

IN CONFIDENCE

MINUTES OF FACTOR VIII STUDY GROUP MEETING HELD IN
THE HEADQUARTERS UNIT, ELLEN'S GLEN ROAD ON THURSDAY,
3RD JUNE, 1982

Present:

Dr J D Cash (in the Chair)
Dr C V Prowse (Secretary)
Dr F E Boulton
Mr A Farrugia
Dr D S Pepper
Mrs B Griffin
Mr J G Watt
Dr R J Perry
Dr P Foster
Mr B Cuthbertson
Dr G S Gabra
Mrs E Porterfield (Notes)

1. INTRODUCTION AND APOLOGIES FOR ABSENCE

There were no apologies for absence.

2. MINUTES OF MEETING HELD ON 30TH MARCH, 1982

Mrs Porterfield passed out copies of a short amendment list which had been suggested by Dr Pepper. It was agreed that the amendments should, in fact, be:-

Page 4, line 7 should read "...+4°C", not "...-4°C"

Page 4, para. 2, item (ii): Omit ".....rather than in Scotland only".

Page 4, last line should read "...NANB virus", not "...B virus"

Page 5, first line should read "...NANB virus", not "...B virus".

3. MATTERS ARISING(a) Assays & Standards Working Party

Dr Prowse was asked to summarise the latest Report from the Working Party (which had been circulated) and present any more up to date data available. He began by recapping the results obtained on assay of artificial and congenital substrate against FFP and British Plasma Standard using automated and manual techniques. These had shown equally good results between the congenital and artificial substrate, but better results using automated techniques than manual. Mr Watt felt that if the assay was to have any credibility outside Scotland this disparity would have to be rectified. Dr Cash suggested that NIBSC should be asked to verify the results obtained. However, after further discussion it was agreed that these studies would be necessary, but at a later date, especially if the work was to be published but that, at the moment, this was not a priority for the Working Party.

Discussion moved on to the assays carried out using PFC automated techniques. Two machines had been used simultaneously and very satisfactory results obtained. It was agreed that the results obtained using automated techniques had been more reliable ("robust").

Sufficient material was now being produced for one 5 litre batch of substrate which would be enough to supply SNBTS needs for the next year. However, problems still exist in relation to Factor V losses in preparation. Mrs Griffin was looking at this to try to establish at what stage losses occur.

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It was agreed that:

- (i) Mrs Griffin would produce 5 litre batch and, when available, samples would be sent to Dr Gabra, Mr McQuillan (PFC) and Dr Prowse for testing;
- (ii) When results available from (i) above a "Wet Workshop" would be organised to familiarise all staff concerned with developments etc. prior to the introduction of the assay. This Workshop would probably be held some time in early autumn.

The question of stability was raised by Dr Foster. Mrs Griffin stated substrate kept at -40°C did not deteriorate but it did at $+4^{\circ}\text{C}$. Mr Watt was concerned re supplies of substrate plasma. He had arranged for PFC supply of 500 vials (1 ml) of FVIII deficient plasma per month from South America and the preliminary evidence on this was satisfactory.

It was agreed that Mrs Griffin, Dr Perry and Mr McQuillan should prepare a specification.

The SOP which Dr Prowse wished to circulate to Centres, prior to introduction of the assay, should be held meantime and circulated prior to the Wet Workshop.

(b) FVIII Yield Working Party

Dr Cash thanked Dr Foster for his comprehensive Report and asked him to speak to same. Dr Foster felt it would be easier if he identified the particular aspects for discussion by using the paragraph numbers in the Report.

Para 2.1

Dr Foster briefly summarised the work currently being carried out in an effort to identify suitable reagent(s) which could be substituted in place of citrate during production of FVIII as it was known that this was a major cause of inactivation of FVIII:C.

Solubility and clotting problems arise if citrate is omitted altogether from the process and, if reduced levels are used, solubility remains a problem. The clotting problems can be counteracted by the addition of heparin, but solubility is more difficult to resolve. The results obtained so far, using Sodium Phosphate and Glycine are not promising. Studies are currently being carried out using maltose, which have shown improved solubility, but it is not yet known if the improvement is sufficient to compensate for the absence of citrate.

Mr Watt pointed out that future licensing problems may occur using maltose, as it was now known there will be no covering EP monograph and therefore manufacturers would have to justify the inclusion of any "heavy" sugar in any parenteral product.

There was also the possibility that maltose may create problems with diabetic haemophiliacs, but since it was known that this substance was used in preference to glucose in the production of "diabetic" foods, it was not thought that this should create difficulties. Dr Boulton was asked to liaise with Dr Foster in relation to possible clinical trials to verify safety and in vivo recovery.

Para 2.2

The points outlined in the Report were summarised. Dr Foster is not satisfied with the clotting assays as a means of measuring FVIII distribution because of enhancement of assay due to presence of zinc in plasma.

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Para 2.2.1

In terms of fibrinogen removal the Working Party were able to specify FVIII losses which would be necessary to achieve a stated arbitrary figure of fibrinogen content.

Para 2.2.2

One aspect causing concern at the moment is FIX/Prothrombin complex. It was essential to remove this to alleviate long term storage problems. This is achieved by $Al(OH)_3$ adsorption.

Dr Cash asked if a FIX antigen assay was required and Dr Pepper intimated that he was pursuing this at the moment.

Para 2.2.3

Work was currently in progress on the removal of zinc from final FVIII product and advice was sought on the levels of zinc which could be tolerated by humans. Dr Cash will write to Dr Peter Brunt on this topic and Dr Boulton will contact Pharmacy to ascertain levels of zinc in diabetic protamine zinc injections. When this information is available it should give some indication of zinc toleration.

Para 2.2.5

Work on the removal of hepatitis virus being carried out in collaboration with Dr Milan Bier at his Phoenix Centre.

Para 2.2.6

Future projects include critical study of the clotting assay and zinc precipitate.

Para 2.3

Mr Farrugia was asked to speak to this section of the Report as he had carried out various studies since the last meeting following the interest shown in Dr Foster's work on crushed plasma of varying age and different freezing rates (para 3, page 4 of minutes of meeting of 28th January, 1982). This work can be summarised as follows:-

One plasma pool was split into 6 or 8 parts, frozen as quickly as possible, the time to reach $-40^{\circ}C$ being recorded. This time ranged from 20 minutes to 4 hours. Plasma was kept at $-40^{\circ}C$ up to 1 day, then thawed. Plasma was processed by thaw-siphon method to cryoprecipitate. Over 3 runs the fast freeze, fast thaw gave an 80% yield in cryoprecipitate and the slow freeze, fast thaw 50% yield of FVIII.

When fast thaw/centrifugation^{was} carried out, both rates of freezing gave 40-50% yield. There was a higher level of VIII:C in supernatant with slow freezing/thaw-siphon than fast thaw/centrifugation and the latter fractions had a lower ionic strength. Higher fibrinogen levels were noted in supernatant fractions from fast thaw/centrifugation processing, but not to any great extent.

Again over 3 runs thaw-siphon material showed no difference in fibrinogen levels on frozen storage up to 7 days at $-15^{\circ}C$ or $-40^{\circ}C$. However, the yield of FVIII:C in cryoprecipitate drops the longer material is stored (both at

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both at -15°C and -40°C . This ranges from 80% (1 day storage) to 60% (7 days storage). Longer storage time revealed higher levels of RAg in cryoprecipitate. Assay of RAg in cryoprecipitate from plasma stored for 1 day and for 7 days revealed a level 2 to 3 times higher for 7 days.

In summary, results show that fibrinogen levels are not different for material stored at -15°C and -40°C , but in both cases FVIII:C drops, together with some evidence of fragmentation of FVIII at 7 days.

The group was extremely interested in these results and Mr Farrugia was asked if he would do 3 more runs, recording daily temperatures. He would bring the results of this latest study to the next meeting of the Group.

The Group considered the introduction of the single pack in view of the new sterile docking device. Mr Watt spoke at length on various options for packs which have been tried and tested over the years and outlined the current development at PFC of a machine to open packs (Lancet, Vol. 1, (1982) p. 909).

(d) FVIII Safety Action Group

Dr Pepper introduced the second Report from this group and outlined the current activity re procurement of animal models. It had been established that a suitable colony sited at PHLs, Colindale would be available to SNBTS for infectivity studies, if required. Experimental design would be devised and costs ascertained.

A colony of Woodchuck ~~monkeys~~ exists in the USA. These animals produce a hepatitis virus which closely resembles the human hepatitis B virus. Unfortunately, these animals cannot be made available to SNBTS.

It is now known that the marmoset animals sited at the Bush estate are not the correct species for the infectivity studies to be carried out. In the long term it is probable that SNBTS colony of Saguinus labiatus would have to be set up. There was prolonged discussion of the feasibility/necessity to set up SNBTS colony if access was available to Colindale colony. There was also a possibility of animals becoming available on the closure of facilities at the University of Aberystwyth. No definite decisions were taken, but Dr Cash and Dr Pepper will remain in close touch regarding developments, bearing in mind future work which may also require access to animal models.

(c) Quality of Plasma Working Party

Dr Gabra was invited to report on reactions to the proposed framework which had been discussed at the last meeting. A copy of the current document was handed to each member of the group.

The question of storage time between separation and freezing was raised by Dr Foster. Dr Gabra was of the opinion that this would be extremely difficult to quantify, but it was agreed that an extra question should be inserted on page 2, under "Separation": "Time from end of centrifugation to initiation of freezing". It was also agreed that in all cases where times were requested, throughout the questionnaire, Minimum, Maximum and Average times should be recorded. There was also a recommendation that Centres should clarify arrangements, if any, for ensuring that materials were processed in time order, i.e. first donation in, first out, etc. Dr Cash knew of a system operational in the Inverness Centre and thought perhaps similar systems could be adopted in other Centres.

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The other recommendations for amendment to the questionnaire were as follows:-

page 3, "Freezing" - Recommended that Maximum, Minimum and Average temperature should be recorded at line 7.

page 5, "Quality Assurance Procedures I" - Dr Pepper was of the opinion that perhaps this should be divided into 2 categories - platelet reduced and CRC. It was, however, agreed that in the meantime this item should remain ISQ.

"Temperature Monitoring" had been inserted since the last draft framework had been presented to the previous meeting. It was recommended that the method of recording temperature should be specified, e.g. daily recording, continuous recording, etc..

page 6, "Quality Assurance Procedures II" - It was not felt necessary to have a section on "Fibrinogen assay" but since questionnaire had already been sent to Centres, it was decided not to delete this item at the moment.

"Microbiological Testing" had been added since the last meeting and it was generally felt that "Method" and "Where test carried out: e.g. BTS or elsewhere" should be inserted under this heading.

A preliminary report, "Documentation of Current Practice in Scotland" was passed to each member of the Group with a request to let Dr Gabra or Dr Boulton have any comments as soon as possible.

4. DATE OF NEXT MEETING

To be fixed by Mrs Porterfield, probably in September/October 1982.