

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

FACTOR VIII Study

'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).

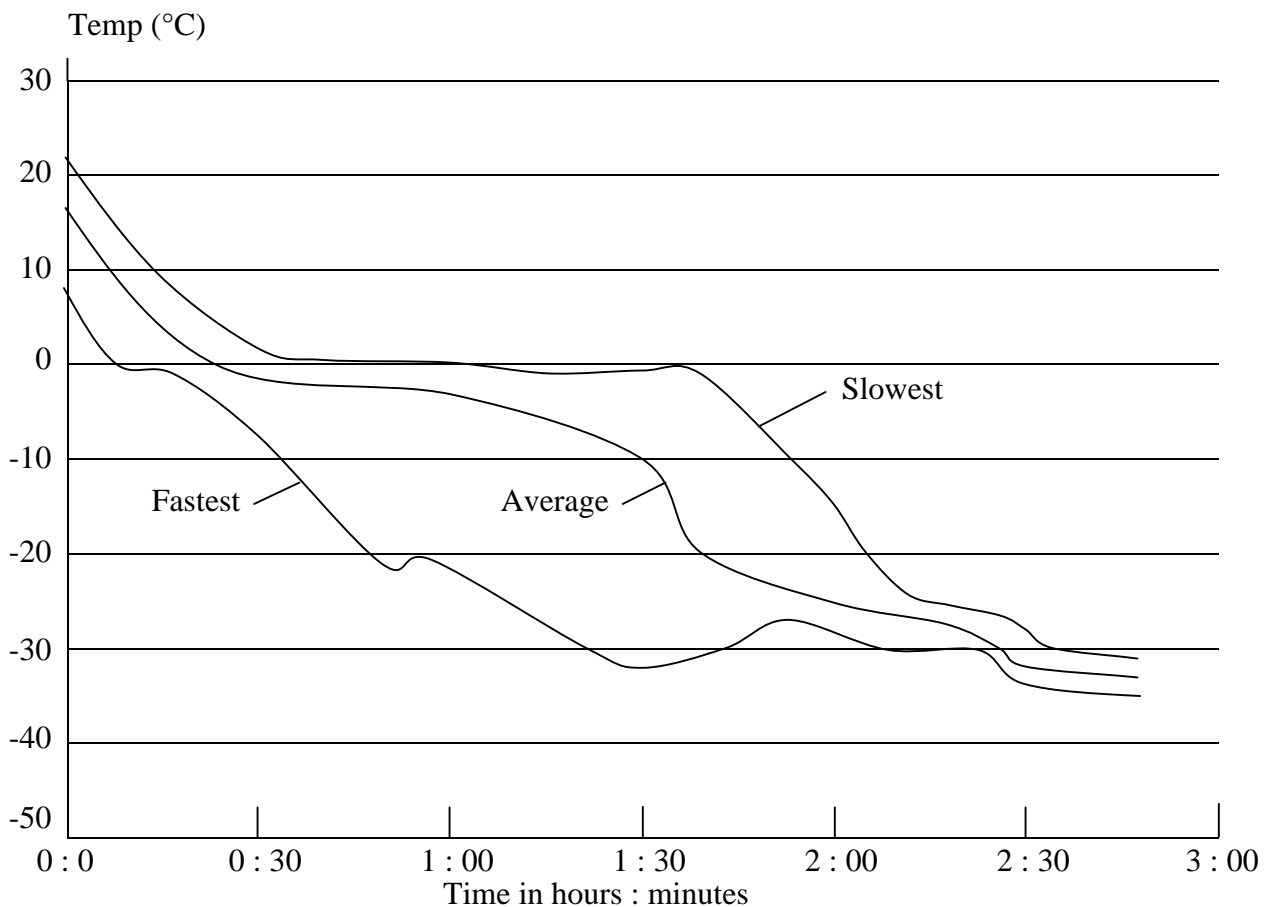


Figure 1 (Freezing rate of plasma in  $-40^{\circ}\text{C}$  Air Blast Freezer, Before September 1, 1990)

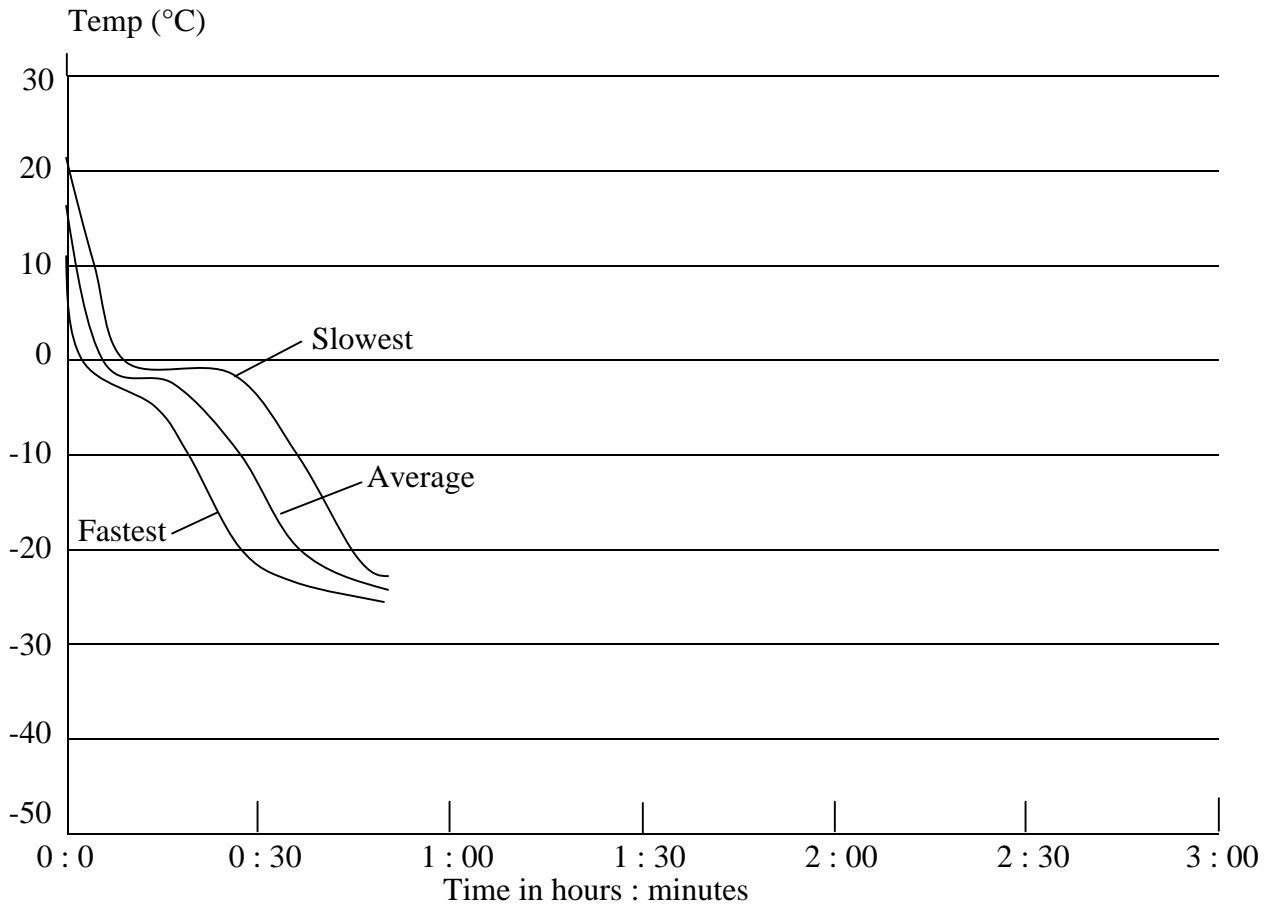


Figure 2 (Freezing rate of plasma in InstaCool Freezer, used after September 1, 1990)

*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: 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

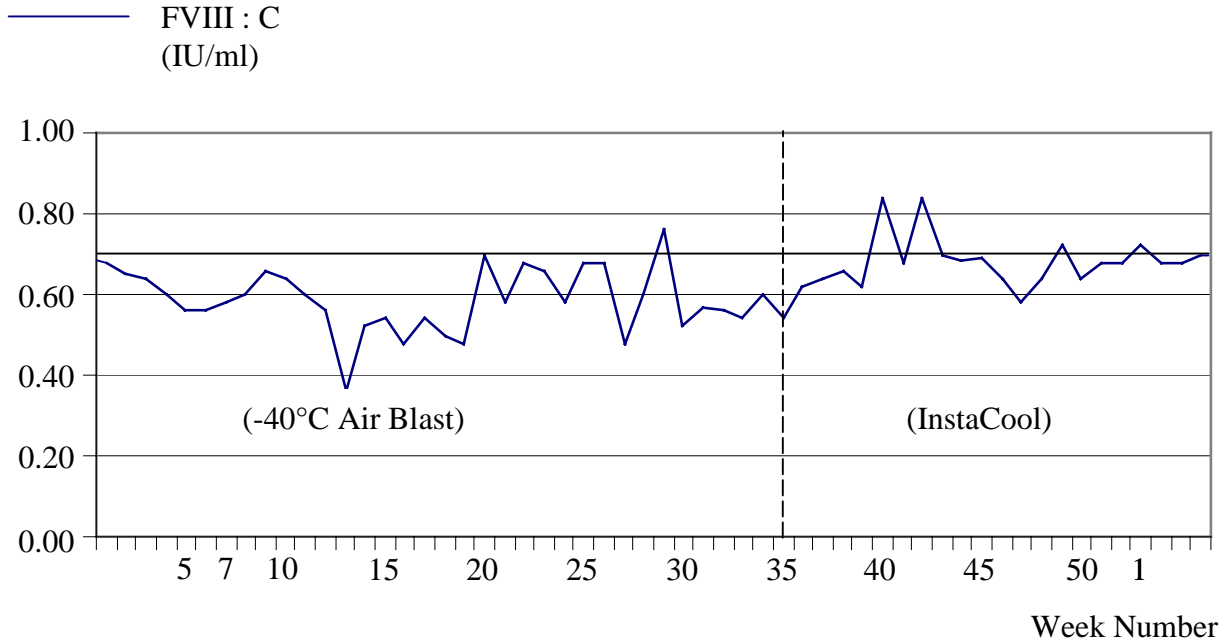


Figure 3a Weekly FVIII Concentration in IU/ml for 1990

### Deliveries FP to CLB

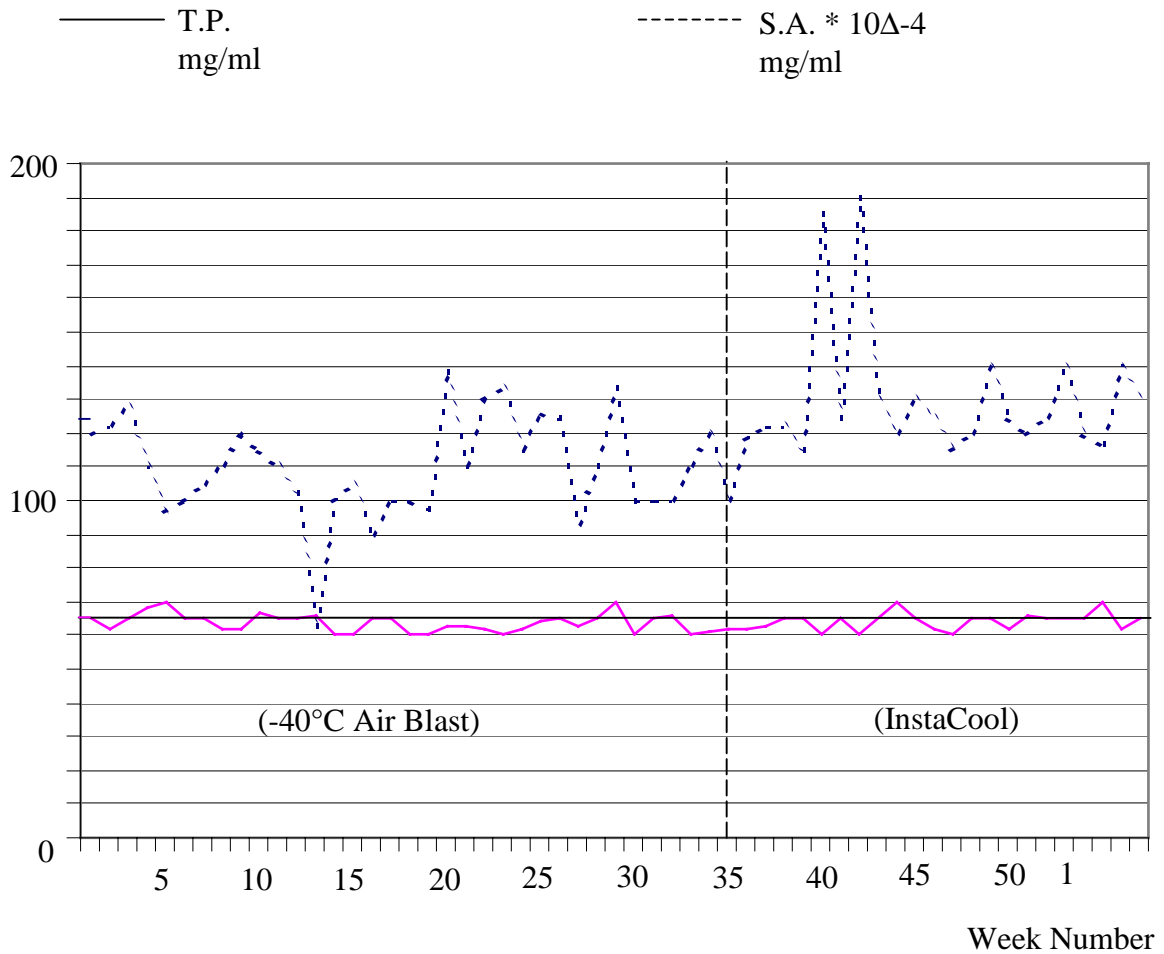


Figure 3b Weekly Concentration of Total Protein in mg/ml  
And Specific Activity for 1990

### 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.

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

Table 1

CLB Measurements of plasma frozen in  $-40^{\circ}\text{C}$  air blast freezer  
From January 1, 1990 to August 31, 1990

<u>Delivery Date</u>	<u>FVIII I.U./ml</u>	<u>T.P. mg/ml</u>	<u>S.A. IU/mg TP (<math>10^4</math>)</u>
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 $\pm$ SD	0.58 $\pm$ 0.08	62.8 $\pm$ 2.9	110 $\pm$ 12

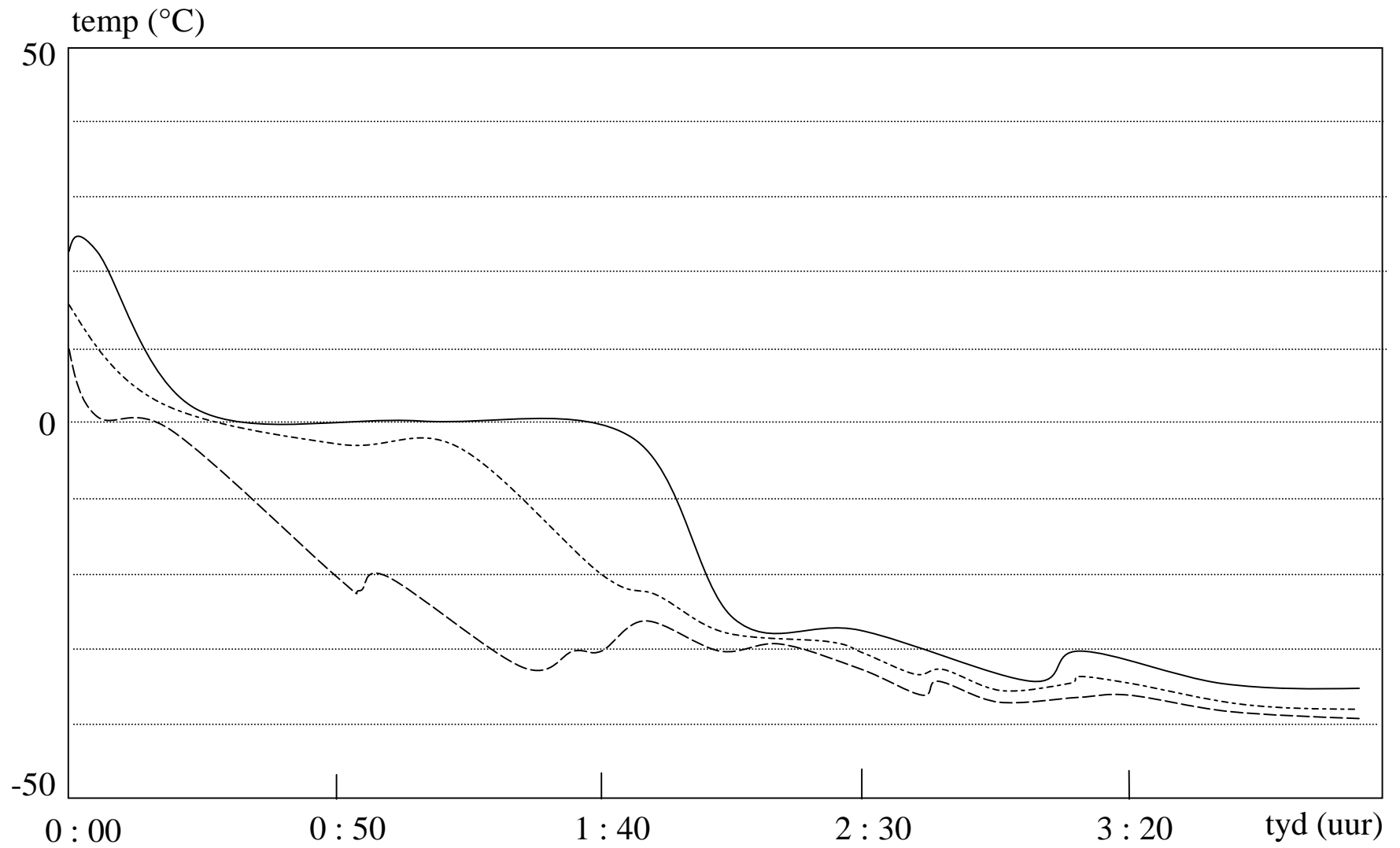
Table 2

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

<u>Delivery Date</u>	<u>FVIII I.U./ml</u>	<u>T.P. mg/ml</u>	<u>S.A. IU/mg TP (10<sup>4</sup>)</u>
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 INSTACOOOL (24-10-90)  
(2 compartimenten gedurende 1 uur)

