THE VALUE OF QUALITY ASSURANCE OF FACTOR VIIIIC ASSAYS IN THE PRODUCTION OF FRESH FROZEN PLASMA FOR FRACTIONATION

INTRODUCTION.

The availability of Factor VIII concentrates to cover the needs of the haemophiliacs in any population is affected by many variables. The quality of fresh frozen plasma (FFP) is of major importance and Factor VIII assays are necessary to monitor its content in raw plasma and its yield in the concentrated product.

It is necessary to ensure that the Factor VIII assays provide meaningful information in order to monitor the quality of FFP at collection in the Regional Centres and through the various processing stages at the protein fractionation unit.

This paper describes and discusses a quality assurance scheme launched to monitor the performance of Factor VIIIIC assay programmes used in the Regional Transfusion Centres of the Scottish National Blood Transfusion Service (SNBTS) that supplies bulk FFP for the Service's Protein Fractionation Centre, with the following objectives:

1. To give participating laboratories the opportunity to monitor the quality of their Factor VIII assays and to determine the source or sources of the differences between assay values.

2. To determine the value of using a single standard and confirm its potency.
3. To evaluate a centrally prepared artificially depleted substrate and to determine its importance in reducing inter laboratory variability in Factor VIII assays.

4. To obtain meaningful assay values by the various Transfusion Centres and so that operational monitoring of the quality of FFP from collection to fractionation.

Design, Methods and Materials

Design

An external quality assurance scheme was set up in which SNBTS Laboratories were sent at quarterly intervals a set of unknown freeze dried plasma samples for Factor VIIIC assay against a Scottish Plasma Standard, using a one stage clotting assay and a Scottish Freeze dried Factor VIII deficient substrate.

Parallel assays of the samples were also requested in which the participating Laboratory's own substrate was used.

The local standard in use by the laboratory was also assayed against the Scottish standard using the supplied substrate.

Coded results were analysed statistically (details, C.P.1)
Methods and Materials

Standard SNBTS/1/85: This was prepared from citrated plasma and freeze dried in 1 ml vials. It was calibrated against the British plasma standard (NIBSAC 11th Plasma Standard). The value was confirmed to be 0.69 iu. (C.P.?)

Artificial Factor VIII Deficient Plasma: This was a mixture of the following, aged human plasma, crude bovine Factor V and human fibrinogen. It was freeze dried in 2 ml vials. (CP? or TMCQ)

Unknown Test Samples: During 1984 and 85, 20 freeze dried plasma samples were circulated to seven SNBTS laboratories.

Results

The Scottish plasma standard was prepared and assayed repeatedly (n = 42) in 1983. Figure 1 shows the calibration and stability assays over 12 months. (New Figure 1 CP)

Local standards were assayed against SNBT/1 and the per cent deviation from the originally assigned values is given in Figure 2. (New Figure 2 CP)

The artificially depleted substrate was used in parallel with the local substrate to assay 57 samples in the 4 exercises over one year and the co-efficient correlation is shown in Fig (3).
(New figures for two years C.P.)

Comparison of the results obtained at different Centres is shown in Fig (4). The graph also shows the 95% confidence limits (as per cent of mean) plotted against the weighted mean potency for each sample. (New figure 4 required CP)

Table (1) shows the mean Factor VIII assay value of a number of samples that have been recirculated under different identification number over the two year period. (How many are these in 1984 and 1985? Table required).

Discussion

The quality of fresh frozen plasma is crucial for the final yield of Factor VIII Concentrate obtained by fractionation. (References)

Effective processing of blood donations into FFP can be monitored by regular random Factor VIII assays of a proportion of the total production. Consistently reliable and accurate assays are essential to assess the overall input of Regional Transfusion Centres into the fractionation plant.

The main objective of this quality assurance scheme was to identify the sources of interlaboratory variation in Factor VIII assay and to propose practical solutions to eliminate or reduce
as much as possible the factors responsible for these differences. Kirkwood et al.1977 analysed the findings of a workshop held at Oxford and concluded that the differences between reagents accounted for the major part of the variation between laboratories.

Theie study also showed there were differences between assays performed by the one and the two stage methods. We did not have to face this problem because all the six participating laboratories used the one stage assay method. One lab only used a manual method while the other five used different semi automated machines.

Part of the scheme was to prepare and supply the two major reagents required for the method. The Scottish standard was prepared from a pool of 5 donations and calibrated by assay repeatedly (n = 45) against 11th British standard. Assays were repeated at 6 and 12 months to ensure that its potency was maintained at 0.69 iu per vial and the value was confirmed in subsequent confirmatory calibrations (Fig. 1).

The artificial factor VIII – deficient substrate was difficult to prepare and a number of methods were tried and a batch was successfully freeze dried to allow the scheme to continue for one year at least. It was prepared along the lines suggested by Nyman (1970) from aged human serum, crude bovine Factor V and human fibrinogen concentrate, and was supplied in two ml vials.
The Centres were asked to determine the Factor VIII content in the unknown samples and any local standards relative to the Scottish standard using a one stage clotting assay. Parallel assays were also requested using the laboratories own locally used substrate.

Comparison of individual results on plasma samples assayed showed good correlation with the locally used substrates and the value was 0.982. (Fig. 3).

This substrate was also used to assay the local Factor VIII standards relative to the Scottish Standard. They showed considerable deviation from their assigned potencies (Fig. 2). This finding confirmed the need for a single standard in order to be able to achieve some degree of conformity in the values assigned to the Factor VIII content of the national Fresh Frozen Plasma pools destined for fractionation.

The average potency of the twenty samples circulated ranged from 0.15 to ? and figure 4 shows the confidence limits of the individual values expressed relative to the mean value obtained by all the laboratories. The overall average confidence limits were 87 to 115% of the mean factor VIII content. These results are remarkable for single assays performed at six Centres.

(Are the results for the second year also good? CP)
A number of samples were redistributed and retested. The mean value of the paired assays are shown in table (1) and confirm the inherent degree of variability and error in this biological assay. This error has to be accepted and considered in all experiments and in the general use of Factor VIIIIC values for comparative studies. (Can the error be quantified? CP).

The following conclusions can ... be drawn from this two year Quality Assurance programme.

1. Meaningful Factor VIII assay values can be obtained at different laboratories when a common standard is used, despite differences in the equipment and methods used in the one stage assay.

2. Artificial Factor VIII deficient substrate was shown to be a viable alternative in place of haemophilic plasma for the quality assurance of fresh frozen plasma.

3. This programme provided evidence of the validity and limits of comparing plasma factor VIII levels determined in regional laboratories and at the central fractionating plant to monitor the content in raw plasma and the yield in the finished product.

Corrected 1st Draft December 1985

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CALIBRATIONS OF SNBTS/1

iu/ml Factor VIII

n 42

1983 1984

1 6 12 6 12

Month/Year

0.9

0.7

0.5