HELENA

Virus Inactivation and Removal in Coagulation Factors Concentrates
BPL/PFC Discussions

Minute of a Meeting at PFC, 8th March 1995

Present: **For PFC:** P. Foster, B. Griffin, H. Hart, A. MacLeod, R. McIntosh (Chair), J. Rudge (part-time)

For BPL: D. Evans, P. Roberts, L. Winkelman (minute)

1. **Agenda**

As in Appendix 1.

2. **Minutes of the Meeting on 16th November 1994**

Agreed.

3. **Matters Arising from the Minutes of 16/11/94**

3.1 Item 2.2, 16/11/94

Final, agreed minute of 30/9/94 has been circulated. RM to confirm that all PFC participants now have the final version.

ACTION: R. McIntosh

3.2 Item 2.3.2, 16/11/94

Chromogenic assays are now in use for HT studies at PFC (and see below for results of assay comparability study).

3.3 Items 2.3.4, 2.5.1, 16/11/94

Resistivity measurements at PFC, DSC measurements at PAFRA and simple thermometry studies at BPL all show a eutectic transition for the Replenate formulation at -23 +/- 1°C. DSC showed a glass transition temperature for 8SM formulation at -32°C. MacKenzie has found a glass transition temperature for Hemofil M (Replenate) formulation at -35°C.

[N.B. The difference between Replenate and 8SM as formulated for FD is in NaCl content: Replenate is 0.15M NaCl; 8SM is 0.6M NaCl. After reconstitution all formulants are at different concentrations because 8SM is reconstituted in 5/2 x fill volume.]

Freezing characteristics of H8 and Replenate are very different. Resistivity curves for H8 do not show the two phase changes that are seen for Replenate.

There may be value in doing further resistivity measurements to sort out the effects of different components.

ACTION: B. Griffin to consider

Data and reports from these studies are collated in Appendix 2.

3.4 Items 2.4.1, 8.4, 16/11/94

PAF has started to review FD/HT data for the four PCC and FIX concentrates in order to determine whether further studies (e.g. assay comparisons etc.) are warranted.

ACTION: P. Feldman

3.5 Item 2.5.2, 16/11/94

BG will have osmolality measurements made on Replenate (and Replenine, if samples are provided). Osmolality will also be measured at BPL.

ACTION: B. Griffin, P. Feldman, G. Sims

3.6 Items 2.7.3, 2.7.4, 16/11/94

See section 4, below.

3.7 Item 6.1, 16/11/94

Samples have been exchanged. Assays are in progress. Data will be compiled and analysed when all results are in.

ACTION: H. Hart, P. Roberts

3.8 Item 6.2, 16/11/95

There is some documented pedigree for the "Lister Institute Strain" of sindbis used at BPL and PFC. Although its original source is not known, there is no reason to believe the virus is not sindbis. Confirmation should be sought from Porton Down.

ACTION: P. Roberts

3.9 Items 6.3, 6.6, 16/11/94

Canine and bovine parvovirus have been exchanged as part of a study to assess effects of terminal heat treatment on the two viruses in Replenate and Liberate. Study protocol attached in Appendix 3.

3.10 Item 6.4, 16/11/94

There are no updates on 8Y virus transmission since publication by Williams et al.

Two recent (end 1994) possible transmissions of B19 by 8Y (in Aberdeen and RFH, London) are being fully investigated.

3.11 Item 6.5, 16/11/94

PR has received EMC virus, but an assay system has not yet been established.

CPV and SV40 virus and assay systems are available from HH if BPL wish to make use of either.

3.12 Item 6.6, 16/11/94

See 3.9 above.

3.13 Items 7.3, 7.4, 7.5, 16/11/94

The BPL SOP for KF measurement has been sent to PFC.

"Very dry" vials, for use as blanks in KF assay, have been prepared at BPL and some sent to PFC.

RWC calibration standards have been exchanged.

A protocol has been agreed (Appendix 4) to compare KF assay at both sites, to tie KF to QB measurements, to identify the true limit of sensitivity, to establish assay cvs and to investigate the "window" below KF sensitivity where QB detects water.

There was a discussion covering the use and usefulness of "Blank" (as dry as possible) and "Control" (empty vials dried in parallel with the product) vials in the KF system. The existing protocol will be amended by DRE if necessary, to clarify any misunderstanding. Results from this exercise should be available for a May meeting.

ACTION: D. Evans, J. Rudge

3.14 Item 8.1, 16/11/94

FVIII process outlines and FVIII recoveries have been exchanged. FVIII recovery in the lyophilised product is similar for the two products.

FIX process outlines and FIX recoveries have not yet been exchanged.

ACTION: A. MacLeod, P. Feldman

3.15 Item 8.2, 16/11/94

The exchange of heated/unheated FVIII products to assess assay variability has been completed (see Appendix 5 for protocol and data).

Both labs assayed vs the 10th BWS (6.3iu/ml). Mean potencies for unheated vials are shown in Table 1.

Table 1: FVIII Potency in Unheated Product

	FVIII iu/ml	
	at BPL	at PFC
Replenate (4231)	100.4	106.9
Liberate (4088)	27.7	29.0

Heating losses were similar at both labs and slightly greater for H8 than for Replenate (Table 2). The close agreement of BPL and PFC chromogenic assays was encouraging. Further sample exchange is not required.

Table 2: Mean FVIII Recovery after Terminal Heat Treatment

	% FVIII Recovery	
	at BPL	at PFC
Replenate:		
80°, 24h	93	101
80°, 72h	95	98
90°, 10h	96	99
100°, 24h	84	86
110°, 4h	87	84
120°, 2h	75	79
H8		
80°, 24h	100	92
80°, 72h	86	82
90°, 10h	108	89
100°, 24h	62	58

Clotting assays (1st and 2st) were also performed on these samples at BPL. These showed a similar loss of FVIII across heat treatment, though 1st assays always showed slightly greater loss across HT than did 2st clotting assays.

BG reported that recent batches of Liberate may be slightly more stable than some earlier batches. As more batches are assessed at each site, data should be collated to expand our understanding of batch-to-batch and product-to-product differences.

ACTION: L. Winkelman, B. Griffin

3.16 Item 8.3, 16/11/94

Experiments to reformulate Liberate in the Replenate formulation (see Appendix 6 for protocol and detailed results) have shown that Liberate is activated during or after buffer exchange into Replenate formulation (tested with and without 4mM $CaCl_2$). More work is needed to unravel the cause(s) of this activation. Investigations will continue to see which component(s) (or non-component) of the Replenate formulation buffer is causing activation. The objective is to re-formulate Liberate into a formulation with freezing characteristics that resemble those of the Replenate formulation.

ACTION: B. Griffin

3.17 Item 8.4, 16/11/94

See 3.4 above.

3.18 Item 8.6, 16/11/94

LW had a brief review of neo-antigen investigations with I. MacGregor and D. Pepper at NSL after the PFC/BPL meeting.

NSL have used the neonatal mouse model for both H8 and H9 and seen no indication of neo-antigen recognition in heated product. Antibodies have been raised in rabbits vs UH and HT samples of each product. Cross-over competitive ELISAs have shown no indication of neo-antigen formation. MAb binding studies with the panel of ESH MAbs have shown some indications of loss of antigenicity post-HT but no indication of increased antigenicity.

BPL are awaiting results of tests of UH and HT Replenate and Replenine in a sheep model.

MAb binding studies at BPL have identified a commercially available MAb to which pasteurised Replenate (unprotected and heated in solution until 50% of clotting activity gone) shows markedly increased binding. Terminally heated Replenate (100°, 24h; 80°, 72h) showed no increased binding to this MAb.

Further discussions are in hand to identify how we can help each other in neo-antigen and molecular characterisation.

ACTION: L. Winkelman

4. Water Activity and the Validation of Terminal (Dry) Heat Treatment

4.1 MCA Interactions

DRE reviewed the history of BPL/MCA interactions concerning "water activity" (WA). When the 8Y licence was reviewed by J. Sloggem (in 1991), he asked that relative humidity (RH) in the vial be measured and related to RWC as measured by KF. BPL did such measurements and produced a 3 point graph which showed an apparent correlation of RH and RWC. The 8Y licence was granted. At that time, BPL consulted F. Franks (PAFRA) about his views on the relevance or not of WA to terminal heat treatment. His view was that WA was not relevant. As the PL was granted, we did not pursue him for a detailed argument.

A recent application for a CTX for terminally heated FXI was turned down for lack of extensive validation of terminal heat treatment (see Appendix 7 for MCA letter). The wording of the rejection expressed a "preference" for water activity measurements but may have left the door open for alternative validation. BPL recognise that there were no sensible validation data presented with the FXI CTX (e.g. FD mapping, batch homogeneity, VI at extremes of RWC). Thus we do not know whether a closely argued case based on the above might have been accepted.

The MCA accepted the VI status of terminally heated fibrinogen in the PFC fibrin sealant CTX (199?).

As we met, MCA visitors were at PFC talking to B. Cuthbertson and PFC Regulatory Affairs staff. Those discussions were germane to our concerns.

4.2 RWC and Water Activity

We discussed our various understandings of water activity and agreed to exchange what relevant published references we have each unearthed (see Appendix 8 for listing).

ACTION: D. Evans, P. Foster

There was not a BPL/PFC consensus on the relevance of WA to terminal heat treatment and alternative terms such as "available water" and "water mobility" were mooted.

We were all agreed that in a sealed system, virus inactivation will be some function of the measured RWC in the vial.

P. Foster tabled some items for discussion (Appendix 9).

4.3 Measuring RWC in Vial

We agreed that the following parameters would affect the RWC in the vial:

1. Composition of solids phase.
2. Structure of the solids phase.

3. Quality and state of closure.
4. Quality of seal.
5. Temperature and time of heating.
6. Composition of gas phase (e.g. presence of moisture).

Other parameters were tabled, but there was not unanimous agreement that these would affect the RWC in the vial:

1. Pressure in the vial (in particular whether or not it was sealed under vacuum).

4.4 Water Function in Vial

We tried to identify parameters that might affect the relationship of total RWC to that function of water which affects VI.

I think we agreed the following:

1. Composition of solids phase.
2. RWC.

4.5 Control During HT

We agreed that we need to be able both to measure and to demonstrate control of the above parameters.

4.6 Correlation of Virus-Spiking Studies with HT of Product

The potential effect of virus culture medium was discussed (would this alter the relationship between RWC and water function?). DRE believes this is the only valid reason for MCA concern regarding WA, and he has drawn up a protocol for some practical work that may address this concern (Appendix 10). PLR and P. Foster were not sure the MCA have thought of this. HH and PLR agreed to think about what might be possible by way of demonstrating that increments of culture medium did not affect RWC and/or VI. The work done on this subject for 8Y is attached (Appendix II). Spiking did not affect RWC as determined by KF or QB.

ACTION: H. Hart, P. Roberts

4.7 Validation of Terminal HT

There was a discussion of what the elements of a validation package could/should be. We agreed that we could measure and demonstrate control of:

1. Temperature of HT.
2. Time of HT.

3. Range of RWC

- Correlation of KF with QB
- Mapping of batch/FD with QB
- Distribution of RWC across batch by QB of representative sample of vials

4. Extremes of formulation, if applicable, e.g. total protein content, if variable

We agreed that we need to demonstrate VI at extremes of RWC (and show positive correlation of VI with RWC).

We agreed that we should set RWC limits on the measured vial(s) from each batch such that all vials in the batch will fall within the range that has been shown in virus studies to give adequate VI for chosen model viruses. The number of vials in which RWC is measured and the RWC limits fixed would be based on validation data.

We agreed that, in addition to VI at the extremes of RWC, stability studies would be required (particularly important at the upper extreme of RWC).

4.8 Joint Approach to MCA

Both BPL (FXI CTX, 8Y relicence) and PFC (Fibrin sealant PL) will need to submit terminal heat validation data to the MCA this year.

We agreed that we would try to prepare a joint rationale for such a validation package and that we should discuss this with the MCA soon. RM agreed to draft a protocol. We will aim to get this agreed for a May meeting.

ACTION: R. McIntosh

5. Next Meeting

No date fixed. Suggested in w/c 15th May or 22nd May at BPL.