CONFIDENTIAL

PROGRESS REPORT FOR FACTOR VIII STUDY GROUP

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CONTENTS

1. PLASMA QUALITY

1.1 Routine FFP

Three plasma samples from each FVIII lot are taken prior to thawing and assayed for FVIII C, FVIIIR:Ag and platelet content. All assays are carried out at Edinburgh BTS.

Table 1 shows the mean FVIII C values for various types of plasma from April 1980 to April 1984. A fall in FVIII content since 1980 is apparent with the minimum value (0.57 iu/ml) being experienced in 1982/83. Although there was some improvement in 1983/84 more recent data (table 2) suggests that very low values are being experienced again (0.54 iu/ml).

1.2 PFC/Law Plasmapheresis Study

1.2.1 Plasma samples were taken at 3 points:- from the donor, before freezing and from the frozen pack before thawing. Samples were assayed for FVIII C, FVIIIR:Ag and FVIII C:Ag. Mean values for VIII C and VIII R:Ag are shown in table 3 (VIII C:Ag has not been included because the "scatter" was so great that the values are suspect).

Samples were not taken from all donors and frozen core samples were not taken from all packs hence the data is presented in two forms.

(i) Where there is a complete set of samples from donor through to frozen core.

(ii) Mean values from all samples.

The data in table 3 shows that higher VIIIC levels were obtained from machine 'pheresis and confirms the view that with 'manual pheresis the 2nd pack contains less FVIII C than the first (although the VIII R:Ag levels are similar)

The levels of donor FVIII are higher in the machine group (were the anticoagulant volumes different?) and the results can therefore be best compared by calculating the % loss of FVIII at each stage (table 4). Losses are much higher in the manual group during processing (ie from donor to pre-freezing) and during freezing (ie from pre-freezing to frozen-core). Comparison of the freezing conditions may show differences, but if not the data suggests that the greater trauma experienced during processing (for the manual group) also results in greater losses during freezing.

The FVIIIR:Ag data is more difficult to interpret. Fairly steady results are obtained from the machine group (probably within the assay variation) but the normal group show an apparent loss of antigen similar in magnitude to the loss of FVIII C. Reasons for this could include:-

(i) Assay error (note the fairly high S.D.).

(ii) Unrepresentative samples.

(iii) Change in molecular size of antigen (ie aggregation).

(iv) Loss of antigen (eg with red cells)

These factors could exist in combination and could therefore exaggerate any true loss of antigen.

1.2.2 Fractionation

Four pools of 'pheresis plasma were processed to the standard intermediate-purity concentrate by PFC production staff (2 pools manual and 2 pools machine). The pool sizes were relatively small (about 150kg) compared to the normal pool size (600 - 1000 kg). This made processing more difficult and the results cannot be strictly compared to routine production lots, this factor may also be responsible (at least in part) for the failure of one manual lot to complete process.

Results from these four pools are listed in table 5. The higher FVIII C content of the machine plasma is reflected in high cryoprecipitate recoveries, but this advantage is lost during further processing (probably by citrate-induced inactivation) with similar final product yields, typical of routine production. The fact that the improved plasma FVIIIC is reflected in the cryoprecipitate will be important for more advanced processes now under development in which the benefit of the higher quality plasma should be retained throughout processing.

1.3 Conclusions On Plasma Quality

From both the routine data collected over 4 1/2 years (tables 1 & 2) and the Law plasmapheresis study it appears that plasma with a satisfactory FVIIIC content (ie >0.8 iu/ml) cannot be consistently achieved by current methods of manual collection.

Satisfactory levels of FVIIIC may be achieved by:-

(i) Automated methods (eg machine plasmapheresis).

(ii) Modification of manual methods to provide positive protection to FVIIIC (eg low citrate anticoagulant, Prowse - unpublished results).

2. FACTOR VIII YIELD (Standard Process)

2.1 Introduction

Laboratory studies have shown that the remaining major loss of yield is caused by citrate induced inactivation of FVIIIC. (Foster, B. J. Haematol,53:343, 1983). This was confirmed in a

split production batch, one half having calcium added to maintain a constant concentration of ionised calcium (NY 771/772 see report of January 1984). These observations were based on results from 1-stage assays and were not confirmed by the 2-stage assay. A clinical evaluation was proposed to resolve this question but it was felt that the difference between NY 771 & 772 (20%) was too small for such a study. It was agreed to prepare a 2nd split batch exagerating the difference between the lots by holding them for as long as possible before dispensing. The use of the chromogenic assay was also proposed to provide further in vitro data.

2.2 Results

2.2.1 (NY 771/772). The chromogenic assay was carried out using 4 vials from each lot (C. Prowse). The results (below) tended to agree with the 1-stage method rather than 2-stage.

ASSAY	FACTOR VIII C (iu/ml		% INCREASE
	NY 771	NY 772 ($+Ca^{2+}$)	
1-stage (PFC), n=2	14.85 ± 0.34	16.44 ± 0.83	10.7
1-stage (EBTS), n=2	12.39 ± 1.05	15.66 ± 0.85	26.4
2-stage (EBTS), n=2	12.34 ± 0.26	11.66 ± 0.32	-5.5
Chromogenic (EBTS) n=4	12.90 ± 0.30	15.00 ± 0.60	16.3

2.2.2 This exercise was repeated with th second set of production batches NY 4013 and NY 4014 (+Ca). both lots were held at room temperature for 4 hours before dispensing and freezing.

ASSAY	FACTOR VIII C iu/ml		% INCREASE
	NY 4013	NY 4014 ($+Ca^{2+}$)	
1-Stage (PFC), n=2	7.62 ± 0.42	11.42 ± 0.33	49.9
1-Stage (EBTS), n=2	7.87 ± 0.00	8.9 ± 0.00	13.1
2-Stage (PFC), n=2	8.89 ± 0.19	10.43 ± 0.09	16.1
2-Stage (EBTS), n=2	8.75 ± 0.00	7.5 ± 0.00	-11.4
Chromogenic	8.22 ±	10.4 ±	26.5
No. vials to inspection	222	225	
FVIIIC/Vial (iu)	163	238	

These results continue to show discrepancies between the assays, including differences between PFC & EBTS as well as between types of assay.

Attempts to arrange a clinical evaluation of this material have not yet been successful. This is dissapointing : if the value of controlling ionised calcium could be confirmed then the world supply of FVIII (estimated to be only 20% of need) could be increased by about 25% virtually overnight.

3. NEW PROCESSES TO IMPROVE QUALITY

3.1 ZHT Process

This process resulted from the observations that zinc could be used to precipitate fibrinogen from FVIII solutions (Foster, Thromb. Haem 50, 117) and that sorbitol could be used as a stabiliser to protect FVIII from inactivation when heated in solution (Macleod, Thromb. Haem. 50:432, 1983).

The process was designed to give a sterile (virus free) product with a reduced fibrinogen content, but with a high enough yield to maintain self-sufficiency.

Following the completion of small-scale laboratory studies (see previous reports) a number of experiments have been carried out at pilot-scale under GMP conditions to determine the equipment, procedures and conditions needed to translate the laboratory performance to full-scale production. The results from these experiments are summarised in tables 6 and 7.

Our current intermediate-purity product has a yield of about 45% from cryoprecipitate hence this is the target that a new process should aim for. The pilot-scale yields attained at each stage are shown in table 6 with the targets to achieve an overall yield of 45% shown in the first column.

3.1.1 Results from zinc precipitation (step 1) are dissapointing compared to the earlier laboratory data. this is considered to be due to the different pilot-scale mixers used being either too small (inadequate mixing giving overshoot during zinc addition) or too severe (giving shear-induced inactivation). Confirmation of this view was given by a full-scale experiment with conditions carefully designed to avoid overshoot or inactivation.

Production Scale Zinc Precipitation of Cryoprecipitate Extract

Plasma pool (kg)	992
Cryo wt (g/l plasma)	9.1
Cryo protein (g/l plasma)	0.9

	RECOVERY OF FVIII C %	RECOVERY OF PROTEIN %	SPECIFIC ACTIVITY (iu/mg)
Redissolved Cryoprecipitate	100	100	0.27
Zinc Supernatant After Filtration	98.9	52.9	0.58

3.1.2 Sorbitol is added as a solid powder and this can cause inactivation due to entrapped air causing foaming. The temperature and rate of addition are critical and specialist equipment is needed to achieve the required degree of control easily. Alternatively a saturated solution of sorbitol can be used without loss of FVIII C at this point (Macleod, unpublished results). However this entails a four fold increase in volume (4 x the cost of sorbitol also) and is only practicable with relatively small process volumes (eg may be suitable for process in section 3.2).

3.1.3 Heating conditions were:

Expt 1 : 10 hrs at 60 deg C followed by 20 mins at 70 deg C.

Expt 2-5: 16.5 hrs at 60 deg C followed by 20 mins at 70 dec C.

These conditions were selected to give a maximum viral kill with about 75% recovery of FVIII C.

3.1.4 Filtration (step 6) was introduced to remove any solids formed during pasteurisation. The formation of solids at the higher temperature (70 deg C) could be prevented by adding extra calcium to the process solution at step 2. Step 6 could therefore be omitted.

3.1.5 Recovery of the NaCl/glycine precipitate was difficult but good results were obtained by carefully adjusting the feed to the sharples centrifuge to achieve a long residence time with proper distribution in the bowl.

3.1.6 Ultrafiltration is used to de-salt and concentrate the FVIII solution. The choice of reagent solutions and equipment is crucial to achieving a high yield, good solubility and an appropriate final product formulation. Progress has been made with this step but further work is still required.

Work on the ZHT process was suspended in October 1984 to give priority to a new process which promises a higher purity product in high yield.

Much of the knowledge gained in the ZHT programme will be valuable in the alternative process and some of the key steps may remain.

3.2 High Purity Product - Further Developments

In July 1983 PFC was invited to collaborate in the scale-up and clinical evaluation of a new procedure for the preparation of FVIII concentrate. Work on this project began at PFC in August 1984 and by October 1984 we were sufficiently impressed with the procedure that the ZHT process (above) was shelved so that maximum effort could be given to the newer method. The details of this are strictly confidential and are covered by confidentiality agreements signed by PFC in October 1983 and August 1984. However the overall process is likely to include some steps developed from the ZHT process. The form of the process is:

(i) Cryoprecipitation.

(ii) Precipitation of cryo extract to reduce fibrinogen load. This will be by zinc precipitation and/or a modified PEG precipitation. (McIntosh, unpublished results).

(iii) New procedure (confidential).

(iv) Pasteurisation. Heating in solution with sorbitol as a stabiliser is the preferred option at the moment but severe heating of the freeze dried powder may be possible (Smith, unpublished results) and may be of interest.

4. HEAT TREATMENT PROGRAMME

At the time of the last meeting of the Study Group our preferred option for viral inactivation was heating in solution, as opposed to heating the freeze dried powder, for the following reasons:

(i) It is likely to achieve a greater degree of viral kill. This is particularly important if relatively heat resistant viruses are to be destroyed (eg HBV).

(ii) Preliminary animal and clinical data from heated dried products suggested little effect on HBV and incomplete inactivation of NANB.

(iii) In theory the procedure is difficult to control both within a batch (eg due to variation in the glass vials) and from batch-to-batch (eg due to variations in moisture content after drying).

Although heating in solution would seem to be still the preferred option recent information concerning HTLV-III has led to the introduction of a dried-heating procedure for the existing product. The decision to pursue this option was based on the following points:

(i) The AIDS virus (LAV, HTLVIII) is relatively heat sensitive (Desmyter ISBT July, 1985) and can be destroyed by the dried-heat method (MMWR 33 589-90, 1985; Jason, Groningen Symp. Nov 1985; Mannucci, unpublished results).

(ii) A number of haemophiliacs treated with SNBTS FVIII were found to have a positive test of anti-HTLVIII .

Much of this information became available at least in a tentative form, at about the same time, resulting in the initiation of a dried-heating study to enable the rapid introduction of a heat treated product. The results of this study are summarised below, (table 8).

TABLE I

FVIII CONTENT OF FFP

PLASMA TYPE	1980/81			1981/82			1982/83			1983/84		
	No. Lots	No. Samples	FVIIIC iu/ml Mean s.d.	No. Lots	No. Samples	FVIIIC iu/ml Mean s.d.	No. Lots	No. Samples	FVIIIC iu/ml Mean s.d.	No. Lots	No. Samples	FVIIIC iu/ml Mean s.d.
All 6 hour	36	104	0.83 ± 0.24	50	138	0.66 ± 0.16	59	171	0.57 ± 0.18	65	195	0.71 ± 0.16
All 18 hour	5	15	0.80 ± 0.23	18	49	0.59 ± 0.18	20	58	0.48 ± 0.35	10	30	0.67 ± 0.25
All 6 + 18 hour	17	50	0.80 ± 0.26	5	13	0.62 ± 0.17	2	6	0.55 ± 0.08	-	-	-
Glasgow 6 hour	11	32	0.90 ± 0.18	16	48	0.68 ± 0.17	21	61	0.51 ± 0.22	5	15	0.68 ± 0.11
Glasgow 18 hour	1	3	0.97 ± 0.11	1	2	0.66 ± 0.04	-	-	-	1	3	0.75 ± 0.03
Edinburgh 6 hour	-	-	-	1	2	0.69 ± 0.9	-	-	-	-	-	-
Edinburgh 18 hour	-	-	-	-	-	-	-	-	-	-	-	-
Belfast 6 + 18 hour	-	-	-	-	-	-	1	3	0.54 ± 0.01	-	-	-
Belfast 18 hour	-	-	-	-	-	-	4	11	0.54 ± 0.03	-	-	-
Inverness 6 hour	1	2	0.58 ± 0.09	-	-	-	-	-	-	-	-	-
Inverness 6 + 18 hour	2	6	0.78 ± 0.17	-	-	-	-	-	-	-	-	-
All types of plasma	58	169	0.82 ± 0.24	73	200	0.64 ± 0.17	81	236	0.55 ± 0.24	75	225	0.70 ± 0.18
Proportion of lots Sampled	78.4%			96.1%			97.6%			100%		

Table 2

FFP QUALITY 1984

DATE PROCESSED	No. Lots		No. Samples	FVIIIC (iu/ml)	FVIIIRAG (iu/ml)
	6 hr	18 hr			
January	7	1	24	0.74 ± 0.17	1.06 ± 0.36
February	7	-	21	0.81 ± 0.12	1.04 ± 0.21
March	4	1	15	0.83 ± 0.12	1.09 ± 0.15
April	6	-	18	0.68 ± 0.14	0.89 ± 0.22
May	3	2	15	0.61 ± 0.13	1.22 ± 0.41
June	5	1	18	0.61 ± 0.11	1.11 ± 0.28
July	7	-	20	0.61 ± 0.12	1.10 ± 0.26
August	4	-	12	0.60 ± 0.10	1.01 ± 0.21
September	2	1	9	0.59 ± 0.08	0.96 ± 0.17
October	5	3	24	0.54 ± 0.12	0.92 ± 0.32
November	1	1	6	0.54 ± 0.09	1.07 ± 0.29
December	-	-	-	-	-
YEAR	51	10	182	0.65	1.03

TABLE 3A

FVIII CONTENT OF PLASMAPHERESIS PLASMA

	FVIII C (iu/ml)			
	DONOR	PRE-FREEZING		FROZEN CORE
		BAG 1	BAG 2	
Matched Samples				
Manual (n=24)	0.80 ± 0.33	0.73 ± 0.21	0.64 ± 0.23	0.45 ± 0.22
Machine (n=41)	0.87 ± 0.28	0.79 ± 0.27	-	0.72 ± 0.35
All Samples				
Manual	0.79 ± 0.28 (n=39)	0.68 ± 0.27 (n=67)	0.61 ± 0.27 (n=66)	0.45 ± 0.22 (n=24)
Machine	0.86 ± 0.31 (n=44)	0.77 ± 0.26 (n=74)	-	0.70 ± 0.31 (n=68)

TABLE 3B

	FVIII R:Ag (u/ml)			
	DONOR	PRE-FREEZING		FROZEN CORE
		BAG 1	BAG 2	
Matched Samples				
Manual (n=24)	0.89 ± 0.51	0.75 ± 0.61	0.73 ± 0.47	0.52 ± 0.41
Machine (n=41)	1.02 ± 0.51	1.06 ± 0.49	-	0.92 ± 0.62
All Samples				
Manual	0.87 ± 0.60 (n=39)	0.65 ± 0.49 (n=67)	0.57 ± 0.58 (n=66)	0.52 ± 0.41 (n=24)
Machine	1.01 ± 0.52 (n=44)	1.00 ± 0.61 (n=74)	-	0.97 ± 0.52 (n=68)

TABLE 4A

PLASMAPHERESIS - STAGE LOSS OF FVIII

	% LOSS FVIII C	
	PROCESSING	FREEZING
Matched Samples		
Manual	14.5	34.3
Machine	9.2	8.9
All Samples		
Manual	18.4	30.2
Machine	10.5	9.1

TABLE 4B

	% LOSS FVIII R:Ag	
	PROCESSING	FREEZING
Matched Samples		
Manual	16.9	29.7
Machine	Nil	13.2
All Samples		
Manual	29.9	14.8
Machine	1.0	3.0

TABLE 5

FRACTIONATION OF LAW 'PHERESIS PLASMA

	MANUAL		MACHINE	
Lot No.	794	4.008	798	4.004
Plasma Quality (no. Samples)	(n=14)	(n=3)	(n=3)	(n=3)
FVIII C (iu/ml)	0.55 ± 0.10	0.57 ± 0.04	0.77 ± 0.24	0.77 ± 0.04
FVIIIR:Ag u/ml	0.87 ± 0.27	0.7 ± 0.16	1.73 ± 0.24	1.07 ± 0.14
Platelets (10^9/l)	13.4 ± 2.8	16.7 ± 5.2	50.3 ± 3.3	21.0 ± 1.6
Pool Size (litres)	147	135	139	142
Cryo wt (g/l plasma)	13.4	5.9	15.0	10.6
FVIII Recovery (iu/l plasma)				
Cryoprecipitate	363.5	176.8	558.5	647.6
After Al(OH) ads.	345.9	142.2	440.7	604.0
Final product	276.3	-	282.0	279.5
		Process Failure		
PRODUCT QUALITY				
Potency (iu/ml)	13.6	-	13.5	14.6
Sp. activity (iu/ml)	0.34	-	0.23	0.32
Solubility (mins)	7	-	26	8

NOTE: Plasma quality assessed from crushed plasma samples.

TABLE 6

ZHI PROCESS. SUMMARY OF PILOT-SCALE EXPERIMENTS

PROCESS STAGE	STAGE EFFICIENCY (ZFVIIIC)					
	TARGET	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
1. Zinc pptn	95	82.9	75.6	71.7	85.8	73.7
2. Citrate addn.	95	94.6	82.1	118.4	89.5	127.4
3. Filtration (0.45 µm)	95	92.3	88.4	95.6	93.7	80.8
4. Sorbitol addn	90	99.4	55.8	81.5	72.1	83.0
5. Pasteurisation	75	67.6	74.2	57.9	81.8	57.3
6. Filtration	100	69.2	93.0	96.4	84.2	100.0
7. NaCl/Glycine pptn	95	50.4	87.9	81.1	94.9	79.9
8. Filtration	95	86.5	67.8	88.4	123.4	63.5
9. Ultrafiltration	90	80.9	69.9	90.2	79.3	57.1
10. Filtration (0.2 µm)	95	77.7	73.5	66.5	72.8	81.9
11. Freeze Drying	95		107.4	77.0	93.6	
	Σ47					

TABLE 7

PRODUCT QUALITY

	EXPT. 1	EXPT. 2	Expt. 3	EXPT. 4	EXPT. 5
Potency (iu/ml)	18.8	15.7	11.6	16.0	6.4
Specific Activity (iu/mg)	1.13	1.20	0.84	0.92	0.68
Fibrinogen (g/l)	12.9	10.3	10.4	13.7	7.3
Solubility (mins)	18	19	27	22	10
Pyrogen (Deg C/3 Rabbits)	0.4	0.3	1.2	1.2	1.7
Zinc (ppm)	3.5	1.4	0.9	0.8	2.0
Sorbitol (%)	0.3	0.2	0.9	0.2	0.3

TABLE 8

HEATING OF FREEZE DRIED FVIII

PRODUCT	N	TEMP. (C)	TIME (Hrs)	% FVIII LOSS	SOLUBILITY (MINS) Before	After
1. Intermediate Purity						
	4	60	24	Nil	15	2/4 slight insol.
	2	60	48	Nil	15	1/2 insol.
	8	60	72	19.3	8.5	8/8 insol.
	6	68	72	32	8.5	6/6 insol.
	2	68	3	Nil	9.5	1/2 insol.
	11	68	2	2.9	8.1	11, 1/46 slight insol.
2. I.P. + Additives (old FVIII)						
Ca + 1.5% Maltose	2	68	6	6	13	12
	2		12	21		1/2 insol.
	2		24	31		1/2 insol.
Ca + 1.5% Sucrose	2	68	6	9	10.2	14.5, 1/2 insol.
	2		12	31		17.5
	2		24	38		2/2 insol.
Heparin + 1.5% Maltose	2	68	6	21	8	9.5
	2		12	26		15.5
	2		24	48		12.5
1.5% Sucrose	2	68	6	8	12.5	12.5
	2		12	28		18.5
	2		24	20		1/2 insol.
2% Sucrose	2	68	12	2	6.7	10.2
	2		18	18		3.5
	2			24	23	4.2
3% Sucrose	2	68	12	7	7	10
	2		18	14		6
	2		24	26		7
1.5% Maltose	2	68	6	13	9.5	8
	2		12	31		13
	2		24	44		2/2 insol.
2% Maltose	2	68	12	29	5.5	4.7
	2		18	29		8
	2		24	34		8
3% Maltose	2	68	12	19	4.5	11.2
	2		18	22		8
	2		24	25		8
3. I.P. (Routine FVIII						
2% Sucrose	4	68	12	11	4.3	4.1
	4		18	13.5		5.5
	4		24	17.5		7.5