

## NOTES

# Use of a New Buffer System with Formalinized Sheep Erythrocytes in the Rubella Hemagglutination-Inhibition Test

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Received for publication 9 December 1970

The increased sensitivity and improved agglutination and settling patterns of formalinized sheep erythrocytes in a new buffer, HEPES (*N*-2-hydroxyethylpiperazine *N'*-2'-ethanesulfonic acid), make this system more suitable for use in the rubella hemagglutination-inhibition test.

In a previous communication (2), we reported the use of formalinized sheep red blood cells (formalinized SRBC) in a rubella hemagglutination-inhibition (HI) test. But it was observed that the sensitivity of formalinized SRBC to agglutination by rubella hemagglutination antigen (HA) was lower than that of pigeon erythrocytes. This necessitated the use of more antigen in the test and caused an apparent decrease in the antibody titer. By using now another buffer system (1, 3), HEPES (*N*-2-hydroxyethylpiperazine *N'*-2'-ethanesulfonic acid), at pH 6.2 instead of DGV (dextrose-gelatine-Veronal) at pH 6.8, the sensitivity of formalinized erythrocytes to agglutination was not only increased to the level of that of pigeon erythrocytes, but the agglutination pattern was also superior to pigeon erythrocytes. Excellent correlation of antibody titers was also obtained between tests with formalinized SRBC in HEPES and the conventional avian erythrocytes in DGV.

Formalinized SRBC were prepared, and rubella antibody was determined as in (2). However, slight changes were made in the test components while using HEPES, so as to bring the test serum nearer to the pH of HEPES before double dilution. The buffer containing 0.025M HEPES (Calbiochem, Australia Pty. Ltd.), 0.14 M NaCl, 0.001 M CaCl<sub>2</sub>, and 0.5% bovine albumin was used for diluting serum, antigen, and formalinized erythrocytes. The test components were: 0.1 ml of serum, 0.05 ml of heparin (750 units/ml), 0.05 ml of 0.2 M manganous chloride, and 0.05 ml of 50% formalinized SRBC. The

supernatant fluid from above was diluted 1:4 with HEPES before using as a final 1:10 dilution (strictly speaking, a 1:9 dilution if the fluid volume of 0.05 ml of 50% cells is taken as 0.025 ml) of serum. For final titration, 0.16% pigeon cells in DGV or 0.20% formalinized SRBC in HEPES were used.

The comparison of hemagglutination titer of the antigen with pigeon erythrocytes and formalinized SRBC in different buffers is shown in Table 1. The increased sensitivity to agglutination of formalinized cells in HEPES is apparent from identical antigen titers with pigeon cells in DGV, whereas the titer was fourfold lower with formalinized cells in DGV. The comparison of HI titers of 102 sera with pigeon cells and formalinized SRBC both in DGV is shown in Fig. 1. The same data were previously presented in tabular form (2). Forty three (41%) of these samples had identical titers in both tests, 41 had twofold and 12 had fourfold lower titers, whereas only 6 had twofold higher titers with formalinized SRBC in DGV. With formalinized SRBC in HEPES, 53 (47%) of 113 sera tested had identical titers, 25 had twofold higher, 6 had more than twofold higher, 28 had twofold lower and 1 had fourfold lower titers when compared to tests with fresh pigeon erythrocytes in DGV (Fig. 2). With formalinized SRBC, HI titers were, therefore, identical or equivalent, i.e., within one dilution of each other in 90 (88%) of 102 in DGV and in 106 (95%) out of 113 sera in HEPES buffer as compared to fresh pigeon erythrocytes in DGV. But the number of samples with higher

TABLE 1. Comparison of titers of rubella hemagglutination antigen (HA) by using pigeon erythrocytes and formalinized sheep red blood cells (formalinized SRBC)

Cells	Concn (%)	Buffer	Antigen	HA titer (units/0.025 ml)
Pigeon	0.16	DGV	Flow Laboratories, lot C961160	256
Pigeon	0.16	DGV	Flow Laboratories, lot C961172	128
Pigeon	0.16	DGV	Wellcome Research, lot K1456	32
Formalinized SRBC	0.20	DGV	Flow Laboratories, lot C961160	64
Formalinized SRBC	0.20	DGV	Flow Laboratories, lot C961172	32
Formalinized SRBC	0.20	HEPES	Flow Laboratories, lot C961160	256
Formalinized SRBC	0.20	HEPES	Flow Laboratories, lot C961172	128
Formalinized SRBC	0.20	HEPES	Wellcome Research, lot K1456	32

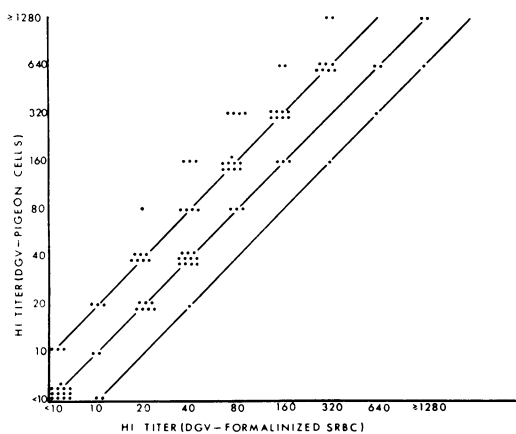


FIG. 1. Comparison of HI titers of 102 sera in tests with pigeon erythrocytes and formalinized sheep erythrocytes, both in DGV buffer. The central of three diagonal lines is the line of identical titer, representing sera whose titers are the same in both tests. The other two lines represent sera with titers one dilution lower or higher than each other in the two tests.

or lower titers was evenly distributed in tests using formalinized SRBC in HEPES, whereas most of the samples had lower titers with formalinized SRBC in DGV. Another useful advantage was the very fine and stable agglutination and inhibition patterns obtained by using formalinized erythrocytes in HEPES buffer. Moreover, the same antigen titer was obtained repeatedly with the same batches of antigen and formalinized cells. We have also used HEPES as eluent for fractionation of serum on a Sephadex G-200 column. The titers of the fractionated samples (into IgM and IgG) as determined by HI tests with formalinized SRBC in HEPES correlated well with the known clinical and other serological evidences in patients with recent rubella infection (*in preparation*). The use of formalinized sheep erythrocytes in HEPES buffer

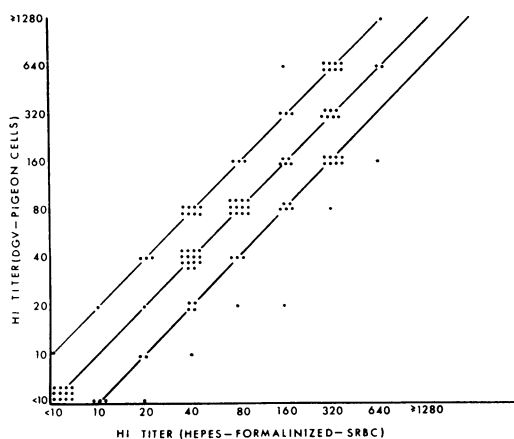


FIG. 2. Comparison of HI titers of 113 sera in tests with pigeon erythrocytes in DGV and formalinized sheep erythrocytes in HEPES buffer. The three diagonal lines have the same significance as in Fig. 1.

is, therefore, expected to simplify further the rubella HI test and make it possible to test larger numbers of people for determination of sero-immunity and for the diagnosis of rubella infection. The increased sensitivity of formalinized SRBC to agglutination by rubella hemagglutination antigen in HEPES buffer effectively counteracts the objection of using such stabilized cells.

One of us (J. D. G.) was supported by a Research Fellowship from ATN Channel 7, Sydney. Equipment provided by N. H. & M. R. C., Australia, was used for this study.

#### LITERATURE CITED

- Good, N. E., G. D. Winget, W. Winter, T. N. Connolly, S. Izawa, and R. M. M. Singh. 1966. Hydrogen ion buffers for biological research. *Biochemistry* 5:467-477.
- Gupta, J. D., and J. D. Harley. 1970. Use of formalinized sheep erythrocytes in the rubella hemagglutination-inhibition test. *Appl. Microbiol.* 20:843-844.
- Liebhaber, H. 1970. Measurement of rubella antibody by hemagglutination inhibition. *J. Immunol.* 104:818-825.