is stable.

Both the fixed cells were stable at 4°C for an observation period of eight weeks. Long shelf life of the fixed cells has been reported earlier (Scott, 1986). The fixed CRBC has several advantages over fresh CRBC in the diagnosis of ND since fixed CRBC can be made readily available and carried to the field for diagnosis of ND.

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XXI Congress of Indian Society for Veterinary Surgery at HPKV, Palampur Concluded : The congress concluded on 19th October 1997 with recommendations on the emerging trends in management of Surgical trauma in animals, to be sent to Veterinary Colleges and Agricultural Universities for implementations. Office bearers of the society were elected at the close of the congress. Onthe whole, the congress was a grand success.