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GLASGOW AND WEST OF SCOTLAND

BLOOD TRANSFUSION CENTRE

REPORT ON THE PRODUCTION OF LYOPHILISED CRYOPRECIPITATE

G. Gabra,

Jan., 1980

INTRODUCTION

The simple method of preparing cryoprecipitate ensures a continuous, reliable supply of Factor VIII prepared at minimum expense in any Transfusion Centre.

The yield of Factor VIII C in cryoprecipitate is around 50% of the content of fresh donor plasma. This can be raised substantially by improving the techniques of preparation.

At the moment fresh plasma is collected in the West of Scotland and sent to Liberton Plasma Fractionation Centre for the production of intermediate factor VIII concentrate.

The figures over the last five years show a continuous increase in fresh donations sent to PFC as shown in the table -

	<u>Number of Fresh Donations</u>	
	<u>Sent to P.F.C.</u>	<u>Processed as Cryo.</u>
1974	4.081	22.642
1975	8.129	25.563
1976	18.222	16.626
1977	23.753	17.932
1978	30.519	19.912

The table also shows the number of units of cryoprecipitate prepared over the same five years and used in the region. They show a small downward trend but the clinical demand for cryo. still has its place. This is not only in Scotland but also in the whole of the U.K. and in many European countries ¹.

The preparation of dried cryoprecipitate is a reliable technique that has now been successfully adopted by many Transfusion Centres outside the United Kingdom ^{2,3,4}. This dried product should have most of the advantages of the dried N.H.S. Factor VIII concentrates; namely the long shelf life, the easy storage and the pre-determined dosage. It also has the added value of being simple and economical to produce and carries reduced hepatitis risk being prepared from

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small pools of donor plasma in short it is an improved method of storing and dispensing cryoprecipitate.

These are the thoughts that led the production team at Glasgow Blood Transfusion Centre to explore the possibility of introducing a lyophilised small pool Factor VIII rich product called "lyophilised cryoprecipitate" by pooling cryoprecipitate from five plasma donations and freeze drying these small pools.

Experimental Stage of Dried Cryoprecipitate Production

The presence of adequate facilities for drying encouraged the production team to embark on an experimental study to produce freeze dried cryoprecipitate.

This had to start by an evaluation of the various techniques in use to prepare dried cryoprecipitate. During the experimental stage early 1978 many practical and technical problems were solved.

A suitable buffer was selected for pooling of the cryoprecipitate. It was chosen after experimental trials to give the best yield of Factor VIII and to be suitable for sterilisation by filtration.

The pooling of 5 units of cryoprecipitate was performed in a laminar flow cabinet to ensure sterility of the product.

It was also decided at this stage that the rapid freezing of the pooled cryo was crucial and that the flasks had to be snap frozen on rollers using a mixture of CO₂ and methanol bath prior to drying.

The lyophilisation of the product was done in the two types of dessicators that we have and it was found that the critical control of the temperature during drying was essential to reduce denaturation of the product. It was decided that the Edwards Model 250 P.S. is far superior to the smaller dessicator Model E.F.6 - where at all times

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the temperature of the bottles was kept below 20°C. Specimens for assay were taken and the results of the assay showed no significant loss in the Factor VIII content of the material when subjected to the suitable drying conditions. This experimental work is unpublished⁵ and some figures from this pilot study are shown on the following table

	<u>Pre-drying</u>	<u>Dried Product</u> <u>2/12 at 4°C</u>
Bottle 1	4.7	1.55
Bottle 2	3.0	1.3
Bottle 3	3.8	3.1
Bottle 4	4.1	3.7

Clearly it was decided to adopt the technique employed for Bottles 3 and 4.

These early experimental studies convinced us at Law Blood Transfusion Centre that the production of dried cryoprecipitate is a feasible proposition and that such a product will have its place as a useful alternative for frozen stored cryoprecipitate.

Early Production Stage of Dried Cryoprecipitate

The findings from the early experimental study led to the setting up of a larger production team to conduct a pilot production study as follows -

1. To confirm the early experimental findings.
2. To investigate the feasibility of applying these findings in the routine production of dried cryoprecipitate in the West of Scotland.
3. To work out the details of a comprehensive scheme for routine production of this product.
4. To lay down comprehensive quality control measures and criteria to qualify this product for therapeutic use according to the recognised pharmacopoeal standards.

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By early 1979, 120 bottles of dried cryoprecipitate were available and ready awaiting clinical trials and the findings were presented as a paper to the West of Scotland blood club in May, 1979⁶.

Many details of this paper are appended at the end of this report.

Method of Preparation of Dried Cryoprecipitate
and Scheme for Routine Production

Fifty bags of fresh frozen plasma are allowed to thaw at 4°C in two thermostatically controlled water baths.

This fast thaw procedure takes approximately 1½ hours.

The bags are removed from the water bath and immediately centrifuged for 10 minutes at 3200 G in a +4°C pre-refrigerated centrifuge.

The supernatant is expressed into the satellite pack leaving only the cryoprecipitate in the bag.

The cryoprecipitate is frozen on a tray of dry ice until pooling or stored at -30°C if drying is to be done at a later date.

Five cryoprecipitate of homologous ABO group are pooled using a 5 tail pooling set as follows:-

50 mls of the buffer (appendices 1 and 2) are introduced into the first bag and the dissolved cryoprecipitate is pushed by gravity into the second bag and so on until the 5th bag. The process is repeated using a second 50 mls of buffer and the 100 mls solution of cryoprecipitate in buffer is finally transferred into the glass bottle MRC Flask (appendix 3) ready for freeze drying.

If the 5 cryoprecipitates required for pooling were stored frozen - they are to be thawed at 37°C in a water bath for a standardised time of 10 minutes to ensure complete dissolution of the cryoprecipitate prior to addition of the buffer for pooling.

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The pooling set is disconnected from the flask and the product is now ready for freeze drying.

Pooling is conducted under strict aseptic conditions in a laminar flow cabinet in a filtered air processing room.

Shell freezing takes 10 minutes in a special French apparatus (UISFROID) where the bottles are placed on rollers and passed through a freezing mixture of Methanol and solid CO₂.

The rubber caps are replaced by gauze caps in the processing room and the bottles are placed in the dessicator. If the frozen material is not dried immediately it is stored at -80°C.

The Edwards model 250 PS is currently in use. Two thermocouple controls are included to monitor the drying procedure.

The total drying time is approximately 70 hours in the secondary dessicator, after which vacuum is broken using nitrogen gas.

Bottles are then re-capped, labelled (appendix 4) and stored at 4°C until despatched for use.

Quality Control Measures and Programme to Monitor the Production of Dried Cryoprecipitate

The product is monitored at all stages of the processing for sterility and all equipment and solutions used are pyrogen free.

Random samples from the finished product are examined for -

(1) Moisture Content

All the batches prepared so far conformed well with the pharmacopoeal limits of moisture content ⁷ of human dried plasma.

(2) Pyrogenicity and Sterility

All the batches prepared passed the rabbit pyrogen testing and showed no bacterial growth. (appendices 5 & 6).

(3) Reconstitution

The material reconstituted easily within 2 minutes in 100 mls of pyrogen free distilled water to give a clear solution.

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Specimens were taken from each bottle at the pooling stage for estimation of the Factor VIII content. This is performed using an artificial substrate and a secondary standard calibrated against a national standard - the clotting times are read on a clotek Hyland machine.

The initial findings were confirmed and the losses of Factor VIII after drying were insignificant.

Ten dried units were assayed at the early production stage and showed an average content of 542 i.u. Factor VIII C per bottle (5.42 i.u./ml).

Two bottles were assayed at the National Institute for Biological Standards and Control and showed even higher content (appendix 7).

The first 39 bottles that are already at Glasgow Royal Infirmary awaiting clinical trial have a mean content of 333.74 ± 251 i.u. of Factor VIII C (3.337 i.u./ml).

Thirty more bottles (pool No. 3089 to 3118) stored at Law awaiting dispatch contain a mean of 339.7 ± 118 i.u. Factor VIII C per bottle (3.397 i.u./ml) but later on it was possible to dry simultaneously small vials taken from the pools and 30 bottles assayed after drying (from these small vials) show a mean content of 406.53 ± 146 i.u. per bottle of Factor VIII C (4.065 i.u./ml).

The specimens taken from some pools after drying have been assayed for the following:

1. Total protein content.
2. Albumin.
3. Fibrinogen.
4. Specific activity.
5. pH.
6. Hemagglutinin content.

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The quality control figures of ten units at the early production stage are shown on the table -

	<u>Mean Values</u>
Factor VIII C	542 ± 232 i.u./bottle
Total protein	27.8 g/L
Albumin	17 g/L
Fibrinogen	4.3 g/L
Specific activity	194 i.u./g protein

The pH of the reconstituted product varied between 7.1 and 7.9 and the product when diluted at 1/64 did not show the presence of any haemagglutinin. These findings conform with the standards of the European pharmacopoea⁸.

Routine Production Stage

This is halted until the results of the clinical trials become apparent but there is enough material on the shelf that has passed the quality control tests and is ready for therapeutic usage.

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Appendices(1) Preparation of Buffer for Pooling

Sodium Chloride	3.4 g	17 g
Trisodium Citrate	6.6 g	33 g
D Glucose	13.0 g	65 g
Glycine	10.0 g	50 g
Pyrogen free distilled water	1000 ml	5000 ml

pH adjusted to 6.5 using 0.1 N HCL before use. This should be sterilised and tested in rabbits for pyrogenicity.

(2) Sterilisation and Dispensing of Buffer in the Flasks

A Pall filtration unit is made up of a clarifying filter and a sterilising filter. This together with a pressure vessel and its rubber tubing are autoclaved. 100 mls of the buffer are dispensed in the Sterile flasks under sterile conditions and random bottles taken for bacteriology and pyrogen testing and pH checking.

(3) Preparation of Bottles

Half size MRC flasks - washed and rinsed in Pyrogen free distilled water - Capped and sterilised by autoclaving - spore test negative.

(4) Label**LYOPHILISED CRYOPRECIPITATE**

PREPARED FROM 1 LITRE OF FRESH HUMAN PLASMA
 RECON. IN 100ml. OF DISTILLED WATER
 STORE AT -20C

DATE OF PREPARATION

(5) Pyrogen testing of Buffer.

GLASGOW & WEST OF SCOTLAND BLOOD TRANSFUSION SERVICE
LAW HOSPITAL, CARLUKE, LANARKSHIRE.

TEL. NO. WISHAW 73318, EXT. 67

REQUEST FOR PYROGEN TEST

FOR OFFICIAL USE ONLY

214

REQUESTED BY: G. MILLIGAN
ADDRESS: BPLAB B7S LAW.

DATE:

PRODUCT: CLIN BUFFER.

CONSTITUENTS: SOD. CITRATE / CLICINE
NALL. / GLUCOSE.

BATCH NO: 9/2/79.

TOTAL UNITS IN BATCH:

QUANTITY SENT: 100 ml.

IF DRIED MATERIAL, STATE DETAILS FOR RECONSTITUTION.

DESPATCHED BY:

TEST DATE: 12.2.79

RABBIT No.	WEIGHT	VOL. INJECTED	MAXIMUM TEMPERATURE RISE
E 257	2.1 Kg	21 mls	0.2°C
E 458	2.8 Kg	28 mls	0.5°C
E 51	3.1 Kg	31 mls	0.1°C

TOTAL TEMP. RISE 0.8°C

REMARKS ON CONDUCT & RESULTS OF TEST:

RESULTS CHECKED BY: *Alwart*

THE PRODUCT PASSES FAILS TO PASS THE TEST FOR PYROGENS (B.P.)
 REQUIRES FURTHER TESTING

(6) Pyrogen Testing of Dried Cryoprecipitate.

GLASGOW & WEST OF SCOTLAND BLOOD TRANSFUSION SERVICE
LAW HOSPITAL, CARLUKE, LANARKSHIRE.

TEL. NO. WISHAW 73316, EXT. 67

REQUEST FOR PYROGEN TEST

FOR OFFICIAL USE ONLY

730

REQUESTED BY: *James G. G.*

DATE:

ADDRESS: *875 LAW.*

PRODUCT: *DRIED CRYOPRECIPITATE.*

5 SAMPLE CRYOPRECIPITATES
CONSTITUENTS: *IN. GLUCOSE / GLUCOSE / TRIS -*
SODIUM CIT / MALL BUFFER
TOTAL UNITS IN BATCH: *APPROX 700 u. F.U.U.*

BATCH NO: *3041/79 (5/3/79)*

QUANTITY SENT:

IF DRIED MATERIAL, STATE DETAILS FOR RECONSTITUTION *RECONSTITUTE IN 100 MLS PYROGEN*
FREE DISTILLED WATER.

DESPATCHED BY:

TEST DATE: *21.5.79.*

RABBIT No.	WEIGHT	VOL INJECTED	MAXIMUM TEMPERATURE RISE
<i>E 846</i>	<i>2.2 Kg</i>	<i>4.4 mls</i>	<i>Nil</i>
<i>E 847</i>	<i>1.7 Kg</i>	<i>3.4 mls</i>	<i>0.1°C</i>
<i>E 848</i>	<i>2.0 Kg</i>	<i>4.0 mls</i>	<i>0.1°C</i>

TOTAL TEMP. RISE

0.2°C

REMARKS ON CONDUCT & RESULTS OF TEST:

Injection = 2 mls per Kg
RESULTS CHECKED BY: *A Watt*

THE PRODUCT

PASSES

~~FAILS TO PASS~~

THE TEST FOR PYROGENS (O.P.)

~~REQUIRES FURTHER TESTING~~

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(?) Report of the National Institute of Biological Standards
and Control - Copy of the result.



NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS AND CONTROL

Holly Hill, Hampstead, London, NW3 6RD

NATIONAL BIOLOGICAL
STANDARDS BOARD

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DPT/MGS/79/238

23rd March 1979

Dr. R. Mitchell,
Director,
Blood Transfusion Service,
Law Hospital,
Carluke,
Dumfries, M.S. SES.

Dear Dr. Mitchell,

We have now had a chance to look at your freeze-dried cryoprecipitate, and the results are as follows:-

Batch Q3006/79

Factor VIII:C = 712 i.u./bottle (95% confidence limits are 649-782 i.u.)

The VIII R:Ag for this batch gave a value of 9.2 units/ml., with an VIII R:Ag/VIII:C ratio of 1.3. The VIII R:CoF assay gave a value of between 7-8 units /ml.

Batch Q3024/79

The VIII:C value for this bottle was 812 i.u.

I think we could say with confidence that your freeze-dried cryoprecipitate is giving you values of around 7-8 i.u./ml. for Factor VIII:C, which I would have thought was highly satisfactory. The VIII R:Ag/VIII:C ratio is also very low.

I would be interested to know what values you have found. If you would like us to carry out some assays on other batches from time to time, we would be very pleased to help.

Kind regards,

Yours sincerely,

Duncan P. Thomas MD FRCP Path
Head, Division of Blood Products

REFERENCES

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- (4) Allian, J.P. (1979). Dose requirement for replacement therapy in Haemophilia A. *Thrombosis and Haemostasis*, 42, 825-831.
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- (8) European pharmacopoeia: PA/PH/Exp. 15B/T (75)1 Com - Amended draft (Nov. 1978). Freeze-dried Human Antihaemophilic Cryoprecipitate, page 2.

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ALBUMIN AND FACTOR VIII

<u>Product</u>	<u>Volume of plasma fractionated</u> (litres/10 ⁶ pop/yr)	<u>Fractionation Yield</u>	<u>Output</u> (10 ⁶ pop/yr)
Albumin	8,000	20g/litre	160 Kg
		24g/litre	192 Kg
VIII	8,000	200 iu/litre	1.6x10 ⁶
		250 iu/litre	2.0x10 ⁶

DONATION INPUT TO ACHIEVE 8,000 LITRES p.a.*

<u>Number of Transfusable Donations</u> (per 10 ⁶ pop/yr)	<u>% Processed in less than 18 hours</u>
40,000	100
50,000	80
60,000	67