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Scottish National Blood Transfusion Service
South East Regional Centre & Department of Transfusion Medicine

Royal Infirmary
Edinburgh
EH3 9HB
Telephone 031-229 2585
Telex 72163
FAX No. 031 229 1069

CVP/JD

17th February 1989

PROTEIN FRACTIONATION CENTRE	
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Refer to	Action taken
C Rooney	✓
B. Cuthbertson	✓
R. Stewart (HQ)	copy circulated ✓

Dr C Rooney
Product Registration
Protein Fractionation Centre
21 Ellen's Glen Road
EDINBURGH
EH17 7QT

Dear Cathy

DRAFT LABORATORY PROTOCOL FOR FACTOR VIII PHARMACOKINETICS
(S8 vs Z8)

The quick answer to your question is that a 10 ml sample at each time-point is enough for the proposed assays. I would suggest use of 1 ml 3.8% trisodium citrate ± HEPES buffer per 9 ml blood (preferably by separate venepuncture) and the following plasma aliquots:

1 ml for 1 stage assay VIII:C
1 ml for 2 stage assay VIII:C
0.5 ml for VIII:Ag assay
0.5 ml for vWf:Ag assay
3 x 1 ml library samples

However, I have a number of other comments:

a) I presume Drug Development is a commercial enterprise - where are they going to recruit 8 haemophiliacs? Are their haemophilia consultants aware of this plan and will it interfere with other trials? Is HIV-status confidential (likely) or can it be determined, will patient identity be apparent? Will viral inactivation data and patient insurance be available? Would the traditional Haemophilia Centre route be easier?

b) It would be preferable to obtain an additional baseline (pre-infusion) sample and, if possible, a 48 hour post-infusion sample.

c) Acetone/cardice has an even lower flash-point than ethanol/cardice. Despite HSE the latter would be preferable but for 1 ml aliquots air cooling in -40°C should be adequate if done promptly.

d) Which laboratory will do the assays?

e) I am unaware of anyone routinely performing a VIII:Ag assay in SNBTS. We have an ELISA that V Hornsey developed using monoclonal antibodies but she is now working on a different project and there is no S.O.P. available at present. I have recently made a fresh batch of reagent and susan Hardie knows this is available.

f) As there are separate standards for plasma and concentrate VIII:C there are different approaches to which standard to use in this kind of study which involves both concentrate (dose) and plasma (from patient). Our, and others, usual approach is to use a plasma standard and predilute the concentrate in deficient plasma. This needs to be defined, or possibly both standards should be used (the word at the end of validation section should be homogeneity).

g) Assays should be performed on the actual vial(s) of concentrate administered to avoid errors in recovery estimates arising from handling at or after reconstitution. Volume infused will presumably be recorded.

h) I presume there is a separate clinical protocol covering e.g. blood pressure, pulse and temperature measurements and assessment of plasma volume.

i) I have copies of programmes on disc allowing analysis of data by AUC methods, possibly a useful addition to the normal logarithmic analysis (IBM-PC: sample printout attached - see Longo et al Thr Res 42 471-476, 1986).

j) I presume you have access to data from our previous studies in Edinburgh - the latest will be the infusions performed in Cardiff with concentrates prepared with and without calcium, and the four patient study in Edinburgh on 12-8 summarised in Dr Boulton's report of 24.6.87.

Good luck.

Chris Prowse

C PROWSE

Enc.