

SMALL POOL HEAT-TREATED INTERMEDIATE PURITY FACTOR VIII CONCENTRATE

Revised Edition

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Small Pool Heat-Treated Intermediate Purity Factor VIII Concentrate

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Introduction

Hemophilia care in underdeveloped and developing countries is extremely inadequate and, in some instances, completely absent. Indeed, the majority of persons around the world who have hemophilia receive virtually no treatment. Total world usage of factor concentrates in 2000 was in the order of 3.7 billion international units (IU). In that year Europe and North America (18.8% of the world's population) consumed 74% of the global supply of all factor concentrates. All other regions of the world (80% of the world's population) used only 26% of the factor supply. In Germany, for example, per capita factor VIII use was 5.5 IU, while in Bangladesh it was 0.004 IU. This vast difference in consumption results not only from the variation in economic resources and availability, but also the lack of diagnosis in the developing world. In 2004, an estimated 75% of people with hemophilia worldwide had not been diagnosed. The majority live in developing countries and will not survive childhood with the current rate of inadequate diagnosis and treatment [1].

The types of products available range from cryoprecipitate (for which viral inactivation is more difficult), intermediate purity heated or solvent-detergent-treated concentrates, high purity heated or solvent-detergent-treated concentrates, to recombinant products. Realistically, the only potential products for developing countries are cryoprecipitate or purchased commercial intermediate purity concentrate. In many developing countries, however, the prevalence of transfusion-transmitted viruses is high in the donor population and, therefore, the risk of infection remains. The average commercial price per IU of intermediate purity factor VIII concentrate in 2002 ranged from +/-US\$0.20 - \$0.55 under normal circumstances². Many cannot afford this cost, considering that minimum on-demand replacement therapy requires 30,000 IU per

patient per annum (equivalent to US\$6000 per annum at the low end of the price range [1].)

We present our experience with the manufacture of a small pool heat-treated intermediate purity concentrate which may be suitable and cost-effective for some countries attempting self-sufficiency and who cannot afford high purity products. The method is essentially that described by Knevelman et al [3] in Holland.

Purity versus Safety

Purity refers to the percentage of the desired ingredient (e.g., factor VIII) in concentrates relative to the other ingredients present. Concentrates on the market vary widely in their purity. Generally, products that are produced at higher purity tend to be associated with low manufacturing yields, due to less von Willebrand factor (the natural carrier protein of factor VIII), and are therefore costlier. The purity of factor VIII concentrates has not been convincingly demonstrated to enhance the safety of these products, as long as adequate viral elimination measures are in place [4].

Materials and Methods

Essential equipment

Centrifuge

(*Beckman J-6B, J-6-HC*, Beckman Instruments Inc., California, U.S.A.)

Laminar Air Flow Sterile Work Station

(*Fibatron Model 6FS WHB6*, Fibatron, Cape Town, South Africa)

Sterilizable Lyophilizer

(*Virtis SRC 50/51*, The Virtis Company, New York, U.S.A.)

Dielectric Sealer

(Terumo Corporation, Tokyo, Japan)

Peristaltic Pump
(Dune Engineering, Sunset Beach,
South Africa)

Temperature-controlled water baths

Chemicals

All buffer components are pharmaceutical grade and buffers are prepared in bulk, sterile-filtered, and tested before release.

Buffer A	0.02M Tris - HCl pH 7.0
Buffer B	2.8 M Glycine; 0.3 M NaCl; 0.025 M Tris -HCl pH 6.8
Buffer C	26.7% NaCl; 1.0 M Glycine
Buffer D	0.01 M Na Citrate; 0.00125 M CaCl ₂ ; 0.01 M Glycine; 0.03 M NaCl; 1.0% Sucrose

Consumables

Transfer Packs

Fenwal Code # AFR 1614
(Baxter Healthcare, Illinois, U.S.A.)

Transfer Sets

FP-0002P
(Capricorn Biologics, Jhb, S.A)

Sterile Filters

Millipak # 40
(Millipore, France)

125 mL Clear Glass Injection Vial

(LSL, Rochdale, U.K)

Assays

Factor VIII was measured by both a one-stage manual clotting assay and a chromogenic method (Chromogenix AB Mölndal, Sweden). Fibrinogen was measured by a manual method according to Clauss [5]. Total protein was assayed by the standard Biuret technique. Osmolarity was measured on a *Slamed Osmometer* (E.S.I., Cape Town) and the electrolyte assays were performed on a *Monarch 2000 Automatic Analyser* (Instrumentation Laboratory, Maryland, U.S.A.).

Production/processing

All purification takes place within sealed blood bags. All stages where the bags or bottles are opened take place in a class 100 hepa-filtered laminar air flow workstation. Bags/bottles are sealed before removal from the workstation at the conclusion of each processing step. Aseptic technique is used throughout.

Stage 1 - Cryoprecipitate production

Cryoprecipitate is produced using a standard ethanol/dry ice process for snap freezing and the cryoprecipitate is extracted by thawing in a 4° C water bath. All these procedures take place in a closed bag system.

Stage 2 - Cryoprecipitate extraction

Antihemophilic factor (AHF) is extracted from the cryoprecipitate with 6 mL of Buffer A per bag of cryoprecipitate. The buffer addition is made into the original thawed bags. After addition of extraction buffer, the bags are sealed with a dielectric sealer. The contents of the bag are mixed and incubated in a water bath at 30° C for at least 10 minutes. Five to 12 bags of cryoprecipitate (each bag contains cryoprecipitate from one donation and therefore each bag represents a single donor) are grouped together such that the required dosage (500 IU factor VIII per vial) can be achieved in the final product. Since we began production we have used on average seven to eight bags to meet this requirement. Each group is allocated a number to which the original donations can be traced. This group is processed together as an entity (sub-lot) throughout, and the group number also becomes the final bottle number.

Stage 3 - Fibrinogen precipitation

Fibrinogen is precipitated by the addition of 25 mL of Buffer B, pre-warmed to 30° C, to each bag of cryoprecipitate. The suspension is mixed and incubated at approximately 30° C for at least 30 minutes, then centrifuged at 4000 rpm for at least 20 minutes at 20° C. After centrifugation, the bags are hung in an inverted position in the laminar flow unit. The supernatants of each group of cryoprecipitate are drained separately into 600-mL transfer packs marked with the appropriate sub-lot number. This supernatant contains a high concentration of factor VIII. The precipitate, mainly fibrinogen, is discarded.

Stage 4 – Factor VIII precipitation

The precipitation of factor VIII from the fibrinogen-poor supernatant is achieved by the aseptic addition of pre-warmed Buffer C at 30° C. The suspension is mixed and incubated at 30° C for at least 30 minutes. After incubation, the bags are left overnight at room temperature. The precipitated factor VIII is isolated by centrifugation at 4000 rpm for at least 30 minutes at 4° C. The supernatant is discarded.

Stage 5 - Reconstitution of factor VIII precipitate

The sedimented factor VIII precipitate is reconstituted by adding 50 mL of Buffer D pre-warmed at 30° C to each numbered 600 mL transfer pack. The buffer is pumped into the bag through a bacterial filter with a peristaltic pump. The bag is sealed and the AHF suspension is incubated at 30° C for at least 30 minutes with periodic swirling or until all the precipitate has dissolved.

Stage 6 - Filling and freeze-drying

The transfer packs are hung inverted in the laminar flow unit. Pre-prepared 125 mL bottles are labeled with the same series of numbers as the transfer packs. The contents of the transfer packs are transferred into the correspondingly numbered bottles via transfer sets. Ten per cent of each bottle is inoculated into thioglycollate broth. Bottles showing microbiological contamination are removed from the batch and discarded.

The bottles containing the liquid factor VIII preparation are shell frozen in a dry ice/ethanol bath and either loaded onto the lyophilizer directly or stored in a -40° C chest freezer. For lyophilization, the rubber bungs are removed from the bottles on the class 100 work station and replaced with sterilized bungs. The bottles are then loaded on the freeze drier onto pre-cooled shelves with a condenser temperature of at least -50° C.

Freeze drying is effected under a constant vacuum of approximately 50 mTorr with temperatures being raised in a stepwise manner through -30° C, 0° C, +30° C, +40° C and +50° C over a period of about 90 hours and sealed under vacuum.

Stage 7 - Viral inactivation

The sealed bottles of dried AHF are transferred to a temperature-controlled water bath and incubated at 80 – 83° C for 72 hours.

Stage 8 - Product release

The batch of product is randomly sampled and analysed for factor VIII clotting activity, electrolyte concentration, pyrogenicity, sterility, and toxicity. If found to comply to all release parameters, it is labelled with the batch number and bottle number before being issued for use.

Pharmacokinetic Studies

Three individuals with severe hemophilia were infused with an average of 2000 IU of factor VIII, and samples were taken pre-infusion and at regular intervals for 24 hours post-transfusion.

Results

From 1992 to date, more than 25,000 bottles of 500 IU factor VIII (FVIII) per bottle have been prepared using this method. The mean values for the different parameters over this period were as follows:

FVIII:c	564 ± 128 IU per bottle
Yield	287 ± 65 IU per litre plasma
Total Protein	3.2 ± 1.26 mg/mL
Fibrinogen	25% ± 14.64% of total protein
pH	6.9 - 0.09
Osmolarity	394 ± 81 mOsmoles/kg
Sodium	163 ± 31.7 mMole/L
Chloride	134 ± 28.8 mMole/L
Potassium	0.8 ± 0.58 mMole/L
Calcium	1.0 ± 0.30 mMole/L
Specific Activity	4.2 ± 2.94 IU/mg TP

Clinical Studies:

Mean recovery	94%
Mean half-life	8.3 hours (chromogenic assay) 13.4 hours (one-stage clotting assay)

Discussion

As indicated earlier, the average commercial price per unit of intermediate purity factor VIII in 2002 was in the range of US\$0.20 – 0.55. Our local product is sold at the lower end of this spectrum at a price equivalent to +/- US\$0.20.

To maintain production, we require four staff. Three are required to work at bench level (i.e., at the laminar flow unit) and are in-house trained to technician level. One staff member is required in a supervisory capacity and must have slightly more advanced scientific training. At this level of staffing and equipment, we are able to produce a yield of approximately 60 bottles of factor VIII per eight-hour shift.

We have been producing this product in the Western Cape region of South Africa for the past 12 years and although it has been produced within an accredited plasma fractionation plant, premises of this degree of pharmaceutical sophistication are not required. The process is dependent on the existence of an organized blood transfusion service able to produce cryoprecipitate, and would require some capital investment in the equipment listed on page 1.

The only mild drawback of the current product is that it is not desalted and concentrated, and therefore, requires reconstitution in 50 mL sterile water to approximate isotonicity and is given through a standard blood transfusion set. The main reason for this is economic, but we are currently investigating a cost-effective approach to overcome this. The mean reconstitution time is 2 to 3 minutes.

We do not have sufficient previously untreated patients to accumulate data demonstrating incontrovertible evidence as to the safety of the process, but the heating process used has a good clinical record, has been validated in a similar system [3], and has been efficacious with other factor VIII products. We have not had any HIV or hepatitis seroconversions in our patients since beginning this program. The clinical recovery and half-life are within acceptable limits, although we cannot explain the different results obtained for the half-life studies with the chromogenic and clotting assays.

This product is suitable for the treatment of von Willebrand disease since it contains

sufficient high molecular weight multimers of von Willebrand factor [6]. This is in line with the findings of Rodegheiro et al [7] who demonstrated that some intermediate purity products retain sufficient high molecular weight multimers to be efficacious when used as replacement therapy for VWD.

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