Technical methods

Storage of reagent platelets for anti-platelet antibody testing in the ^{51}Cr platelet lysis assay

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The ^{51}Cr platelet lysis assay (Aster and Enright, 1969) is a clinically useful test for detecting specific anti-platelet antibodies and anti-drug antibodies that interact with platelets. In a clinical laboratory setting, it is extremely useful to have available a method of long-term platelet storage for use in this assay. This is especially true when platelets from rare Pl^{A1} negative donors or from paroxysmal nocturnal haemoglobinuria (PNH) patients are needed as test reagents. We describe a rapid and simple technique for freezing platelets that may be used as reagents in the ^{51}Cr release test system.

¹EDTA Vacutainer Tubes, B&D.

²ACD Vacutainer Tubes, B&D.

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Testing followed the procedure of Cimo *et al.* (1977). In brief, platelets were isolated from EDTA anticoagulated whole blood,¹ labelled with ^{51}Cr , treated with bromelain, and reacted with antisera. Fresh human serum was added as a source of complement, and after a 2-hour incubation the reaction was stopped by the addition of 0.5% EDTA in saline. This mixture was centrifuged, and by counting an aliquot of supernatant and the button in a gamma scintillation counter, the percentage of released ^{51}Cr was calculated.

To determine the constraints of sample acquisition, the effect of holding the EDTA sample at room temperature (22–24°C) before platelet separation and testing was evaluated. As seen in Fig. 1, it was possible to hold blood samples for up to three days before testing without loss of the ability to identify the Pl^{A1} antigen. It was also noted (data not cited) that fresh acid citrate dextrose (ACD) anticoagulated blood² was as acceptable as fresh EDTA blood; however, room temperature storage of ACD blood was not evaluated.

EDTA anticoagulated whole blood up to 3 days old was used to prepare platelets for freezing. Platelets were separated from the specimens by centrifugation in a Sorval RC-3 at 300 *g* for 10 minutes at room temperature. The platelet-rich

Fig. 1 Effect of room temperature storage of EDTA whole blood before platelet separation and testing. Platelets from four different Pl^{A1} positive donors were tested with a 1:2 dilution of anti- Pl^{A1} antiserum. Vertical bars represent the range of % lysis of platelet preparations from four subjects tested up to 4 days of age. The negative control range was determined by testing six normal sera: ● anti Pl^{A1} serum; □ normal control serum. Vertical bars indicate range.

plasma was centrifuged at 2300 *g* for 7 minutes at room temperature, and the platelet-poor plasma was discarded. Platelet buttons were resuspended to 10⁵/ml in 5% dimethyl sulphoxide in heat-inactivated fetal calf serum on ice. The resuspended platelets were then placed in Beckman microfuge tubes and placed in a -76°C freezer. Thawing took place by allowing the platelets to come to room temperature (22-24°C), and then washing with platelet wash solution (Silvergleid *et al.*, 1977). Washed platelets were resuspended in phosphate-buffered saline at 10⁵/ml. In Fig. 2, it is seen that frozen platelets were

Fig. 2 % lysis of fresh (less than 24 hours) versus frozen (within 72 hours after drawing platelets) for three different PI^{A1} positive specimens tested with a 1:2 dilution of anti-PI^{A1} serum; ● anti PI^{A1} serum; □ normal control serum. Vertical bars indicate range.

equivalent to fresh platelets in the detection of the PI^{A1} antigen. Also PI^{A1} negative platelets were still non-reactive after the freezing and thawing process. Platelets from a PNH patient were frozen (at 48 hours of age) and thawed (three months after freezing) following the above procedure. Testing using anti-PI^{A1} serum showed a titre of 16 against frozen platelets from the PNH donor and a titre of 4 against frozen bromelain-treated normal platelets. Testing

using an anti-quinidine antibody indicated a titre of 64 with the same frozen platelets from the PNH donor and a titre of 16 with frozen bromelain-treated normal platelets. These data support Aster's earlier reports of the increased sensitivity of platelets from PNH patients as test reagents for some anti-platelet antibodies (Aster and Enright, 1969). Bromelain-treated normal platelets from four subjects were thawed after 8-12 months' storage at -76°C. The platelets showed the same titres with anti-PI^{A1} sera (4) as they did when tested fresh. They also showed the same titres (16) with anti-quinidine antibody, frozen versus fresh. These data demonstrate that freezing platelets for use in the ⁵¹Cr release assay is a feasible and simple technique. The availability of stable reagents should permit greater utilisation of this clinically useful test for anti-platelet and platelet interreactive anti-drug antibodies.

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References

- Aster, R. H., and Enright, S. E. (1969). A platelet and granulocyte membrane defect in paroxysmal nocturnal hemoglobinuria: usefulness for the detection of platelet antibodies. *Journal of Clinical Investigation*, **48**, 1199-1210.
- Cimo, P. L., Pisciotto, A. V., Desai, R. G., Pino, J. L., and Aster, R. H. (1977). Detection of drug-dependent antibodies by the Cr⁵¹ platelet lysis test: documentation of immune thrombocytopenia induced by diphenylhydantoin, diazepam, and sulfisoxazole. *American Journal of Hematology*, **2**, 65-72.
- Silvergleid, A. J., Haffleigh, E. B., Harabin, M. A., Wolf, R. M., and Grumet, F. C. (1977). Clinical value of washed-platelet concentrates in patients with non-hemolytic transfusion reactions. *Transfusion*, **17**, 33-37.

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