

V. SUMMARY

The recommended technique for fibrin glue preparation from CPD blood 1 unit, which provide 200 ml platelet poor plasma (PPP) was as followed.

(1) One hundred and seventy-five milliliters of CPD-plasma for fibrinogen preparation was frozen at the temperature below -20°C for 18-24 hours. The frozen plasma was thawed at 4°C for 6-10 hours. The thawed plasma was refrozen again at temperature below -20°C for 18-24. The refrozen plasma was thawed at 4°C for 6-10 hours. The thawed plasma was centrifuged at 3,000 rpm for 10 minutes at 4°C . The supernatant was removed by using a pipette, leaving about 12 ml of the mixture of precipitate and supernatant. The mixture was added with EACA in the final concentration of 7.5 mg/ml for fibrinogen solution. The fibrinogen solution was frozen at -20°C or lyophilized and stored at -20°C until used.

(2) Twenty-five milliliters of CPD-plasma for thrombin preparation were diluted to 250 ml with distilled water. The pH of diluted plasma was adjusted to 5.3 with 2% acetic acid. It was centrifuged at 3,000 rpm for 10 minutes at 4°C . The supernatant was discarded. The precipitate was dissolved in 6.25 ml of 0.85% NaCl. The pH of mixture was adjusted to 7.0 with 2% Na_2CO_3 . Seventy-five microliters of 0.25 M CaCl_2 was added and the coagulated fibrin was removed as it formed. It was stood at 4°C for 2 hours for completely thrombin formation. The crude solution was purified by adding one volume of absolute ethanol to one volume of thrombin solution at room temperature. Thrombin was extracted from the precipitate with 6.25 ml of 0.85% NaCl. The saline extract was centrifuged

at 3,000 rpm for 10 minutes at 4 °C. The supernatant was added with CaCl₂ solution and gentamicin in the final concentration of 40 mmol/l and 2 mg/ml, respectively, for thrombin solution.

- (3) The appropriate ratio for fibrin glue application was 2:1 of fibrinogen and thrombin solution.