COMMUNICATIONS

Letters to the Editors

Platelet monolayer formation in the solid-phase red cell adherence method using fresh frozen plasma

A rapid and simple solid phase red cell adherence (SPRCA) technique for platelet antibody screening has been previously described using antithrombocyte globulin (ATG) to bind platelets to microtiter wells and form a stable platelet monolayer.[1,2] Because ATG (DAKO, Denmark) is no longer available, an alternative platelet binding method has been established. We have made a simple modification to the original ATG SPRCA method by diluting group O platelet-rich plasma in inert group O expired fresh frozen plasma (FFP) to give a final platelet concentration of 50–60 $\times$ 10^9L^{-1}. Microtiter wells (NUNC, Denmark) were treated with 200 μL of methanol (BDH, Merck, Australia) for 5 minutes and dried. Fifty microliters of diluted platelets were added to each microtiter well and the microtiter plate was centrifuged at 150 g for 3 minutes, followed by incubation at 37°C for 15 minutes. Thereafter, the modified SPRCA method followed the method described earlier for the ATG SPRCA method.[2] Excess nonadhered platelets were removed by washing $\times$ 3 with 200 μL of 0.2% bovine serum albumin in phosphate-buffered saline (0.2% BSA/PBS), then 50 μL of serum and 100 μL of 1.9% glycine were added, followed by incubation at 37°C for 15 minutes. The plates were washed $\times$ 4 with 200 μL of 0.2% BSA/PBS, then 50 μL of anti-human globulin (CSL, Australia) and 50 μL of 0.6% antiglobulin control cells (CSL) diluted in Celpresol (CSL) were added. The microplates were then centrifuged at 90 g for 2 minutes. Presence or absence of red cell effacement was observed for result determination.

We compared the results of the modified SPRCA with that of the original method to ensure adequate platelet binding and appropriate test results. Thirty-nine sera containing known platelet antibodies were tested including 30 anti-HLA, 3 anti-HPA-1a, 1 anti-HPA-3a, 1 anti-HPA-5a, and 4 quinine-associated antibodies. These known platelet antibodies had been identified previously using a monoclonal antibody immobilization of platelet antigen (MAIPA) technique. In addition, 28 unknown sera from bone marrow transplant (BMT) recipients were examined.

Identical results were obtained by the two platelet-binding methods, with the exception of one known HLA antibody found positive only by the modified SPRCA. All 28 BMT sera were found to be negative by both binding methods.

Titers of seven platelet antibodies were determined, including two HPA-1a antibodies and five HLA antibodies. The seven platelet antibodies had titers from 1 to 32. The titers of three antibodies were found to be the same by both platelet-binding methods. The titer of one antibody was twofold higher by ATG SPRCA, whereas the remaining three platelet antibodies were twofold higher by the modified SPRCA method.

The results obtained were comparable for the two platelet-binding methods both in the ability to detect positive and negative sera and the titer of the antibodies. The strength of red cell effacement for the positive reactions was also found to be concordant. The performance time was identical for both assays.

In conclusion, FFP is an inexpensive and simple alternative to ATG for performance of the SPRCA method. The effective platelet binding in this modified method may be associated with adherence of fibrinogen, albumin, or other plasma proteins present in FFP between the platelets and the microtiter well.[3]

References

1. Rachel JM, Sinor LT, Tawik OW, et al. A solid-phase red cell adherence test for platelet crossmatching. Med Lab Sci 1985;42:194–5.
2. Lown JAG, Ivey JG. Evaluation of a solid phase red cell adherence technique for platelet antibody screening. Transfus Med 1991;1:163–71.
3. Edmunds LH. Blood surface interactions during cardiopulmonary bypass. J Card Surg 1993;8:404–40.

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