Production of freeze-dried human antihaemophilic cryoprecipitate

G MILLIGAN, R GRAHAM, S HANRATTY,* W MUIR, R MITCHELL

*From the Glasgow and West of Scotland Regional Blood Transfusion Centre, at Law Hospital, Carluke, Lanarkshire and the *Blood Transfusion Service Board, Pelican House, Dublin*

SUMMARY Concentration of factor VIII from fresh plasma by cryoprecipitation remains the basis for preparation of products used to treat haemophilia A.

This paper describes the preparation of a factor VIII concentrate from small plasma pools in transfusion centres with drying facilities.

The dried concentrate from one litre of plasma dissolves very well in 50 or 100 ml of distilled water and contains around 500 IU per bottle. The specific activity per mg protein is 0.19 IU and the fibrinogen concentration is half that in frozen cryoprecipitate. This method of drying causes no appreciable loss in the factor VIII activity and little denaturation as shown by the factor-VIII-related antigen/factor VIII activity ratio of 1.7.

Human factor VIII is available in the United Kingdom as a blood component, cryoprecipitate, prepared by regional transfusion centres and as a plasma fraction in a freeze-dried concentrate form produced by National Health Service protein fractionation units and commercial sources.¹

Cryoprecipitate has been widely used and has proved acceptable in the treatment of patients deficient in factor VIII. Cryoprecipitation, as described by Pool and Shannon,² is a simple, inexpensive and efficient method of producing a readily available source of factor VIII in transfusion centres.

Unfortunately, in contrast to the freeze-dried products, the component varies in potency from pack to pack, needs careful storage at temperatures below -20°C , has a relatively short shelf life and must be thawed and pooled before administration to patients. A dried cryoprecipitate has most of the advantages associated with the blood fraction and has been adopted in many countries outside the United Kingdom.³

This paper describes a method of producing dried cryoprecipitate at the West of Scotland Blood Transfusion Centre, in collaboration with the Blood Transfusion Board, Dublin.

Material and methods

A donation of blood is collected into a triple plastic

blood collecting pack containing citrate phosphate dextrose anticoagulant. Each donation is screened for HBsAg by a sensitive radioimmunoassay test (Abbott Ausria II Radioimmunoassay Test). Approximately 220 ml of plasma is separated into one of the satellite packs within 6 h of collection after centrifugation at 4°C (4875 *g* for 10 min). The pack containing the concentrated red cells (Haematocrit 0.7 l/l) is detached and the plasma pack immediately frozen in aluminium cassettes in an alcohol solid carbon dioxide mixture controlled at -70°C for a minimum of 20 min (Fig. 1). The frozen plasma is stored at -30°C until required for cryoprecipitate production.

Cryoprecipitate is prepared by thawing the plasma in a 4°C thermostatically controlled waterbath until only a small amount of ice remains in the pack. Twenty-four units of plasma thaw in approximately 90 min. The packs are removed from the bath and immediately centrifuged at 4°C (3200 *g* for 10 min). Most of the supernatant plasma is carefully transferred into the remaining satellite pack using a plasma expessor (Travenol Laboratories, Thetford, England). By this technique the loss of factor VIII in supernatant plasma is minimised.

The cryoprecipitate is processed under laminar air-flow conditions in groups of five donations. Each group is allowed to liquefy at 22°C for 10 min after which they are connected by means of pooling set FKC 2250 (Travenol Laboratories) (Fig. 2), to a 250 ml bottle containing 100 ml of filter-sterilised pyrogen-free glycine buffer (Table 1).

Fig. 1 *Aluminium cassette for freezing fresh plasma packs.*

Fig. 2 *Five lead pooling set.*

Table 1 *Constituents of glycine buffer*

Sodium chloride	3.4 g
Trisodium citrate	5.5 g
D-glucose	13.0 g
Glycine	10.0 g
Distilled water to	1000.0 ml
Adjust pH to 6.5 with 0.1 M HCl	

Approximately 50 ml of buffer is introduced into the first pack. This is agitated manually and the contents transferred to the rest of the packs in turn and finally retained in the fifth. This procedure is repeated using the remaining buffer in the bottle. The total contents of the fifth pack are transferred to the

bottle. The pooling set is disconnected from the bottle and the bottle recapped. The residual material in the pooling set is retained for sterility tests⁴ (approximately 5 ml). The bottles are shell frozen and stored at -70°C prior to lyophilisation, using a Usifroid Shell Freezer (L'Air Liquide (UK) Ltd, London, England).

During the fully monitored drying process, the temperature of the product is not allowed to exceed 20°C .⁵ The total drying time is 72 h. Before final sealing, oxygen-free nitrogen gas is introduced into the bottles. The bottles are labelled and stored at 4°C , and 6% of the total load was randomly selected for sterility and quality control.

Results and discussion

The product is a whitish fine powder which readily reconstitutes in 100 ml or 50 ml of distilled water within 3 min. Ten bottles of an initial production of 120 were submitted to quality control. The results are shown in Table 2 and are compared with similar analyses carried out on routinely produced conventional frozen cryoprecipitate. The mean factor VIII content of the bottles was found to be 542 IU, the 90% confidence range being 310-774 IU. No appreciable loss of factor VIIIC concentration occurred in the production of the product. The reduction in the protein content leads to a noticeable increase in the specific activity. The reduced protein and fibrinogen content make the dried material considerably superior to frozen cryoprecipitate in the treatment of haemophilia A.

Table 2 Comparison of freeze-dried human anti-haemophilic cryoprecipitate with frozen cryoprecipitate

Analysis	Dried cryoprecipitate	Routine frozen cryoprecipitate
Factor VIIIC (IU/ml)	5.42	5.79
Total protein (mg/ml)	27.80	60.00
Specific activity (IU factor VIII/mg protein)	0.194	0.08
Fibrinogen (mg/ml)	4.34	8.446
Factor VIII: fibrinogen ratio (IU/mg)	1.25	0.69
Fibrinogen/unit factor VIIIC (mg/unit)	0.80	1.46
Sterility	Sterile	Sterile

A further five bottles were independently assayed and all contained >500 IU factor VIII per bottle. In addition to tests shown in Table 2, these five bottles were assayed for factor VIII-related-antigen and factor VIII ristocetin co-factor and

average results of 9.2 u/ml and 12.8 u/ml respectively were obtained. The mean factor VIII-related-antigen/factor VIIIC ratio was found to be 1.7. This ratio is favourable⁶ and suggests little denaturation of factor VIII during processing.

No evidence of bacterial contamination was noted in the samples obtained during bottling or after final drying.

The product of 17500 donations (3500 bottles of freeze-dried cryoprecipitate) prepared by the Blood Transfusion Service Board, to date have been administered to haemophilia A patients at the National Haemophilia Treatment Centre, Dublin. The material was found as efficacious as conventional frozen cryoprecipitate and no adverse reactions were reported. A clinical trial is presently being conducted in Glasgow and West of Scotland and the results will be offered for publication at a later date.

This product combines the advantages of simplicity, high recovery of factor VIII, small pool size, stability at 4°C with convenience of administration to patients.

Our thanks are due to Dr DP Thomas and Dr TW Barrowcliffe, National Institute for Biological Standards and Control, Holly Hill, London, for performing various factor VIII assays and to our Medical and Technical colleagues for both constructive advice and technical assistance.

References

- Watt JG. Plasma fractions. In: Cash JD, ed. *Clinics in haematology*. London: Saunders, 1976;5, 1:106-9.
- Pool JG, Shannon AE. Production of high potency concentrates of anti-haemophilic globulin in a closed bag system. *N Engl J Med* 1965;273:1443-7.
- Report on the European Public Health Committee in the preparation and use of coagulation factor VIII and IX for transfusion. CDSP (79) 52. Strasbourg: Council of Europe, 1979.
- European Pharmacopoeia (1978). Test for Sterility. Revised text, July, 1978.
- Vermeer C, Southe BAM, Ates G, Brummelhuis HGJ. Contributions to the optimal use of human blood: VIII. Increase in the yield of factor VIII in four-donor cryoprecipitate by an improved processing of blood and plasma. *Vox Sang* 1976;30:1-22.
- Yang HC, Duffy C. Factor VIII procoagulant and antigen content of antihaemophilia factor (AHF) concentrates. *Transfusion* 1978;18, 6:747-9.

Requests for reprints to: Dr R Mitchell, Director, Glasgow and West of Scotland Blood Transfusion Centre, Law Hospital, Carluke, Lanarkshire ML8 5ES, Scotland.