Communication

Viral haemagglutination of glutaraldehyde-fixed sheep erythrocytes

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Des globules rouges de mouton stabilisés par traitement à la glutaraldéhyde ont été employés avec succès dans les tests d'hémagglutination par le virus de la maladie de Newcastle et celui de la grippe équine. La fixation a permis de conserver les globules rouges pendant 21 jours, sans perte de leur pouvoir agglutinant. La meilleure stabilité était obtenue lorsque les cellules étaient congelées à -114°C dans la phase gazeuse de l'azote liquide.

Mots clés : Ovin - Grippe équine - Maladie de Newcastle - Epreuve d'hémagglutination - Erythrocyte - Congélation - Cameroun.

Introduction

Haemagglutination (HA) and haemagglutination-inhibition (HI) tests are commonly used for detecting viral antigens and antibodies. Whether coated or not, fresh erythrocytes can only be used for a few days before they become haemolysed. In order to make them a more convenient reagent, they can be fixed or stabilized by agents such as formalin (9), pyruvic aldehyde (6), sulphosalicylic acid (4) and glutaraldehyde (2, 7). The aim of the present study was to test the viral agglutinability of sheep erythrocytes after stabilisation of the cells with glutaraldehyde.

Materials and Methods

Sheep blood was collected in an equal volume of Alsever's solution. Erythrocytes were separated by triple centrifugation at 700 g for 10 minutes in 20x their volume of Alsever's solution. Packed erythrocytes were then diluted in phosphate-buffered saline (PBS) at pH 7.3 to a concentration of 2.5 %.

Fixation with glutaraldehyde was by an adaptation of the methods described by MAEDA (7) and SATO *et al.* (8). Twenty-five ml of the 2.5 % red blood cell suspension was mixed with 3 ml of 2.5 % glutaraldehyde in PBS and then kept at 37°C for one hour with gentle magnetic stirring. The erythrocytes changed from bright red to chocolate brown. The fixed cells were then washed three times in PBS before preparing a one per cent suspension in PBS containing 0.2 % bovine serum albumin and 0.02 % sodium azide.

Aliquots of the fixed erythrocyte suspension were stored at 25°C, 4°C, -20°C and -114°C in the vapour phase of liquid nitrogen. The pH of other aliquots was changed from 7.3 to 9.6 and 4.5 before storage at 4°C. Some fixed cells were resuspended in saline and stored at 4°C. The stability of the erythrocytes under these conditions was monitored by HA tests using Newcastle disease and equine influenza viruses until haemolysis occurred. HA tests were carried out as described by ALLAN and GOUGH (1). Differences were tested for significance by paired t-tests (3).

Results

The glutaraldehyde-fixed sheep erythrocytes remained stable under various conditions and gave consistent HA titres with samples of the virus stabilates for at least 21 days (table I). There was no agglutination in the absence of the virus. The difference between the titres at days 0 and 21 by the fixed cells was not significant ($p > 0.1$) regardless of the virus used. After 50 days, a highly significant deterioration ($p < 0.01$) occurred in all of the erythrocyte aliquots except those kept in the vapour phase of liquid nitrogen (-114°C). After 50 days, cells kept at 25°C, at -20°C and those suspended in saline had haemolysed. Erythrocytes kept at 4°C at a pH of 7.3, 4.5 and 9.6 still agglutinated but gave decreased titres. The fixed cells remained stable for over three months in liquid nitrogen. No marked change in the HA titre of the viruses used occurred during this period (table I).

Discussion and conclusion

The tests showed that glutaraldehyde-fixed sheep erythrocytes remained stable and gave consistent HA titres for at least 21 days. Thereafter the cells deteriorated except those stored in the vapour phase of a liquid nitrogen refrigerator. This is in agreement with the finding of DENG *et al.* (5) who used pyruvaldehyde-fixed sheep erythrocytes coated with duck plague virus antibodies. The stability of glutaraldehyde-fixed cells and their ability to agglutinate only in the presence of virus can enable a small diagnostic laboratory that only occasionally runs HA and HI tests to maintain on tap a supply of the erythrocytes ready for use.

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TABLEAU I Agglutination titres of Newcastle disease and equine influenza viruses using stored glutaraldehyde-fixed sheep erythrocytes

storage conditions	storage period (days) and HA titre*				
	0	7	14	21	50
PBS (pH 7.3), 4°C	(a) 1024	1024	1024	1024	256
	(b) 512	512	512	256	128
PBS (pH 4.5), 4°C	(a) 1024	1024	512	1024	256
	(b) 512	512	512	512	256
PBS (pH 9.6), 4°C	(a) 1024	1024	1024	1024	256
	(b) 512	512	512	512	64
PBS (pH 7.3), 25°C	(a) 1024	1024	1024	512	L
	(b) 512	512	512	128	L
PBS (pH 7.3), —20°C	(a) 1024	1024	1024	1024	L
	(b) 512	512	512	256	L
Saline, (pH 7.2), 4°C	(a) 1024	1024	1024	1024	L
	(b) 512	512	512	512	L
Liquid N_2 (—114°C),	(b) 512	512	512	512	512

** Expressed as the reciprocal of the 100 per cent end-point dilution.*
L = haemolysis.
a = Newcastle disease virus.
b = Equine influenza virus.
All tests were carried out in duplicates.

References

1. ALLAN (W.H.), GOUGH (R.E.). A standard haemagglutination-inhibition test for Newcastle disease. 1. A comparison of macro and micro methods. *Vet. Rec.*, 1974, **95**: 120-123.

2. AVREMEAS (S). Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry*, 1969, **6**: 43-50.

3. BAILEY (N.T.J.). Statistical methods in Biology. London, Hodder and Stoughton, 1983.

4. BRECHT (H). Properties of erythrocytes stabilized with sulphosalicylic acid and their use in an indirect haemagglutination test with influenza virus RNP-antigen. *J. Immun.*, 1948, **101**: 18-24.

5. DENG (M.Y.), BURGESS (E.C.), YUILL (T.M.). Detection of duck plaque virus by reverse passive haemagglutination test. *Avian Dis.*, 1984, **28**: 616-628.

6. LING (N.R.). The attachment of proteins to aldehyde-tanned cells. *Br. J. Haemat.*, 1961, **7**: 299-302.

7. MAEDA (A.D.). Studies on the immune response to orf. Ph.D. Thesis. University of Edinburgh, 1980.

8. SATO (K.), INABA (Y.), TOKUHISA (S.), MIURA (Y.), KANEKO (N.), ASAGI (M.), MATUMOTO (M.). Detection on bovine coronavirus in faeces by reverse haemagglutination. *Arch. Virol.*, 1984, **80** : 23-31.

9. WHITMAN (J.E.), HETRICK (F.M.). An indirect haemagglutination test for detecting antibody to infectious bovine rhinotracheitis virus. *Cornell Vet.*, 1965, **55:** 613-622.

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Sheep erythrocytes stabilized by treatment with glutaraldehyde were used successfully in haemagglutination tests with Newcastle disease, and equine influenza viruses. The fixation enabled storage of the cells for at least 21 days without altering their agglutinable properties. Stability was best when the cells were stored at -114°C in the vapour phase of liquid nitrogen.

Key words: Sheep - Newcastle disease - Equine influenza - Haemagglutination test - Erythrocyte - Freezing - Cameroon.