SNB.007.4052

MEMO

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From	To
GRO-C	P. Kotin
Our Reference / Your Reference	5.1 19.84

I attach a copy of our VIII dry-heating results to date, having removed sections which are of internal interest only — i.e. those re private coagulation assays received. Please let me know if I can send anything of particular value.

The SDS-PAGE patterns of wet-heated VIII (and presumably dry-heated human VIII) are astonishingly similar to dry-heated VIII.

PROTEIN FRACTIONATION CENTRE
Received 10/1/84
File No FVIII 2.167

Refer to	Action taken
PJV	
Bob Berry	
Alex Bruce	

GRO-C

MSERS PD8

PRSE0003743_0001

2. Strategy

In the absence of new laboratory or clinical evidence of the likely effect of heat on NANBH, heat conventional 8CRV concentrate for the maximum time at the maximum temperature compatible with a <10% apparent loss of factor VIII:C activity, and the appearance of no other undesirable characteristics.

3. Methods

Vials of freeze-dried factor VIII (8CRV/7) from various batches, sealed under nitrogen or vacuum, were heated, submerged to their necks in a water bath for increasing periods at a series of temperatures. Either limited tests for factor VIII:C and solubility, or full pharmacopoeial QC, were carried out on the reconstituted vials.

4. Results

4.1 It was confirmed that a Pt 100 probe in evacuated vials reached the final temperature within about 1h of immersion, but it is difficult to be precise about the temperature of the dry porous plug of protein. The technique is too difficult to use as a control for routine pasteurising.

4.2 Table 1 and Fig. 1 show the promising preservation of factor VIII:C and solubility of one batch (8CRV 1445). Data reproduced from my memo of July 1983.

Table 1 (from experiments 8H9, etc., July 1983)

Hours	Percent recovery of factor VIII:C after heating				
	60°	70°	75°	80°	90°
0	100	100	100	100	100
2				98	80
4				96	82
8					
10	96	94	99	84	+*62
24	99	99, 92	88	*73	+*72
48		100			Insoluble
72		*88			

*darkening of dry plug
+some insolubility

4.3 In experiment 8H13 we heated a variety of HL and 8CRV batches for 24h only at 60°, 70° and 80°. All batches except the original 8CRV 1445 lost more factor VIII:C activity at 70° than at 60° (8-13%, mean 11%, compared with 0-9%, mean 2%) and took longer to re-dissolve. All samples heated at 80° lost >25% of factor VIII:C and became much less soluble.

4.4 More carefully controlled comparisons (8H17, 8H 23) were made of seven batches, heated at 60° for (a) 24h and (b) 72h, chosen as (a) the most conservative conditions we could consider for virus inactivation and (b) the most severe conditions likely to cause insignificant damage to the product. Results are given in Table 2.

Table 2

	Batch	Condition	Re-solution times min.			F.VIII:C recovery %	
			Control	24h	72h	24h	72h
A	8CRV RD 1	Vacuum	5	5	5	87	95+
B	8CRV RD 1	Nitrogen	5	5	5	97	85
C	HLA 2984	Vacuum	11	15	14	101	90+
D	HLA 3082	Vacuum	15	15	15	84(94)*	83(92)*
E	HLB 2881	Vacuum	6	7	8	106	94
F	HL 2833	Vacuum	8	14	17	110	98
G	8CRV 1445	Vacuum	5	-	5	-	97+

*Bracketed values are corrections on evidence that the control vial was re-dissolved in a reduced volume.

Determinations of both factor VIII:C and solubility time have a high coefficient of variation but data(+) are more secure through replication. In A the lower factor VIII:C recovery after 24h than after 72h seems improbable. There is a suggestion that heating under nitrogen is more destructive of factor VIII:C than heating under vacuum but we have insufficient paired batches to prove the point yet. HL 2833 is an isolated example of a batch significantly extending solubility time after heating at 60°, but most of the damage has been done already by 24h; we could not therefore contemplate committing every batch of 8CRV and HL to dry heating until further work could be done on sources of batch to batch variation in this respect.

4.5 Full pharmacopoeial QC of three paired, heated and unheated, batches of 8CRV. The batches used were
(A) 8CRV RD 1. A pool of recent 8CRV rejects, redissolved and re-dried, sealed under vacuum.
(C) HLA 2984. Pyrogen failure, sealed under vacuum.
(G) 8CRV 1445. PFL "reference batch", sealed under vacuum.
Factor VIII:C and solubility results are included in Table 2.

Table 3 summarises their performance in all tests (except sterility) routinely carried out on finished batches, with and without dry heating at 60° for 24h.

Table 3

	8CRV RD 1		HLA 2984		8CRV 1445	
	Unheated	72h 60°	Unheated	72h 60°	Unheated	72h 60°
Pyrogenicity (°/3)	0.5	0.25	3.05	3.35	0.85	1.05
Ab. Toxicity	Pass	Pass	Pass	Pass	Pass	Pass
HbsAg (cpm)	71	81	88	76	70	45
Sol. 20° (min)	5	5	11	14	5	5
Stability 20° (h)	>3	>3	>3	>3	>3	>3
pH	7.03	6.91	7.02	6.90	7.27	7.19
F.VIII:C (iu/ml)	15.5	14.7	15.1	13.6	14.2	13.8
Sp. Act. (iu/mg)	0.44	0.41	0.32	0.30	0.41	0.41
Total protein (g/l)	35.0	35.5	46.7	44.9	34.4	33.3
Clottable protein (%)	51	55	58	58	46	47
F.VIIIR:Ag (u/ml)	47.5	45.3	66.1	82.4	48.0	45.8
PKA (% Ref. 2)	22.6	37.4	21.1	36.5	14.8	21.6
Anti-A (u/ml)	115	118	379	346	130	123

Tests for inorganic components are not quoted.

The batches covered a good range of pyrogen test levels; none was seriously affected by heating.

pH fell in each case by about 0.1 pH units on heating, which is surprising in view of the fact that the vials were sealed. We usually see a rise in pH on drying and none of the alkaline buffer components is known to be unstable at 60°, even in solution.

The only other parameter to change significantly was PKA, but the rise was modest and would not threaten to put the product into a risk category on present evidence.

SDS/PAG electrophoresis of reduced and unreduced samples was carried out by both L. Winkelman at PFL and R. Baker at BPL. Heated and unheated samples were indistinguishable after reduction. Much of the protein in unreduced samples did not enter the running gel and in each case the bands of highest MW present in the unheated samples were accentuated in the heated sample. These results suggest increased disulphide bonding on heating. Supernatants from non-crosslinked clots were identical. RB saw four bands from 60-105,000 MW in cross-linked clot supernatants from the unheated sample, not seen in the heated sample or a control FC fibrinogen; this might suggest some aggregation of non-fibrinogen proteins on heating.