Laboratory techniques

Polybrene technique for red cell antibody screening using microplates

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SUMMARY The manual polybrene technique was adapted as a microplate test for antibody screening to determine its sensitivity and specificity and compared with conventional tube testing using various antibodies and serologically inert sera. Equivalent results were obtained for both techniques and it is concluded that this adaptation of the polybrene technique is useful in pretransfusion testing.

The manual polybrene technique, originally described by Lalezari and Jiang,¹ is a rapid and sensitive method for the detection of a wide range of red cell antibodies. The technique has been recommended for use in pretransfusion testing,²⁻⁴ but it has so far only been described as a tube test for antibody screening, which limits the number of tests performed at any one time.

Although the technique has been applied to microplates for phenotyping,⁵ this modification has not been described for routine antibody screening. This report outlines a microplate adaptation of the technique which facilitates batching of red cell antibody screening tests. The technique was evaluated for sensitivity and specificity by parallel testing with the tube test, using a variety of antibodies and serologically inert sera.

Material and methods

Reagents for the polybrene technique were prepared according to the formulae outlined by Lalezari and Jiang.¹ Polybrene was obtained from Sigma Chemicals St Louis, Missouri.

The tube polybrene test was performed according to the method of Lalezari and Jiang,¹ except that the tubes were only examined macroscopically for agglutination after rolling them on the work bench until the non-specific aggregation in the negative control had dispersed.

MICROPLATE POLYBRENE TEST

For the 0.01 ml of antibody screening cells (Abtectcell, Commonwealth Serum Laboratories, Melbourne, Australia), 0.02 ml of test serum, and 0.2 ml of low ionic medium were added to the wells of a round

bottom microplate (Titertek, Flow Laboratories). After one minute of incubation the plate was centrifuged at 650 g for two minutes in a general purpose centrifuge (Clements GS2000, Australia) fitted with a microplate carrier. The plate was inverted over a sink and the supernatant flicked out. One drop of 0.05% polybrene solution was added to each well. After mixing and centrifugation the supernatant was discarded and one drop of glucose citrate resuspension solution added. The plate was agitated at a speed setting of "5" for 10 seconds on a Titertek microplate shaker (Flow Laboratories) and the wells examined for agglutination under a low power illuminated magnifier. Agglutination was graded on a 0-4+ scale (Ortho Diagnostic Systems Consultation Service Ontario, Canada).

Patient sera containing previously identified antibodies had been stored at -15°C for varying periods of time. Sera without antibodies were routine samples tested within five days of receipt.

Table Reactivity of antibodies by tube and microplate manual polybrene tests

Antibody	No	Equivalent reactivity	Weaker by plate method	Stronger by plate method
E	18	16	1	1
Fy ^a	16	16	—	—
C _w	15	14	—	1
K	13	13	—	—
c	10	10	—	—
Jk ^a	7	6	—	1
S	6	5	1	—
C	5	5	—	—
Jk ^b	3	3	—	—
D	14	13	—	1
s	1	1	—	—
e	1	1	—	—
Total	109	103	2	4

Results

The table shows the comparative reactivity of antibodies by the tube and microplate methods. Equivalent results were obtained for both techniques. One hundred sera previously shown to contain no alloantibodies produced negative results by both methods.

Discussion

The ability of the manual polybrene technique to detect rapidly a wide range of red cell antibodies makes it useful for pretransfusion testing.²⁻⁴ Steane *et al* suggested that it could be used for crossmatching when preceded by a conventional antibody screen.³ Pettit and Kronenberg proposed the test as an alternative to enzyme tests in antibody screening protocols.⁴ Crawford *et al* showed that microplate techniques were suitable for batch processing of routine serological procedures.⁶ Adaptation of the manual polybrene technique to microplates has so far only been described for phenotyping, using selected antisera.⁵ The use of a microplate polybrene technique for antibody screens facilitates the application of this technique for batch processing in larger hospital laboratories, particularly in antenatal screening.

Performance of the manual polybrene technique in microplates for antibody screening confers additional advantages. The final examination for agglutination is simpler with the microplate method because of the mechanically standardised resuspension of cells in the

end stage. In contrast, using the tube technique, variation occurs in the time taken for dissociation of non-specific aggregation in different tubes in a batch and in the degree of vigour used to resuspend the cells manually.

Application of a microplate polybrene technique to antibody screening expands the potential role of polybrene procedures in pretransfusion testing.

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