



ORIGINAL

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Via Federal Express

Dockets Management Branch (HFA-305)  
Food and Drug Administration  
5630 Fishers Lane, Room 1061  
Rockville, MD 20852

**REFERENCE: Docket No. 98N-0673: Final Rule: Revisions to the Requirements Applicable to Blood, Blood Components and Source Plasma**

To Whom It May Concern:

THERMOGENESIS CORP. appreciates the opportunity to comment on the recent direct final rule of the Center for Biologics Evaluation and Research (CBER) removing, revising or updating certain blood and plasma regulations.

In general, we find the revisions of the Center for Biologics Evaluation and Research helpful and non-controversial, but are concerned by one proposed revision and would be grateful for the Agency's consideration of the following question and comments with respect to **Section 640.34 (b): Fresh Frozen Plasma**.

**Summary of the Question:**

Under the proposed revision, all different methods of freezing plasma to be in compliance. This would include some methods that result in plasma undergoing a slow change of phase (liquid to solid) during freezing. It is widely accepted that such slow transitions will yield lower Factor VIII recoveries (please find some data on this below).

Thus, if slower freezing methods become used, the volume of plasma needed to generate the Factor VIII required will increase, which would increase the number of different plasma units that need to be processed. This would bring about an increased exposure of Factor VIII recipients to allogenic plasma donors.

• **Existing Text:**

The plasma shall be separated from the red blood cells, **frozen solid within 6 hours after phlebotomy**, and stored at  $-18^{\circ}\text{C}$  or colder.

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- **Proposed Revision:**

The plasma shall be separated from the red blood cells, *frozen solid within the time frame specified in the directions for use for the specific device* and stored at  $-18^{\circ}\text{C}$  or colder.

- **THERMOGENESIS CORP.'s Suggested Revision:**

The plasma shall be separated from the red blood cells, *placed within the freezer or device within 6 hours after phlebotomy, be completely frozen to  $-30^{\circ}\text{C}$  within 60 minutes*, and stored at  $-30^{\circ}\text{C}$  or colder.

**Rationale Behind Our Recommended Revision:**

FDA is considering these changes to the CFR in order to make the rules "more consistent with the blood industry and to remove unnecessary or outdated requirements." However, data to be presented below indicates that the proposed revision to the regulation would result in a reduction in the Factor VIII level of the FFP and, thus constitute a reduction in the efficacy of both FFP and Cryoprecipitated AHF. To the extent that human Factor VIII is used in therapy, these reductions will have at least two deleterious effects: first, to increase the cost, as more units of plasma will have to be used to produce the Factor VIII needed and second, to increase the exposure of Factor VIII recipients to allogeneic plasma donors by a number proportional to the reduction in yields.

The basis for this assertion is as follows:

1. The knowledge that Factor VIII yield in plasma is increased in plasma frozen at a high rate of speed compared to the Factor VIII yield in plasma frozen slowly has been available for more than a decade. Carlebjörk et al<sup>1</sup> noted that in four consecutive experiments Factor VIII:C recoveries averaged approximately 60% higher in the most rapidly frozen plasma ( $-40^{\circ}\text{C}$  ethanol bath) than the slowest frozen plasma ( $-25^{\circ}\text{C}$  cold box).
2. I have personally measured the core temperature of plasma bags placed at various locations within a  $-25^{\circ}\text{C}$  walk-in freezer (a method of freezing which may be allowed under the wording of the proposed revision) and found that the time needed to completely freeze the "cores" of the plasma units to  $-25^{\circ}\text{C}$  may vary between 2 hours and 10 hours depending on the location and the number of units being frozen at one time.

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<sup>1</sup> Carlebjörk, et al. *Freezing of Plasma to Obtain Better Yield of Factor VIII:C*. Scandinavian Journal of Haematology. 1984;40(33):127-128 (APPENDIX A).

Even in specialized air blast freezers, placement within the freezer can substantially effect freezing rate – and Factor VIII yield. Perhaps the largest single study of the relationship between freezing rate of plasma and Factor VIII yield was the validation report by the Red Cross Blood Bank in The Hague, The Netherlands (Stichting Rode Kruis Bloedbank 's-Gravenhage e.o., Dr. J.A. van der Does, Medical Director) which compares the effects on the yields of Factor VIII in FFP and Cryoprecipitated AHF of fast freezing to 0°C (~40 minutes) versus slow freezing to –20°C (~100 minutes) – APPENDIX B.

The s'Gravenhage (The Hague) study concluded that an average 32% improvement in Factor VIII yield in Cryoprecipitate may be obtained simply by accelerating the rate of freezing (to –20°C) from a mean of 100 minutes to a mean of 40 minutes.

The study also concluded that an average 17% increase in Factor VIII yield in FFP could result from this same acceleration in freezing speed.

It is important to note that the s'Gravenhage study relied upon more than ten times the number of samples reported by the study of Moroff et al<sup>2</sup> which the FDA considered in allowing freezing plasma within 8-hour instead of within 6-hours, for the preparation of FFP.

Not surprisingly, the 5<sup>th</sup> edition of the European Guide to the Preparation, Use and Quality Assurance of Blood Components, 1999 (APPENDIX C) require that fresh frozen plasma (FFP) be completely frozen within 1 hour and presents the same rationale to support their guidelines, namely, that Factor VIII losses occur inevitably with the slow freezing of plasma.

The following extracts of the Guide illustrate the reasons for this recommendation.

**Chapter 7: Freezing and thawing of plasma**

*Section 7.1 Rationale*

“To achieve the highest yield of Factor VIII, plasma should be frozen to –30°C or below.”

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<sup>2</sup> Moroff, et al. *Effect of an 8-Hour Holding Period on In Vivo and In Vitro Properties of Red Cells and Factor VIII Content of Plasma After Collection in a Red Cell Additive System.* Transfusion. 1990;30(9):828-32.

*Section 7.2 Methods of freezing*

“When freezing plasma, the rate of cooling must be as rapid as possible and ideally should bring the core temperature down to  $-30^{\circ}\text{C}$  or below within 60 minutes. . . Experience has shown that it sometimes takes several hours in an environmental temperature of  $-30^{\circ}\text{C}$  and heat transfer by air. The time must be reduced to less than one hour, and if possible, less than half an hour. . .”

**Chapter 14: Fresh frozen plasma**

Methods of preparation

*a. Whole blood*

“Plasma may also be separated from platelet rich plasma. Freezing should take place in a system that will allow complete freezing within one hour to a temperature below  $-30^{\circ}\text{C}$ .”

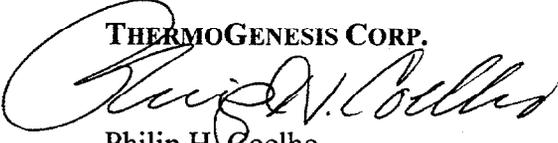
*b. By apheresis*

“Plasma may be collected by manual or automated apheresis. The freezing process should commence within six hours of completion of the procedure in a system which allows complete freezing within one hour to a temperature below  $-30^{\circ}\text{C}$ .”

In conclusion, THERMOGENESIS CORP. respectfully requests a reconsideration of the ruling on **Section 640.34 (b): Fresh Frozen Plasma** to avoid returning to an older and less efficient preparatory system that will reduce human plasma Factor VIII yields as well as increase costs and allogeneic exposure risks to the recipients. The latter in particular would seem to run counter to the Agency's otherwise totally consistent policy of reducing the risk of disease transmission by blood and blood products towards a zero-risk goal.

Sincerely,

**THERMOGENESIS CORP.**

  
Philip H. Coelho  
Chairman/CEO

PHC/mr  
Enclosure

**APPENDIX A**

## Freezing of Plasma to Obtain Better Yield of Factor VIII:C

G CARLEBJÖRK & M BLOMBÄCK

Department of Blood Coagulation Disorders, Karolinska Hospital, Stockholm, Sweden

Plasma was frozen in two types of ampoules with different freezing equipments. Changes in temperature were recorded with thermocouples and the quality of frozen plasma determined by analysis of F VIII:C. Temperature decreased rapidly to the freezing point where it stayed during the phase change to ice and was followed by a second rapid decrease down to the final temperature. The time for duration at the freezing point decreased with thinner plasma layers. Freezing was accomplished faster with -40°C ethanol baths than with different types of freezers. Use of flat 750 ml ampoules resulted in shorter freezing times than with cylindrical 1,500 ml ampoules. The factor VIII:C recovery in frozen plasma increased with faster rate of freezing. The purity and recovery of F VIII:C in cryoprecipitates increased with fast freezing of plasma.

*Key words:* Cryoprecipitate, F VIII:C, Freezing, Plasma quality

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In Sweden like in many other countries the amount of donated blood has not been enough to cover the demand for production of therapeutic derivatives, especially F VIII concentrates. This shortage has mainly been filled by import of plasma and/or concentrates from different countries. As a result of the recommendation by the World Health Organisation that all countries ought to be self-supporting with blood products, the Swedish Government has initiated a research program in this field. The research is supported by The National Swedish Board for Technical Development and several groups are studying different parameters with the aim towards self-sufficiency.

We have noted that freezing techniques at different bloodbanks are not uniform and have therefore started an investigation to clarify the influence of freezing on plasma quality.

### METHODS

F VIII:C recalcification method was performed as described earlier (Carlebjörk et al 1983). Fibrinogen was assayed with an immunodiffusion method.

To find out the basic characteristics, plasma was frozen in two types of ampoules (flat 750 ml and cylindrical 1,500 ml) and the temperature changes followed with adapted thermocouples and a recorder.

Freezing was accomplished with -25°C and -80°C freezers and with two types of -40°C ethanol baths. Cores were drilled out of the frozen ampoules and analyzed for F VIII:C.

Fresh plasma from 3 double plasmapheresis was pooled and transferred to three 750 ml ampoules. The ampoules were frozen with different types of freezing machines. Cryoprecipitates were processed individually and aliquots withdrawn for analysis of F VIII:C and fibrinogen.

### RESULTS

When plasma is frozen in an ampoule the temperature falls rapidly down to the freezing point (with the same speed all over the ampoule). At the freezing point, where the phase change from liquid to

of starting plasma  
is similar, however,  
method gave a final  
activity above  
ing of protein in all  
ed. Although best  
plasma is clearly pre-  
of highly purified  
pared within 24  
o six months can  
actionation. The  
plasma available for  
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solid takes place, a temperature plateau is formed. The plateau is caused by the need of large energy transport at the conversion and indicates the necessity of effective freezing machines to minimise the delay in temperature decrease.

After the phase change, the temperature decrease continues rapidly down to the final freezing temperature.

In the two compared ampoule types freezing is completed faster with the flat 750 ml ampoule than with the cylindrical 1,500 ml ampoule as a consequence of faster energy transportation in thinner layers.

Comparison of different freezing machines reveals that energy is transported more effectively in ethanol baths than in freezers. A new type of ethanol bath with circulation and constant ethanol level turned out to be superior to a conventional bath without these facilities.

Faster freezing rate results in higher recovery of F VIII:C in plasma. The recovery depends mainly on the time for phase change at the freezing point.

Cryoprecipitate quality expressed as IU F VIII:C/mg fibrinogen increases with faster plasma freezing rate. The same tendency can be seen in all experiments (Table 1). The total recovery of F VIII:C in cryoprecipitates from quick frozen plasma was high.

It is concluded that freezing conditions are important in the handling of plasma and that it should be carried out rapidly preferably in flat vessels with effective ethanol baths.

#### ACKNOWLEDGEMENTS

This study was supported by grants from the National Swedish Board for Technical Development.

#### REFERENCES

- Carlebjörk G, Blombäck M & Åkerblom O (1983). Improvement of plasma quality as raw material for F VIII:C concentrates. Storage of whole blood and plasma and interindividual plasma levels of fibrinopeptide A. *Vox Sang* 45, 233-242.

TABLE 1.

Purity of cryoprecipitate IU F VIII:C/mg fibrinogen.

Freezing of plasma in	Experiment				
	1	2	3	4	$\bar{x}$
-40°C ethanol bath	0.90	0.68	0.86	0.84	0.82
-80°C freezer	0.85	0.52	0.68	0.81	0.72
-25°C freezer	0.51	0.36	0.48	0.65	0.50

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**APPENDIX B**

Stichting Rode Kruis  
BLOEDBANK 's-GRAVENHAGE e.o.

FACTOR VIII Study

's-Gravenhage  
February 25, 1991

- Postadres donorcentra, Postbus 61204-2506 AE 's-Gravenhage Donorcentra: Den Haag, Voorburg, Zoetermeer (070-3651900) en Delft (015-615211)
  - Directie en overige afdelingen-(post)adres: Leyweg 297, 2545 CJ 's-Gravenhage-tel. 070-3676854
  - Telefax: 070-3676326 Bankrekeningnummer 64.23.55.126 Rabobank 's-Gravenhage

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## Subject

Quality control measurements of the Factor VIII yields were recorded for the plasma processed from 1989 until January 17, 1991. A change in freezing method from a  $-40^{\circ}\text{C}$  air blast freezer to an InstaCool freezer occurred on September 1, 1990, and the quality control measurements were examined to detect if a change in Factor VIII levels occurred with the change in freezing method.

## Abstract

The Red Cross Society Blood Bank, The Hague, processes blood delivered from satellite collection enters and produces several types of plasma products, two of which are: 1) Lyophilized and heat-treated Factor VIII rich cryoprecipitate supplied directly to local hospitals and, 2) frozen plasma (FP) supplied to the Central Laboratory (CLB) for fractionation. Until August 31, 1990, all plasma was frozen in a  $-40^{\circ}\text{C}$  air blast freezer. Beginning September 1, 1990, until January 17, 1991, all plasma was frozen in an InstaCool freezer. Throughout the reporting period records were kept of the Factor VIII levels of the plasma products. The plasma frozen after September 1, 1990 showed significant increases in Factor VIII yields in both the FP and Lyophilized and heat-treated cryoprecipitate compare to those same products that were derived from the plasma frozen before September 1, 1990.

## Methods and Materials

All plasma sent in the form of FP to the CLB used in the production of the lyophilized and heat-treated cryoprecipitate was separated from whole blood collected at satellite centers and delivered for processing to the Red Cross Blood Bank, Hague. All plasma used throughout this testing period was separated from the red blood cells and entered the freezing chamber within 4 – 6 hours of collection. All Factor VIII measurements were performed by a Model KC-10 from Baxter Instruments. It is a one-step coagulation assay with use of FVIII deficient plasma (Made by the University of Leiden) and actin from Baxter Instruments.

## Freezing Rate Measurements

The measuring of temperature rate drops of plasma frozen in both the  $-40^{\circ}\text{C}$  air blast freezer and the InstaCool freezer were performed with a Yokogawa HR2500 hybrid datalog system. It has 60 thermocouples of copper-constantan. The range of temperature that can be measured is  $-200$  to  $+400$  degrees Celsius.

Each thermocouple is validated and adjusted to PT100 calibrated thermometer. Our thermocouples are adjusted within  $\pm 0.3$  degree Celsius of the PT100 value.

The temperature data are logged with a Hewlett Packard Portable XT and Slidewrite software is used to collect specific data and draw pictures.

With the use of this equipment, core measurements were recorded from 8 bags evenly distributed throughout each full load of plasma bags placed in the  $-40^{\circ}\text{C}$  air blast freezer and the InstaCool freezer. The bags averaged 300 ml in plasma. The slowest, fastest and average freezing rates are shown on Figures 1 & 2 below (See Table 1 & 2).

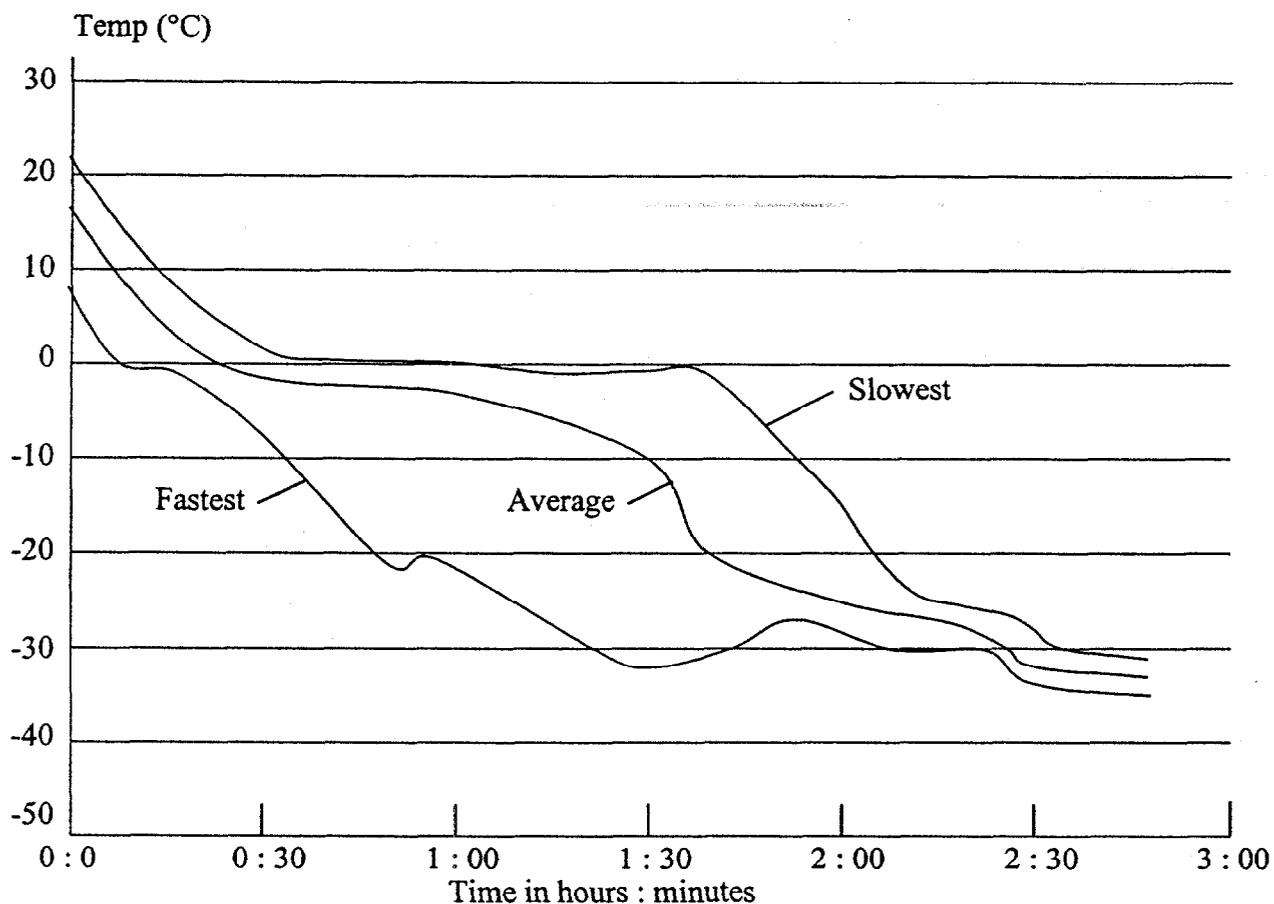


Figure 1 (Freezing rate of plasma in  $-40^{\circ}\text{C}$  Air Blast Freezer, Before September 1, 1990)

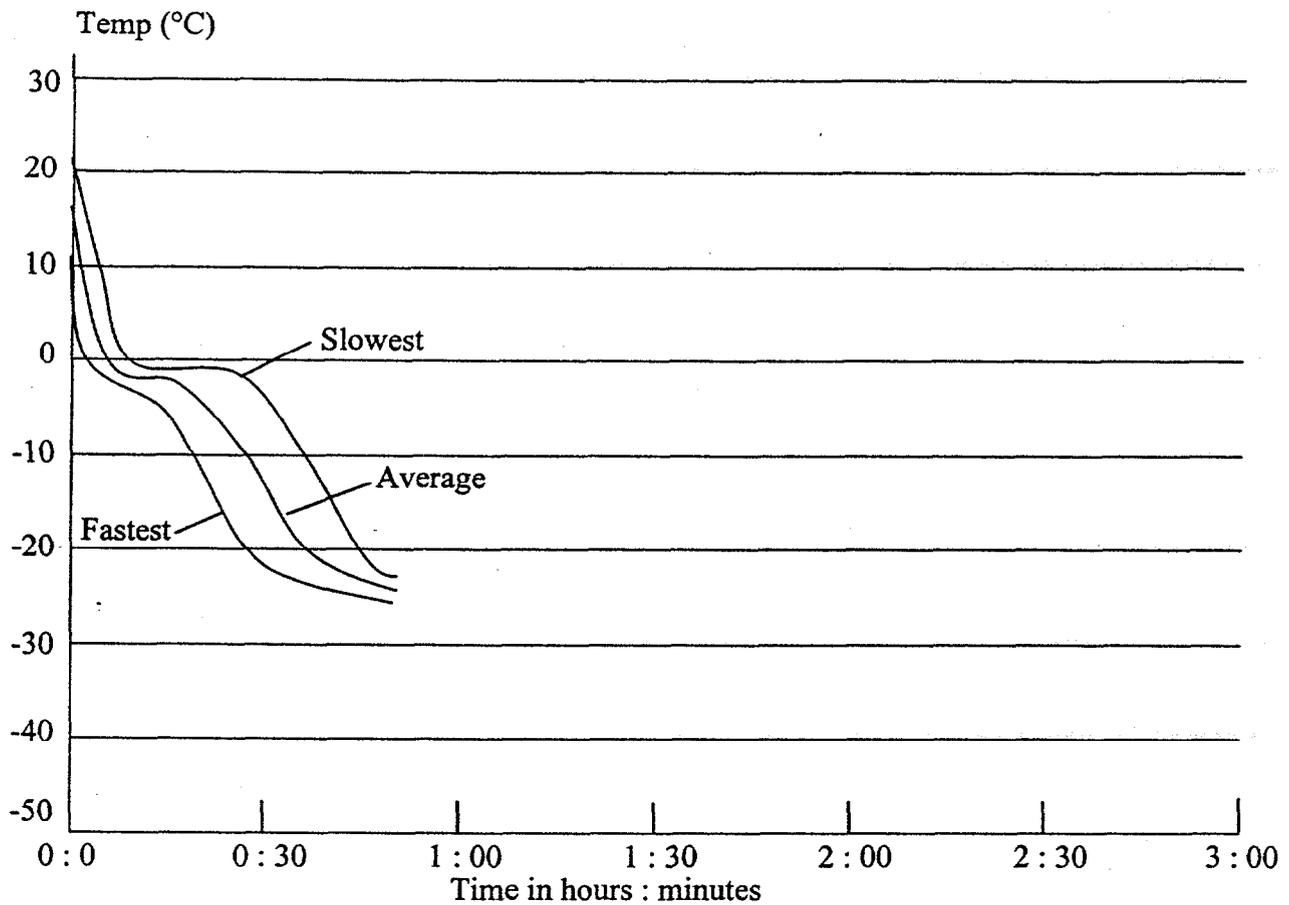


Figure 2 (Freezing rate of plasma in InstaCool Freezer, used after September 1, 1990)

*Factor VIII Rich Cryoprecipitate Powder*

The method of production of a pool of lyophilized and heat-treated cryoprecipitate is as follows:

1. 40 donors each contribute 1 bag of whole blood which when centrifuged delivers plasma of 300 ml  $\pm$  10 ml.
2. These 40, 300 ml  $\pm$  10 ml bags of plasma enter the freezing chamber within 4 – 6 hours of whole blood collection and, after freezing, are stored at  $-30^{\circ}\text{C}$ .
3. Within 3 days to 2 weeks, the 40 frozen bags are then thawed in a  $4^{\circ}\text{C}$  water bath (about 3 hours).
4. The  $4^{\circ}\text{C}$  thawed bags of plasma are then centrifuge at 4,000 rpm for 10 minutes to concentrate the precipitated cryo.
5. The cryo-poor plasma is then expressed away from each bag leaving 4 ml  $\pm$  2 ml of cryo in each bag.

6. When the approximately 4 ml cryo is removed from each bag, the inside of each bag is rinsed with approximately 5 ml 0.9% NaCl water – creating from each bag a volume of about 9 ml. Therefore, the cryo + NaCl water from 40 bags would total approximately 360 ml. Syntamin buffer is then added to bring the total volume of the pool to 500 ml.
7. The 500 ml pool is then distributed into 10 small glass bottles each holding 50 ml, all of which constitutes a single batch. Therefore, each small glass bottle holds the cryoprecipitate from 4 bags of plasma (approximately 1,200 ml plasma).
8. All batches are lyophilized and heat treated (72 hours @ 60°C).
9. Prior to lyophilization and heat treating, the Factor VIII of the entire pool was measured.
10. After the lyophilization and heat treatment, each bottle of concentrate was measured for Factor VIII.

#### *FP for Fractionation*

The CLB performs an incoming inspection on all shipments of plasma received for fractionation. The center core of frozen plasma is removed from a statistically significant number of bags, and then tested for FVIII in IU/ml, total protein in mg/ml and Specific Activity in IU/mg T.P. The correlation between the protein levels in the center core sample plasma and the whole shipment has been historically validated by the CLB.

The quality of FP delivered to the CLB is measured by the CLB and expressed as specific activity:  $\text{IU FVIII/mg total protein} \times 10^{-4}$ . A value of specific activity equal to or greater than 100 is considered Quality A. A value of specific activity between 80 and 99 is considered Quality B and specific activity less than 80 is considered Quality C. The CLB pays the highest compensation for Quality A plasma, lessor compensation for Quality B plasma, and the lowest compensation for Quality C plasma.

From January 1, 1990, until August 31, 1990, all plasma sent to the CLB was frozen in a -40°C air blast freezer. From September 1, 1990 until January 17, 1991 all the plasma was frozen in the InstaCool freezer.

## Results

### *Factor VIII Rich Cryoprecipitate Powder*

	Average FVIII in IU $\pm$ S.D.		% diff
	1989 and 1990 up to Sept. 1, 1990	After Sept. 1, 1990	
	n = 109 pools (40 bags/pool)	n = 30 pools (40 bags/pool)	
Pools (500 ml)	4300 $\pm$ 1000	5700 $\pm$ 900	+ 32%
Lyophilized & heat-treated end product in each bottle (10 bottles/pool)	330 $\pm$ 30	390 $\pm$ 30	+ 18%

Summary Chart 1

### *FP for Fractionation*

	01-01-90 ~ 01-09-90	01-09-91 ~ 01-17-91	% diff
Number of deliveries (approx. 100 kg/delivery)	33	22	
FVIII (IU/ml)	0.58	0.68	+ 17%
Total protein (mg/ml)	62.8	63.5	
Specific activity	110	126	+ 14%

Summary Chart 2

Period January 1, 1990 – August 31, 1990 (33 deliveries of approximately 100 Kg each ) (See Table 1)

	<u>Highest</u>	<u>Lowest</u>	<u>Spread</u>	<u>Average</u>	<u>S.D.</u>
FVIII (IU/ml)	0.75	0.47	0.28	0.58	0.08
T.P. (mg/ml)	66.6	57.3	9.3	62.8	2.9
S.A. (IU/mgTP)	134	88	46	110	12

Period September 1, 1990 – January 17, 1991 (22 deliveries of approximately 100 Kg each)

	<u>Highest</u>	<u>Lowest</u>	<u>Spread</u>	<u>Average</u>	<u>S.D.</u>
FVIII (IU/ml)	0.84	0.57	0.27	0.68	0.07
T.P. (mg/ml)	68.3	57.1	11.2	63.5	2.8
S.A. (IU/mgTP)	174	112	62	126	16

Deliveries FP to CLB

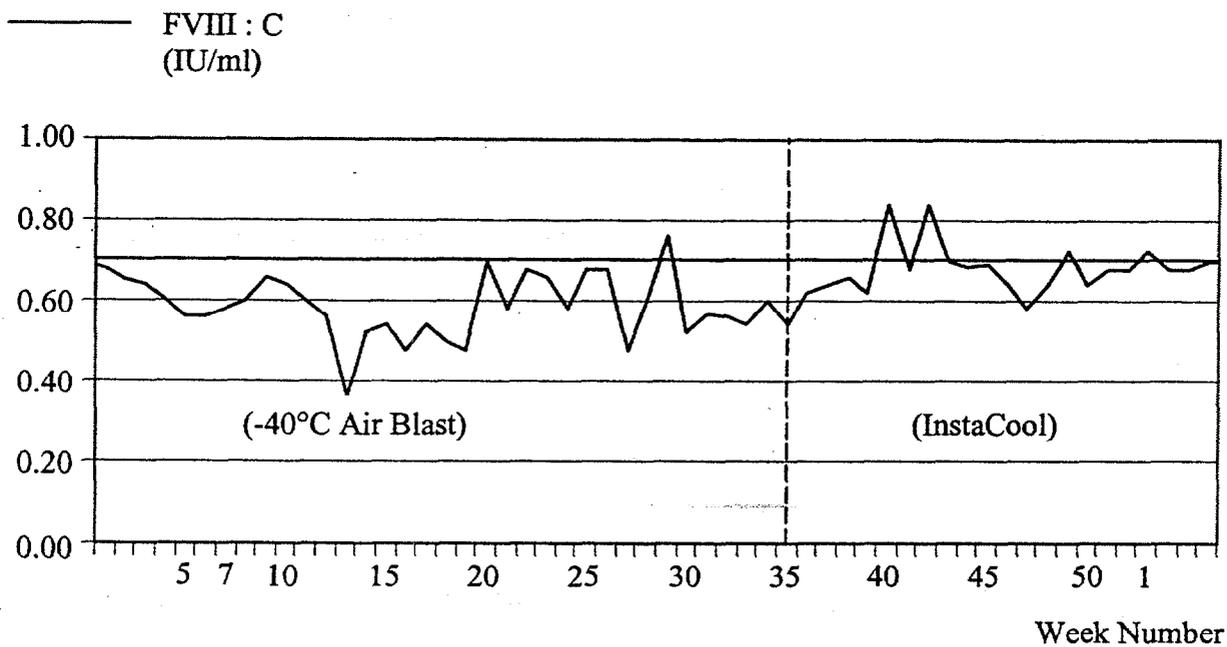


Figure 3a Weekly FVIII Concentration in IU/ml for 1990

## Deliveries FP to CLB

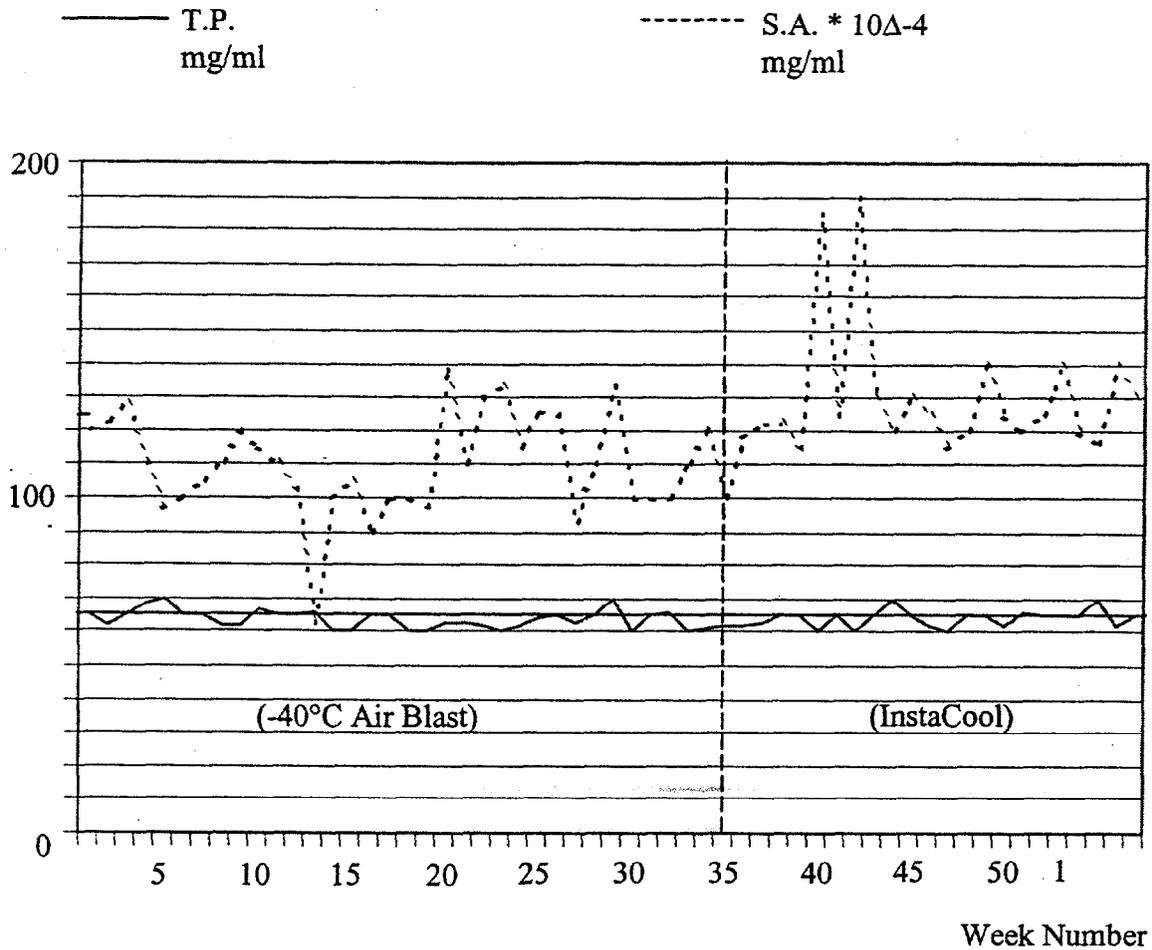


Figure 3b Weekly Concentration of Total Protein in mg/ml  
And Specific Activity for 1990

### Discussion

#### *Factor VIII Rich Cryoprecipitate Powder*

The Factor VIII rich, lyophilized and heat treated cryoprecipitate is being supplied to local hospitals by the Red Cross Blood Bank, The Hague, and this product has been accepted by the Dutch Association of Hemophiliacs for treatment of Hemophilia A.

#### *Frozen Plasma for Fractionation*

The quantity of FVIII expressed in IU/ml increased while the plasma was frozen in the InstaCool freezer. Interestingly, the change in freezing method did not result in a change in the concentration of total protein in the plasma.

During the test period up to August 31, when the plasma sent to the CLB was frozen in the -40°C air blast freezer, 6 of the 33 batches fell into the Quality B category, and resulted in a lower level of compensation from the CLB for that plasma.

With the 22 batches of plasma that were frozen in the InstaCool freezer, every batch received a Quality A rating and compensation.

### Conclusions

Simultaneously with the change in freezing method from a -40°C air blast freezer to an InstaCool freezer, the Red Cross Blood Bank, Hague, and the Central Laboratory each, independently, observed a significant quality improvement in the plasma obtained from whole blood donations as measured by FVIII levels.

Dr. J.A. v.d. Does, internist

CLB Measurements of plasma frozen in -40°C air blast freezer  
 From January 1, 1990 to August 31, 1990

Table I

Delivery Date	FVIII IU/ml	T.P. mg/ml	S.A. IU/mg TP (10 <sup>4</sup> )
01-04	0.68	66.2	121
01-11	0.65	62.3	123
01-18	0.65	64.2	119
01-25	0.59	66.6	105
02-01	0.56	68.6	96
02-08	0.56	65.9	100
02-22	0.60	62.5	113
03-01	0.65	63.2	121
03-08	0.63	66.7	111
03-15	0.58	63.6	108
03-22	0.56	64.0	103
04-05	0.54	60.8	105
04-12	0.53	60.7	103
04-19	0.48	64.6	88
04-26	0.54	62.8	101
05-03	0.49	58.1	100
05-10	0.48	58.6	97
05-17	0.71	62.7	134
05-25	0.56	61.8	107
05-31	0.67	61.7	128
06-07	0.65	58.7	131
06-14	0.57	61.4	110
06-21	0.66	62.9	124
06-28	0.66	63.8	122
07-05	0.47	62.3	89
07-12	0.57	62.8	107
07-19	0.75	68.2	130
07-26	0.50	58.7	101
08-02	0.56	64.6	102
08-09	0.55	65.8	99
08-16	0.52	57.3	107
08-23	0.60	60.2	118
08-30	0.51	60.9	99

Ave ± SD

0.58 ± 0.08

62.8 ± 2.9

110 ± 12

CLB measurements of plasma frozen in InstaCool Freezer  
 From September 1, 1990 to January 17, 1991

Table 2

Delivery Date	FVIII IU/ml	T.P. mg/ml	S.A. IU/mg TP (10 <sup>4</sup> )
09-06	0.61	61.9	116
09-13	0.63	62.2	120
09-20	0.65	64.0	120
09-28	0.62	64.3	112
10-04	0.84	58.5	169
10-11	0.66	63.5	123
10-18	0.84	57.1	174
10-25	0.71	66.1	125
11-01	0.68	67.8	118
11-08	0.69	63.0	129
11-15	0.64	61.5	123
11-22	0.57	60.2	112
11-29	0.63	65.5	113
12-06	0.72	64.6	132
12-13	0.64	61.8	122
12-20	0.66	66.4	117
12-28	0.66	64.0	122
01-03	0.73	64.1	134
01-08	0.66	65.9	118
01-10	0.66	68.3	114
01-15	0.70	62.5	132
01-17	0.71	64.7	129

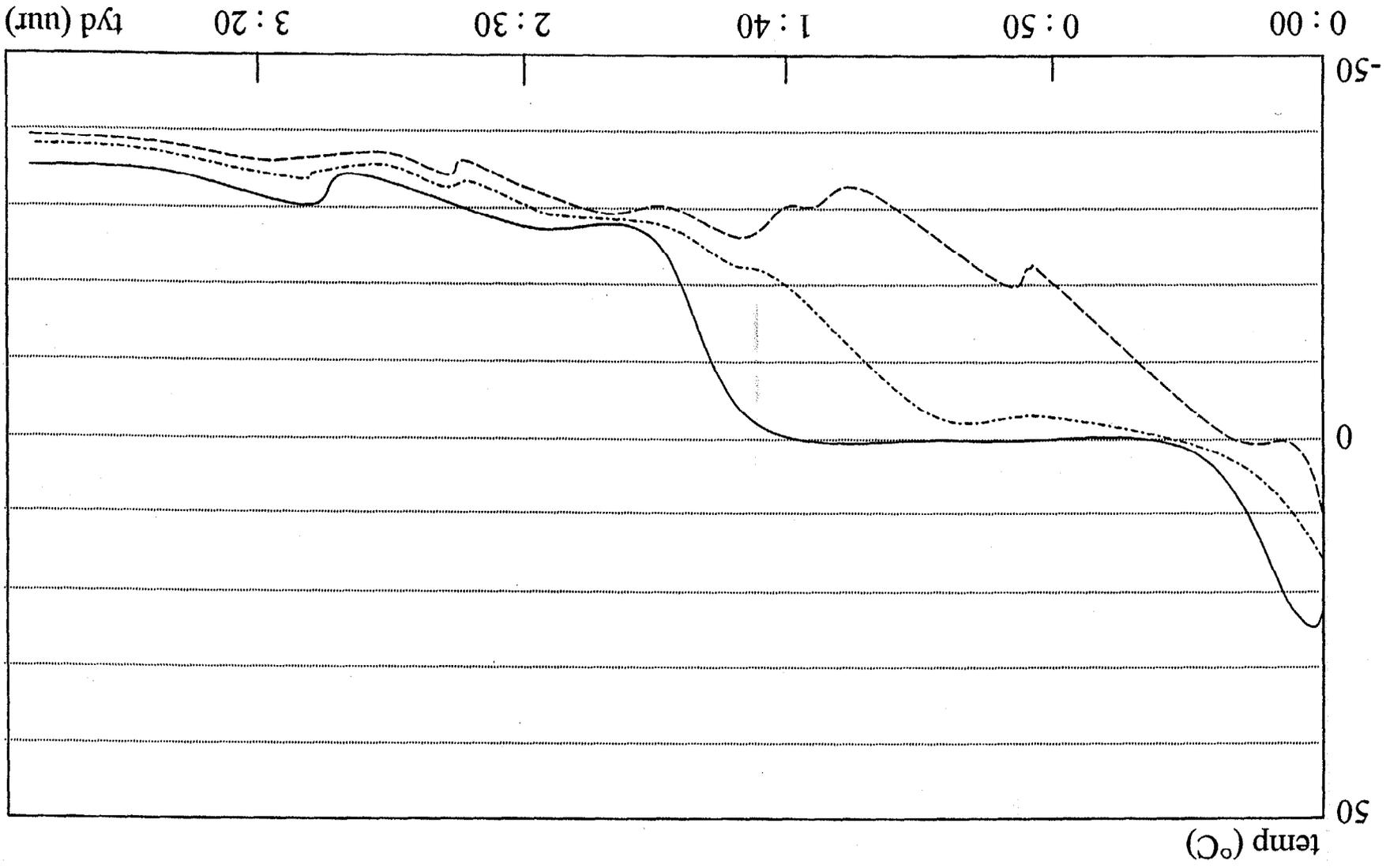
Ave ± SD

0.68 ± 0.07

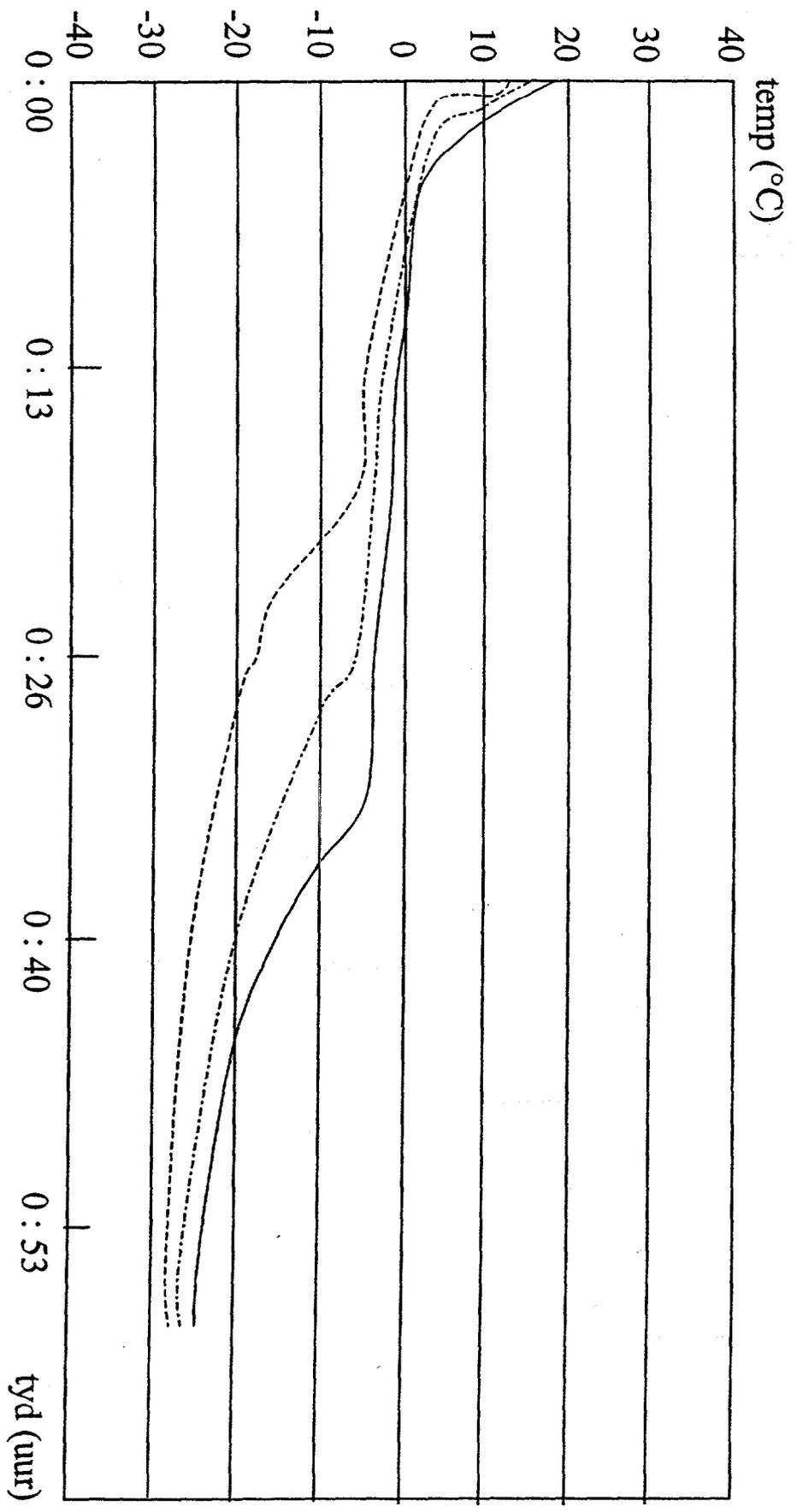
63.5 ± 2.8

126 ± 16

VALIDATIE BLAASVRIEZER (LEYWEG)  
(weergave plasma temperatuur)



VALIDATIE INSTACCOOL (24-10-90)  
(2 compartimenten gedurende 1 uur)



Stichting Rode Kruis  
BLOEDBANK 's-GRAVENHAGE e.o.

FACTOR VIII Study

's-Gravenhage  
February 25, 1991

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## Subject

Quality control measurements of the Factor VIII yields were recorded for the plasma processed from 1989 until January 17, 1991. A change in freezing method from a  $-40^{\circ}\text{C}$  air blast freezer to an InstaCool freezer occurred on September 1, 1990, and the quality control measurements were examined to detect if a change in Factor VIII levels occurred with the change in freezing method.

## Abstract

The Red Cross Society Blood Bank, The Hague, processes blood delivered from satellite collection enters and produces several types of plasma products, two of which are: 1) Lyophilized and heat-treated Factor VIII rich cryoprecipitate supplied directly to local hospitals and, 2) frozen plasma (FP) supplied to the Central Laboratory (CLB) for fractionation. Until August 31, 1990, all plasma was frozen in a  $-40^{\circ}\text{C}$  air blast freezer. Beginning September 1, 1990, until January 17, 1991, all plasma was frozen in an InstaCool freezer. Throughout the reporting period records were kept of the Factor VIII levels of the plasma products. The plasma frozen after September 1, 1990 showed significant increases in Factor VIII yields in both the FP and Lyophilized and heat-treated cryoprecipitate compare to those same products that were derived from the plasma frozen before September 1, 1990.

## Methods and Materials

All plasma sent in the form of FP to the CLB used in the production of the lyophilized and heat-treated cryoprecipitate was separated from whole blood collected at satellite centers and delivered for processing to the Red Cross Blood Bank, Hague. All plasma used throughout this testing period was separated from the red blood cells and entered the freezing chamber within 4 – 6 hours of collection. All Factor VIII measurements were performed by a Model KC-10 from Baxter Instruments. It is a one-step coagulation assay with use of FVIII deficient plasma (Made by the University of Leiden) and actin from Baxter Instruments.

## Freezing Rate Measurements

The measuring of temperature rate drops of plasma frozen in both the  $-40^{\circ}\text{C}$  air blast freezer and the InstaCool freezer were performed with a Yokogawa HR2500 hybrid datalog system. It has 60 thermocouples of copper-constantan. The range of temperature that can be measured is  $-200$  to  $+400$  degrees Celsius.

Each thermocouple is validated and adjusted to PT100 calibrated thermometer. Our thermocouples are adjusted within  $\pm 0.3$  degree Celsius of the PT100 value.

The temperature data are logged with a Hewlett Packard Portable XT and Slidewrite software is used to collect specific data and draw pictures.

With the use of this equipment, core measurements were recorded from 8 bags evenly distributed throughout each full load of plasma bags placed in the  $-40^{\circ}\text{C}$  air blast freezer and the InstaCool freezer. The bags averaged 300 ml in plasma. The slowest, fastest and average freezing rates are shown on Figures 1 & 2 below (See Table 1 & 2).

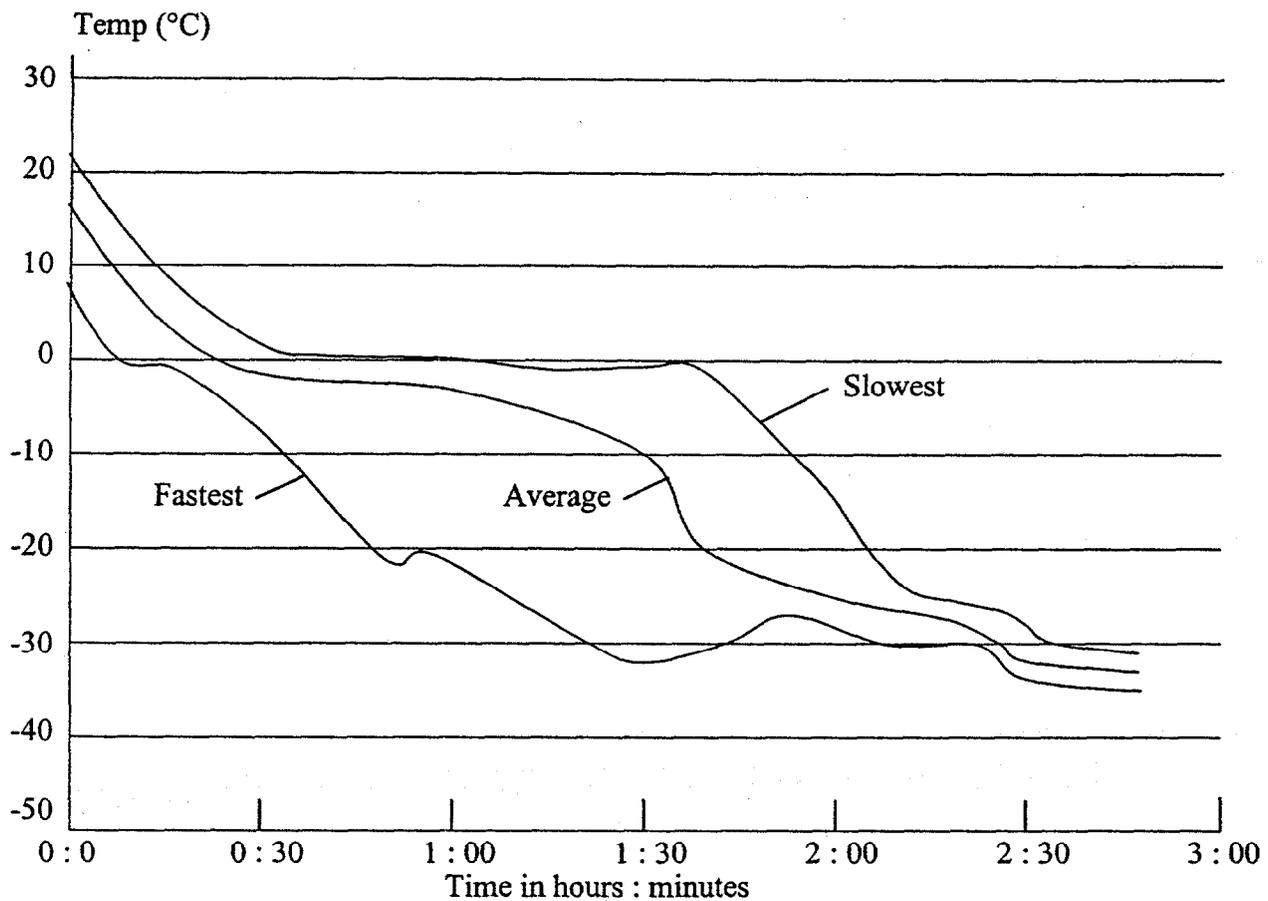


Figure 1 (Freezing rate of plasma in  $-40^{\circ}\text{C}$  Air Blast Freezer, Before September 1, 1990)

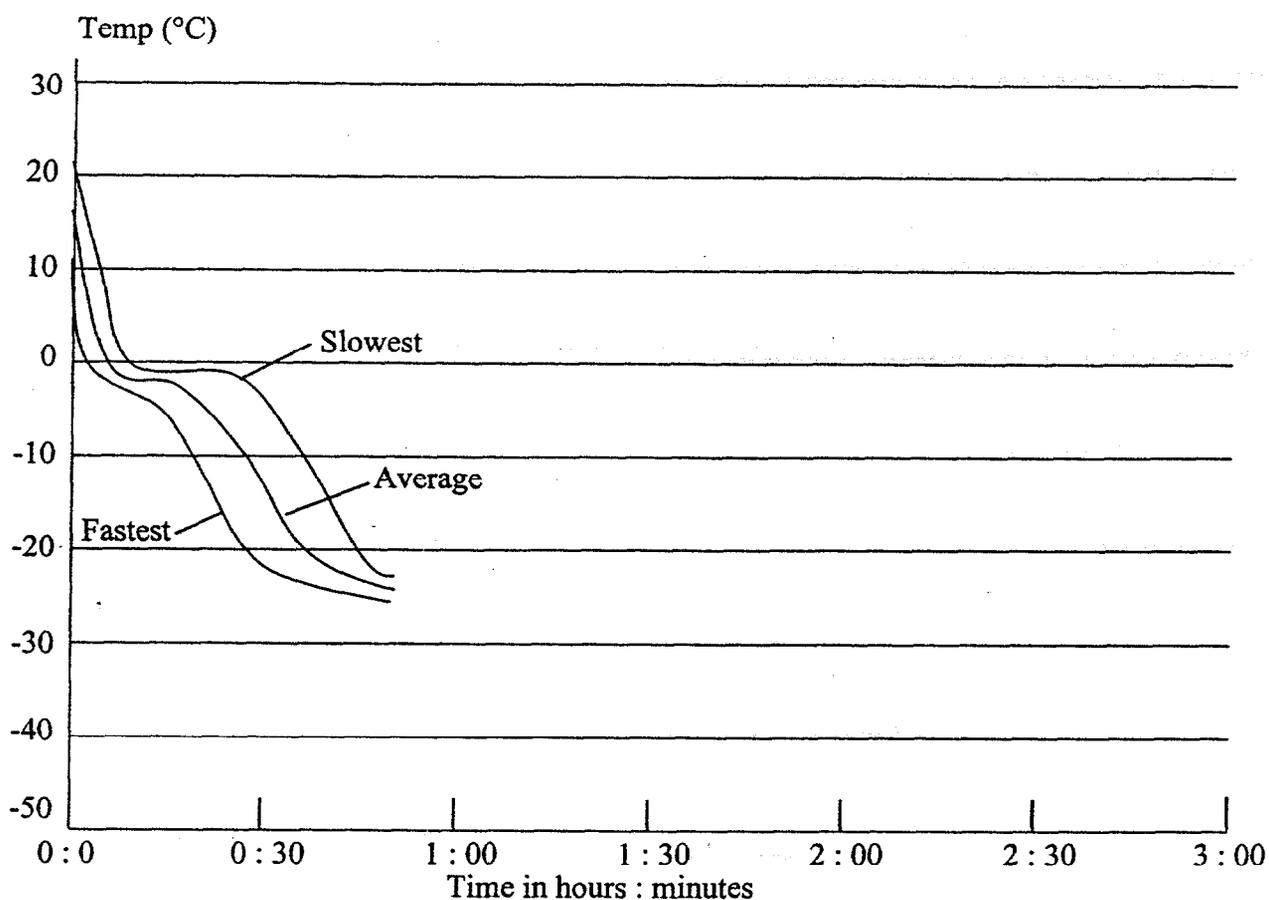


Figure 2 (Freezing rate of plasma in InstaCool Freezer, used after September 1, 1990)

### *Factor VIII Rich Cryoprecipitate Powder*

The method of production of a pool of lyophilized and heat-treated cryoprecipitate is as follows:

1. 40 donors each contribute 1 bag of whole blood which when centrifuged delivers plasma of 300 ml  $\pm$  10 ml.
2. These 40, 300 ml  $\pm$  10 ml bags of plasma enter the freezing chamber within 4 – 6 hours of whole blood collection and, after freezing, are stored at  $-30^{\circ}\text{C}$ .
3. Within 3 days to 2 weeks, the 40 frozen bags are then thawed in a  $4^{\circ}\text{C}$  water bath (about 3 hours).
4. The  $4^{\circ}\text{C}$  thawed bags of plasma are then centrifuge at 4,000 rpm for 10 minutes to concentrate the precipitated cryo.
5. The cryo-poor plasma is then expressed away from each bag leaving 4 ml  $\pm$  2 ml of cryo in each bag.

6. When the approximately 4 ml cryo is removed from each bag, the inside of each bag is rinsed with approximately 5 ml 0.9% NaCl water – creating from each bag a volume of about 9 ml. Therefore, the cryo + NaCl water from 40 bags would total approximately 360 ml. Syntamin buffer is then added to bring the total volume of the pool to 500 ml.
7. The 500 ml pool is then distributed into 10 small glass bottles each holding 50 ml, all of which constitutes a single batch. Therefore, each small glass bottle holds the cryoprecipitate from 4 bags of plasma (approximately 1,200 ml plasma).
8. All batches are lyophilized and heat treated (72 hours @ 60°C).
9. Prior to lyophilization and heat treating, the Factor VIII of the entire pool was measured.
10. After the lyophilization and heat treatment, each bottle of concentrate was measured for Factor VIII.

#### *FP for Fractionation*

The CLB performs an incoming inspection on all shipments of plasma received for fractionation. The center core of frozen plasma is removed from a statistically significant number of bags, and then tested for FVIII in IU/ml, total protein in mg/ml and Specific Activity in IU/mg T.P. The correlation between the protein levels in the center core sample plasma and the whole shipment has been historically validated by the CLB.

The quality of FP delivered to the CLB is measured by the CLB and expressed as specific activity: IU FVIII/mg total protein  $\times 10^{-4}$ . A value of specific activity equal to or greater than 100 is considered Quality A. A value of specific activity between 80 and 99 is considered Quality B and specific activity less than 80 is considered Quality C. The CLB pays the highest compensation for Quality A plasma, lessor compensation for Quality B plasma, and the lowest compensation for Quality C plasma.

From January 1, 1990, until August 31, 1990, all plasma sent to the CLB was frozen in a -40°C air blast freezer. From September 1, 1990 until January 17, 1991 all the plasma was frozen in the InstaCool freezer.

## Results

### *Factor VIII Rich Cryoprecipitate Powder*

	Average FVIII in IU $\pm$ S.D.		% diff
	1989 and 1990 up to Sept. 1, 1990	After Sept. 1, 1990	
	n = 109 pools (40 bags/pool)	n = 30 pools (40 bags/pool)	
Pools (500 ml)	4300 $\pm$ 1000	5700 $\pm$ 900	+ 32%
Lyophilized & heat-treated end product in each bottle (10 bottles/pool)	330 $\pm$ 30	390 $\pm$ 30	+ 18%

Summary Chart 1

### *FP for Fractionation*

	01-01-90 ~ 01-09-90	01-09-91 ~ 01-17-91	% diff
Number of deliveries (approx. 100 kg/delivery)	33	22	
FVIII (IU/ml)	0.58	0.68	+ 17%
Total protein (mg/ml)	62.8	63.5	
Specific activity	110	126	+ 14%

Summary Chart 2

Period January 1, 1990 – August 31, 1990 (33 deliveries of approximately 100 Kg each ) (See Table 1)

	<u>Highest</u>	<u>Lowest</u>	<u>Spread</u>	<u>Average</u>	<u>S.D.</u>
FVIII (IU/ml)	0.75	0.47	0.28	0.58	0.08
T.P. (mg/ml)	66.6	57.3	9.3	62.8	2.9
S.A. (IU/mgTP)	134	88	46	110	12

Period September 1, 1990 – January 17, 1991 (22 deliveries of approximately 100 Kg each)

	<u>Highest</u>	<u>Lowest</u>	<u>Spread</u>	<u>Average</u>	<u>S.D.</u>
FVIII (IU/ml)	0.84	0.57	0.27	0.68	0.07
T.P. (mg/ml)	68.3	57.1	11.2	63.5	2.8
S.A. (IU/mgTP)	174	112	62	126	16

Deliveries FP to CLB

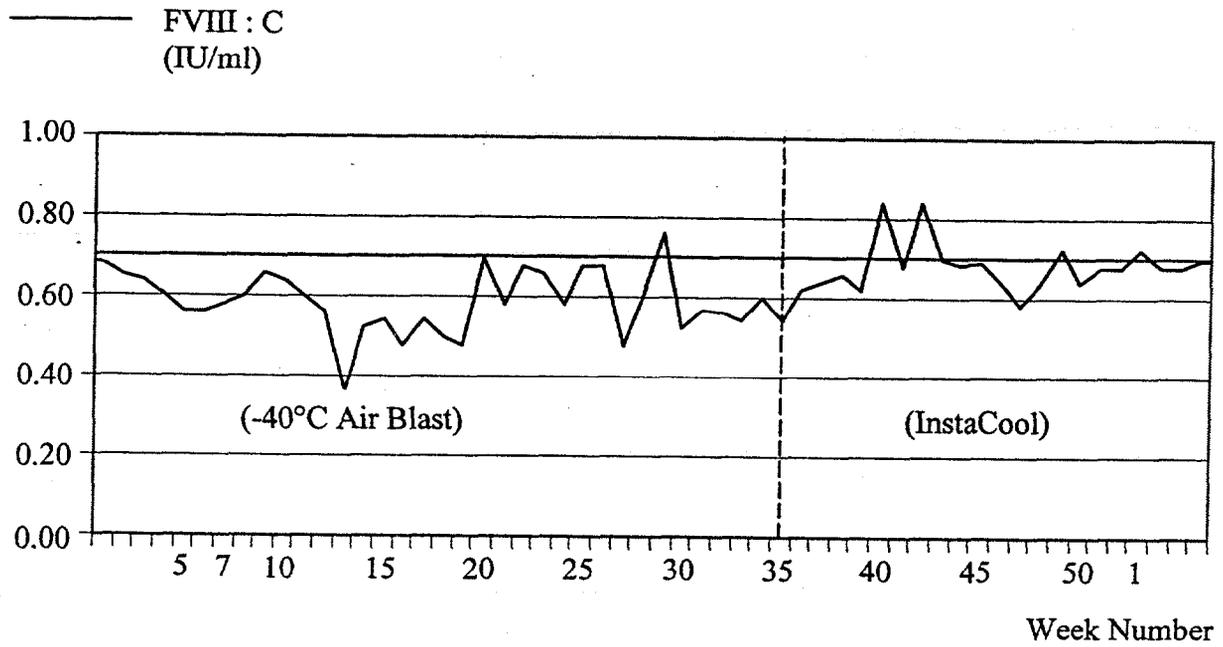


Figure 3a Weekly FVIII Concentration in IU/ml for 1990

### Deliveries FP to CLB

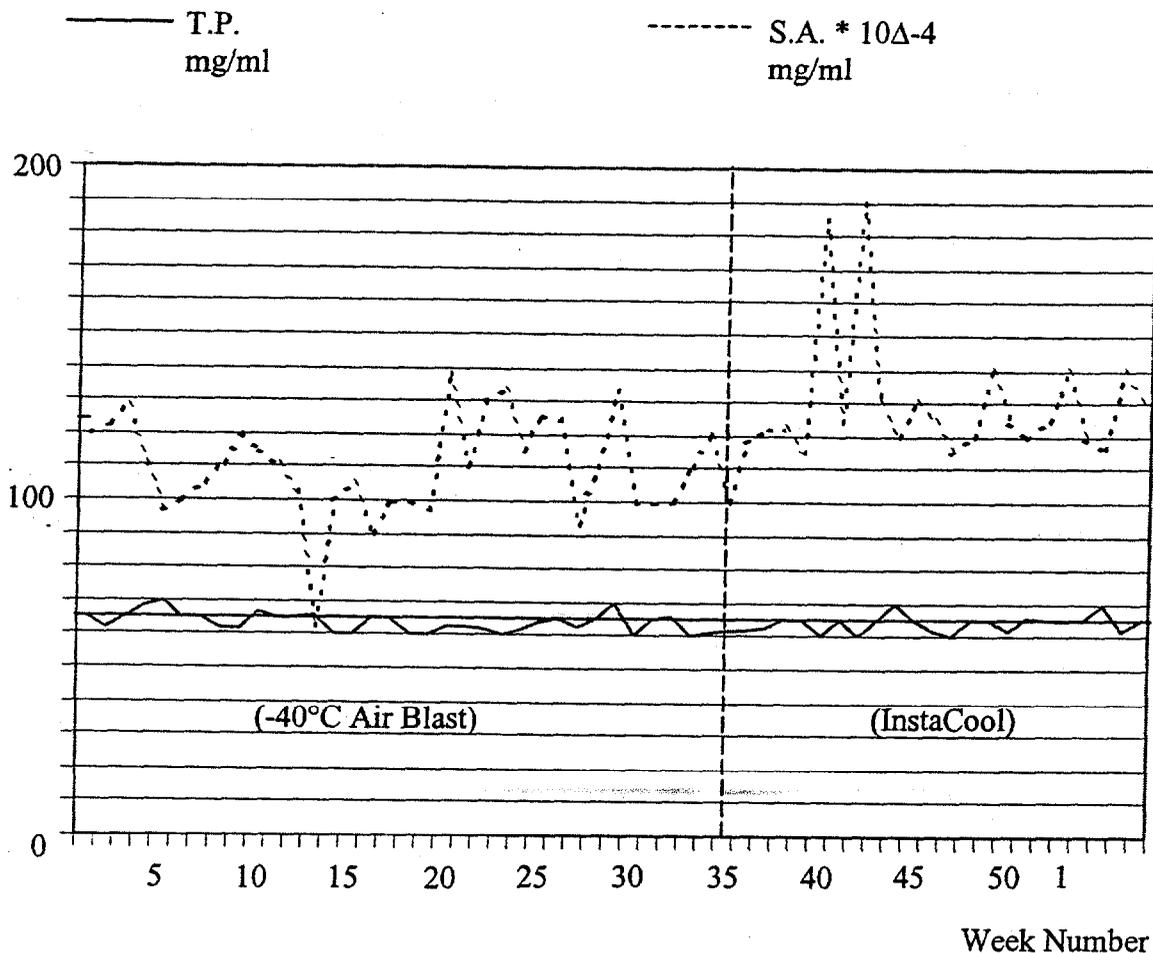


Figure 3b Weekly Concentration of Total Protein in mg/ml  
 And Specific Activity for 1990

### Discussion

#### *Factor VIII Rich Cryoprecipitate Powder*

The Factor VIII rich, lyophilized and heat treated cryoprecipitate is being supplied to local hospitals by the Red Cross Blood Bank, The Hague, and this product has been accepted by the Dutch Association of Hemophiliacs for treatment of Hemofilia A.

#### *Frozen Plasma for Fractionation*

The quantity of FVIII expressed in IU/ml increased while the plasma was frozen in the InstaCool freezer. Interestingly, the change in freezing method did not result in a change in the concentration of total protein in the plasma.

**APPENDIX C**



**Guide to the preparation,  
use and quality assurance of  
blood components**

5th edition



Council of Europe Publishing  
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French edition:

*Guide pour la préparation, l'utilisation et l'assurance de qualité des composants sanguins*

ISBN 92-871-3804-4

## Foreword

Founded in 1949, the institutions and now with increasing cooperation of Europeans.

Within this context of Europe has consisted of an ethical issue relating to blood and tissues.

With regard to blood transfusion in the 1950's. From the outset, the promotion of voluntary blood and blood products.

The first result of this was the Exchange of Therapeutic Reagents in 1958. It was followed by the reagents (European Treaty Series, No. 84) and the Council of Europe ensure good quality of blood.

Since then, the Council's ethical, social, scientific and legal recommendations are binding on the State governments proposing Recommendations include Health Authorities in the 15 which contains as to assurance of blood components.

Work on Recommendations of Experts on Quality Assurance in blood. The Committee produced a report of immediate success and Ministers adopted it as Recommendation No. R (95) 15.

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*Albania, Andorra, Austria, France, Germany, Greece, Hungary, Moldova, Netherlands, Norway, Sweden, Switzerland, "the former"*

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## 7. Freezing and thawing of plasma

### 7.1 Rationale

Freezing is a critical step in the conservation of plasma Factor VIII. During freezing, pure ice is formed and the plasma solutes are concentrated in the remaining water. When the solubility of the solutes is exceeded, each solute forms crystals but may be influenced by the used anticoagulants. Further studies on this aspect are ongoing.

The ice formation depends on the rate of heat extraction, whereas the diffusion rates of the solutes determine their displacement. At slow freezing rates, the diffusion of solutes copes with the rate of ice formation; solutes are increasingly concentrated in the middle of a plasma unit.

Since all solutes are displaced simultaneously, the Factor VIII molecules are exposed to a high concentration of salts for a prolonged time and thus inactivated. At a high freezing rate, the ice formation overtakes the solute displacement and small clusters of solidified solute are homogeneously trapped in the ice without prolonged contact between highly concentrated salts and Factor VIII.

To achieve the highest yield of Factor VIII, plasma should be frozen to  $-30^{\circ}\text{C}$  or below.

Decrease of Factor VIII during freezing occurs when the solidification of plasma takes more than one hour. This can be monitored by measuring the total protein content of a core sample of the frozen plasma; this protein concentration should be identical with the total protein content of plasma before freezing. An optimal freezing rate is obtainable when a heat extraction of 38 kcal per hour per unit of plasma is achieved, and can be monitored by the use of thermocouples.

In order to effectively incorporate these techniques into a coherent daily routine, the blood bank staff has to be familiar with the thinking behind the technique as well as its potential limitations and pitfalls.

### 7.2 Methods of freezing

When freezing plasma, the rate of cooling must be as rapid as possible and ideally should bring the core temperature down to  $-30^{\circ}\text{C}$  or below within 60 minutes. If this is not possible, the minimum acceptable rate of freezing should bring the core temperature down to  $-30^{\circ}\text{C}$  within 4 hours.

Experience has shown that it sometimes takes several hours in an environmental temperature of  $-30^{\circ}\text{C}$  and heat transfer by air. The time must be reduced to less than one hour and if possible, less than half an hour, for example by the following means:

- plasma should be presented in a regular configuration to maximise exposure to the freezing process (e.g. bags laid flat or in formers if vertical);
- immersion in an environment at very low temperature;
- if a liquid environment is used, it should have been shown that the container cannot be penetrated by the solvent.

### 7.3 *Methods of thawing*

Frozen units should be handled with care since the bags may be brittle. The integrity of the pack should be verified before and after thawing to exclude any defects and leakages. Containers which leak must be discarded. The product should be thawed immediately after removal from storage in a properly controlled environment at  $37^{\circ}\text{C}$  according to a validated procedure. After thawing of frozen plasma, the content should be inspected to ensure that no insoluble cryoprecipitate is visible on completion of the thaw procedure. The product should not be used if insoluble material is present. In order to preserve labile factors, plasma should be used immediately following thawing and never beyond 6 hours. It should not be refrozen.

Thawing of the plasma is an inevitable part of some of the current viral inactivation processes. The final component, having been refrozen after treatment, should be used immediately following thawing for clinical use and not further refrozen.

## 8. **The use of an open system and devices for sterile connections**

It is suggested that any new development in component preparation involving an open system should be subjected to intensive testing during the developmental phase for maintenance of sterility.

Other than this, routine sterility testing as an ongoing quality control measure is thought to be of limited value. Blood components prepared by an open system should be used as quickly as possible.

Components prepared in systems using fully validated sterile connecting devices may be stored as if prepared in a closed system.

## Chapter 14: Fresh frozen plasma

### Definition

A component for transfusion prepared either from whole blood or from plasma collected by apheresis, frozen within a period and to a temperature that will adequately maintain the labile coagulation factors in a functional state.

### Properties

This preparation contains normal plasma levels of stable coagulation factors, albumin and immunoglobulins. It contains a minimum of 70% of the original Factor VIIIc and at least similar quantities of the other labile coagulation factors and naturally occurring inhibitors.

If fresh frozen plasma is to be used as source material for the preparation of fractionated products, reference should be made to the European Pharmacopoeia monograph on plasma for fractionation. Fresh frozen plasma should not contain irregular antibodies of clinical significance.

### Methods of preparation

#### *a. Whole blood*

Plasma is separated from whole blood collected using a blood bag with integral transfer packs, employing hard spin centrifugation, preferably within 6 hours and not more than 18 hours after collection. Plasma may also be separated from platelet rich plasma. Freezing should take place in a system that will allow complete freezing within one hour to a temperature below  $-30^{\circ}\text{C}$ . If plasma is to be prepared from a single pack whole blood donation, adequate sterility precautions must be adopted.

Where Health Authorities allow, plasma may also be separated from whole blood, which immediately after donation has been rapidly cooled by special device between  $+20^{\circ}\text{C}$  and  $+22^{\circ}\text{C}$  and held at that temperature for up to 24 hours, depending on the nature of the anticoagulant used.

#### *b. By apheresis*

Plasma may be collected by manual or automated apheresis. The freezing process should commence within six hours of completion of the procedure in a system which allows complete freezing within one hour to a temperature below  $-30^{\circ}\text{C}$ .

c. *Viral inactivation*

Viral inactivation and/or quarantine of this component is a requirement in some countries.

**Labelling**

The labelling should comply with the relevant national legislation and international agreements. The label in the container states:

- name of component;
- nature of component, i.e. from a whole blood donation or by apheresis. The volume should be stated and the composition of the anticoagulant used;
- donation number;
- the ABO group;
- the Rh (D) group specifying "Rh (D)-positive" if D positive or "Rh (D)-negative" if D negative;
- the producer's name and address (clear text or code);
- whether quarantined or virus inactivated.

The following additional information may be placed on the pack or alternatively on a container surrounding the pack (i.e plasma container carton):

- date of preparation;
- storage temperature and expiry date;
- instructions for restorage, thawing and administration including the use of a 170-200  $\mu\text{m}$  filter.

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**Storage and stability**

The stability on storage is dependent on the storage temperature available. Optimal storage temperature is at -30 °C or lower and the following are the permitted storage times and temperatures:

- 24 months at below - 30 ° C;
- 6 months at -25 °C to -30 °C;
- 3 months at -18 °C to -25 °C.

**Quality assurance**

**Table 14(a): Quality control**

Parameter to be checked	Quality requirement (specification)	Frequency of control	Control executed by
ABO, Rh (D)*	Grouping	all units	grouping lab
HIV-Abs*	Negative by approved screening test	all units	screening lab
HBsAg*	Negative by approved screening test	all units	screening lab
ALT* (when required)	Not elevated (as specified by national authorities)	all units	screening lab
HCV-Ab*	Negative by approved screening test	all units	screening lab
HBC-Abs* (when required)	Negative by approved screening test	all units	screening lab
Syphilis* (when required)	Negative by screening test	all units	screening lab
HTLV-Abs* (when required)	Negative by screening test	all units	screening lab

\* Unless performed on whole blood used as the source.

Table 14(b): Quality control

Parameter to be checked	Quality requirement (specification)	Frequency of control	Control executed by
Volume	stated volume $\pm$ 10%	all units	processing lab
Factor VIIIc	> 0.7 I.U./ml	every two months. a) pool of 6 units of mixed blood groups during first month of storage. b) pool of 6 units of mixed blood groups during last month of storage.	QC lab
Residual cells*	red cells: $< 6.0 \times 10^9/l$ leucocytes: $< 0.1 \times 10^9/l$ platelets: $< 50 \times 10^9/l$	1% of all units with a minimum of 4 units/month	QC lab
Leakage	no leakage at any part of container e.g. visual inspection after pressure in a plasma extractor, before freezing and after thawing	all units	processing and receiving laboratory
Visual changes	no abnormal colour or visible clots	all units	"

\* Cell counting performed before freezing. Low levels can be achieved if specific cellular depletions are included in the protocol.

**Note:** If fresh frozen plasma is regularly used as a source of a component other than Factor VIIIc, appropriate estimations should be performed on representative sample units to ensure continuing efficiency of the preparative procedure.

### Transport

Storage temperature should be maintained during transport. The receiving hospital should ensure that packs have remained frozen during transit. Unless for immediate use, the packs should be transferred at once to storage at the recommended temperature.

**Indications for use**

Fresh frozen plasma may be used in coagulation disorders, particularly in those clinical situations in which a multiple coagulation deficit exists and only where no suitable virus inactivated stable product is available.

Fresh frozen plasma may be used in the treatment of thrombotic thrombocytopenic purpura (TTP).

Its major use is as source material for plasma fractionation.

**Precautions in use**

Fresh frozen plasma should not be used simply to correct a volume deficit in the absence of a coagulation deficit nor as a source of immunoglobulins.

Fresh frozen plasma should not be used where a suitable virus inactivated alternative product is available.

Fresh frozen plasma should not be used in a patient with intolerance to plasma proteins.

ABO blood group-compatible plasma should be used.

The product should be used immediately following thawing. It should not be refrozen.

Before use the product should be thawed in a properly controlled environment and the integrity of the pack should be verified to exclude any defects or leakages. No insoluble cryoprecipitate should be visible on completion of the thaw procedure.

**Side-effects**

- Citrate toxicity can occur when large volumes are rapidly transfused;
- Non-haemolytic transfusion reactions (mainly chills, fever and urticaria);
- Viral transmission (hepatitis, HIV, etc.) is possible despite careful donor selection and screening procedures;
- Sepsis due to inadvertent bacterial contamination;
- Transfusion related acute lung injury.

Control executed by
processing lab
QC lab
QC lab
processing and receiving laboratory
"