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Standardization of Factor VIII

I. CALIBRATION OF BRITISH STANDARDS FOR FACTOR VIII CLOTTING ACTIVITY

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Summary. Calibration of successive British Standards for Factor VIII clotting activity against the International Standard (concentrate) has brought to light substantial discrepancies among laboratories and between assay methods. These discrepancies were less in assays of concentrate standards than in calibration of plasma standards. Standardization of reagents in the two-stage assays substantially improved agreement among laboratories using this method. Standardization of the phospholipid reagent and haemophilic substrate separately had little effect on variation among laboratories performing one-stage assays. Standardization of reagents did not alter the basic discrepancy (approximately 20%) between the one-stage and two-stage assay methods. Omission of the aluminium hydroxide adsorption step from the two-stage method reduced the discrepancy between the two methods to less than 10%. Discrepancies did not occur when one plasma standard was assayed against another. Improvements in the stability of VIII:C in freeze-dried plasma now make it feasible to establish a long-term plasma reference standard.

The establishment of the first International Standard for Factor VIII in 1971 (Bangham *et al*, 1971) was a major step forward in standardization of measurements of factor VIII clotting activity (VIII:C). The International Unit, as defined by this Standard, and its replacement, the second International Standard (Barrowcliffe & Kirkwood, 1978), is now widely used as a yardstick for measurement of VIII:C, particularly in therapeutic concentrates.

In view of the long-term reference nature of these materials, they cannot be used as working standards and are intended mainly for calibration of national and local standards. For measurement of VIII:C in patients' plasmas, a plasma standard is the most appropriate and, in the U.K., national plasma standards for VIII:C have been established, each successive plasma being carefully calibrated against the International Standard (Bangham & Brozović, 1974). Because these national standards are used relatively widely and because of the instability of factor VIII in freeze-dried plasma (Bangham *et al*, 1971), each plasma standard has a relatively short life. The consequent need for frequent calibration of new standards against the International Standard has highlighted considerable discrepancies between laboratories and between assay methods.

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Such discrepancies in biological assays are particularly common when dissimilar materials, such as plasma and concentrate, are assayed against each other. The problem is, however, particularly acute for factor VIII because of a substantial discrepancy between the two methods of assay, the one-stage and two-stage methods (Kirkwood & Barrowcliffe, 1978).

Because of these assay discrepancies, it has become clear that a plasma standard would be unsuitable for assay of therapeutic concentrates. A parallel series of concentrate Working Standards has therefore been developed in the U.K. for use by the British manufacturers and control laboratories; these working standards have also been calibrated against the International Standard. The problem of calibration of the plasma standards remains, however, and in a series of studies we have investigated some of the causes of the assay discrepancies, and attempted to improve agreement between laboratories by standardizing various aspects of the assays. This report presents the results of the various collaborative studies of both plasma and concentrate standards, and an analysis of some of the features of the assays which give rise to discrepancies between laboratories and methods.

MATERIALS

Concentrate Standards

The bulk material used for the British Working Standards for Factor VIII Concentrate (76/540 and 79/506) was intermediate purity factor VIII prepared by the method of Newman *et al* (1971) and kindly supplied by the Plasma Fractionation Laboratory, Oxford, and the Blood Products Laboratory, Elstree, Herts. The freeze-dried concentrates were reconstituted in double distilled water, distributed into 1 ml amounts into 4000 glass ampoules, and freeze-dried under conditions used for international biological standards (Campbell, 1974).

The second International Standard for Factor VIII was prepared in a similar fashion (Barrowcliffe & Kirkwood, 1978).

Plasma Standards

The bulk material for the 4th, 5th, 7th and 8th British Standards for Factor VIII (Plasma) was freshly collected acid–citrate–dextrose plasma (420 ml blood to 76 ml anticoagulant) kindly supplied by the Blood Transfusion Centres at Oxford and Edgware, Middx. After centrifugation, the individual plasma donations (20–24) were buffered by addition of either MOPS (morpholino-propane sulphonic acid) or HEPES (hydroxyethyl-piperazine ethane sulphonic acid) to a final concentration of approximately 0·05 M. The plasma was then centrifuged again, pooled to give 4–4·5 litres, distributed at 4°C into 4000 glass ampoules, and freeze-dried by the same method as for the concentrate standards. The total time from collection of blood to freeze-drying of plasma was 10–12 h.

The material for the 6th British Standard was fresh citrate–phosphate–dextrose plasma (420 ml blood to 63 ml anticoagulant) collected at the Blood Transfusion Centre, Edinburgh. The unbuffered plasmas were pooled after a single centrifugation, distributed into 4000 rubber-stoppered vials and freeze-dried at the Protein Fractionation Centre, Edinburgh.

For both concentrate and plasma standards each individual donation and the final freeze-dried standards were tested for hepatitis B surface antigen and found to be negative.

Standard reagents

Phospholipid	NIBSC reagent 76/521, a freeze-dried extract of human brain prepared by a modified Folch procedure.
Haemophilic plasma	Collected at the Oxford Haemophilia Centre from a severe haemophiliac and freeze-dried at the Oxford Haemophilia Centre.
Bovine factor V	NIBSC reagent 76/559, from bulk material supplied by Diagnostic Reagents Ltd, Thame, Oxon.
Human serum	NIBSC reagent 76/557 prepared as described by Austen & Rhymes (1975), and freeze-dried at the Blood Products Laboratory, Elstree, Herts.
Factor VIII combined reagent	NIBSC reagent 78/513, prepared by combining the above phospholipid factor V, and serum reagents, after activation of the serum, and freeze-drying the mixture.

ASSAY METHODS

In all collaborative studies, laboratories used their own assay reagents and methods, which were all variations of either the one-stage (Hardisty & Macpherson, 1962) or the two-stage assay (Biggs *et al*, 1955). The main differences between laboratories were in the source of phospholipid (one-stage and two-stage assays), preparation of serum (two-stage assays) and the contact activating period (one-stage assays).

Stability Studies

The stability of several of the plasma standards was assessed from the results of accelerated degradation tests. Ampoules were stored at 4°C, 20°C and 37°C for periods of 0·5–2 years, then assayed against ampoules stored continuously at −20°C. From these data the predicted losses of potency when stored at −20°C, 4°C and 20°C were calculated using a maximum likelihood method of estimating the Arrhenius equation relating degradation rate to temperature (Kirkwood, 1977).

COLLABORATIVE STUDIES

In all studies, each laboratory was asked to perform at least six assays of the proposed standard, against the International Standard, using their own reagents and techniques. Two series of studies were carried out, one for calibration of plasma standards, and the second for calibration of concentrate standards. Brief details of each collaborative study are as follows:

Plasma Standards

Study No. 1 (1973/74). Calibration of the 4th B.S. against the 1st I.S. Three laboratories used one-stage assays and three used two-stage assays.

Study No. 2 (1975). Calibration of the 5th B.S. against the 1st I.S. Samples of the 4th B.S. were included to check for loss of potency. Two laboratories used one-stage assays and five used two-stage assays.

Study No. 3 (1977). Simultaneous calibration of 6th and 7th B.S. against 2nd I.S. Three laboratories used one-stage assays and five used two-stage assays. In a second part the assays were repeated with standardized phospholipid (one-stage method) and standardized serum, phospholipid and factor V (two-stage method).

Study No. 4 (1979). Calibration of the 8th B.S. against the 2nd I.S. Samples of the 7th B.S. were also included to check for loss of potency. Four laboratories used one-stage assays and four used two-stage assays. The study was carried out in three parts, to investigate standardization of reagents and the influence of aluminium hydroxide (Al $(OH)_3$) adsorption.

Part 1: Local reagents and normal assay techniques, i.e. samples adsorbed in the two-stage methods, but not in the one-stage.

Part 2: Standard reagents—combined reagent for two-stage assays, common haemophilic plasma for one-stage. Samples adsorbed in both one-stage and two-stage methods.

Part 3: Standard reagents as for Part 2. Samples not adsorbed in either one-stage or two-stage method.

To standardize the $Al(OH)_3$ adsorption step as much as possible, all laboratories used the same $Al(OH)_3$ suspension, and both plasma and concentrate samples were adsorbed, the concentrate being diluted in haemophilic plasma before adsorption.

Concentrate Standards

Study No. 5. Calibration of the 1st British Working Standard (B.W.S.) concentrate, against the 2nd I.S. Three laboratories used two-stage assays and one laboratory used one-stage assays.

Study No. 6. Calibration of the 2nd B.W.S. concentrate against the 2nd I.S. The same laboratories took part as in Study No. 5, with the addition of another one-stage laboratory. Samples of the 1st B.W.S. were included to check for loss of potency.

Statistical Methods

All assays were analysed as parallel-line bioassays relating clotting time (with log transformation as appropriate) to log dilution. Assays showing significant deviations from parallelism or linearity were rejected as statistically invalid. The number of assays rejected was not more than 9% of the total number in any study. The log potency estimates from the valid assays were combined to give an overall log potency estimate for each laboratory. Homogeneous sets of log potency estimates were combined by taking a weighted mean, the weights being the reciprocals of the variances of the estimates. Heterogeneous sets of log potency estimates were combined by taking the unweighted mean.

RESULTS

Calibration of Plasma Standards

The results of the studies of the various plasma standards are illustrated in Fig 1. For ease of comparison, each laboratory's potency estimate has been calculated as a percentage of the overall geometric mean. The mean potencies are shown in Table I, with 95% confidence limits. In each study there were statistically significant ($P<0{\cdot}05$ at least) differences between the individual laboratories' estimates of potency; the size of these differences varied from study to study, being particularly marked for Study No. 3, where the potency estimates for the 6th B.S.

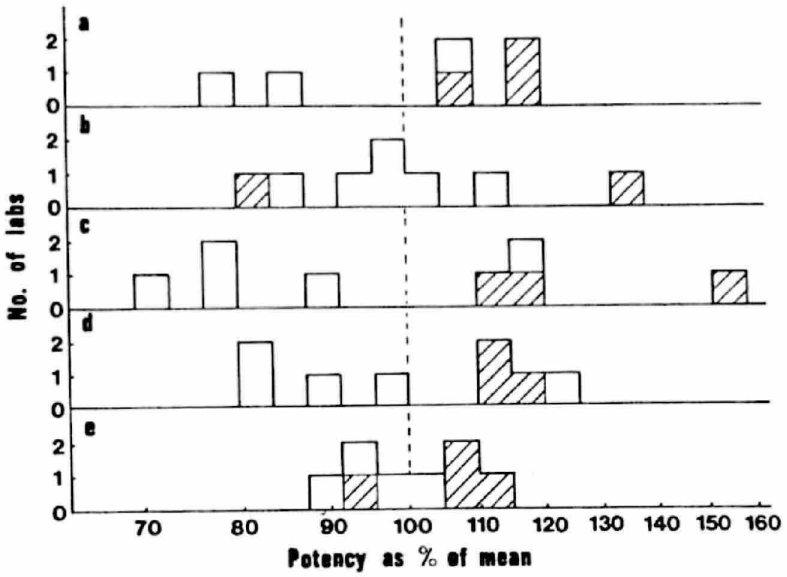

FIG 1. The histograms show the distribution of laboratory mean potency estimates for: (a) 4th B.S.; (b) 5th B.S.; (c) 6th B.S.; (d) 7th B.S.; (e) 8th B.S. All these preparations were freeze-dried plasmas and all assays were performed against freeze-dried concentrate (1st or 2nd International Standard). For each standard, the potencies are given as a percentage of the mean. Diagonal shading distinguishes the one-stage results from the two-stage results (unshaded).

encompassed a two-fold range. By contrast, the potency estimates within each laboratory were often remarkably reproducible.

Calibration of Concentrate Standards

The inter-laboratory variation in calibrating the two concentrate working standards is illustrated in Fig 2, and the geometric mean potencies are shown in Table II. The differences between laboratories were smaller than those seen with the plasma standards though, in Study No. 6 (2nd B.W.S.) in particular, they were still highly significant.

Re-assay of Standards

Two of the plasma standards, the 4th and 7th B.S., were assayed again in subsequent studies, 1·9 and 1·7 years, respectively, after their original calibration. The 1st B.W.S. concentrate was

TABLE I. Potencies of British Standards for Factor VIII (Plasma)

Standard	*Code No.*	*Mean potency (iu/ampoule)*	*95% Confidence limits*
4th	73/619	0·72	0·60–0·86
5th	75/510	0·58	0·51–0·65
6th	77/525	0·73	0·59–0·91
7th	77/520	0·58	0·51–0·67
8th	78/506	0·55	0·51–0·58

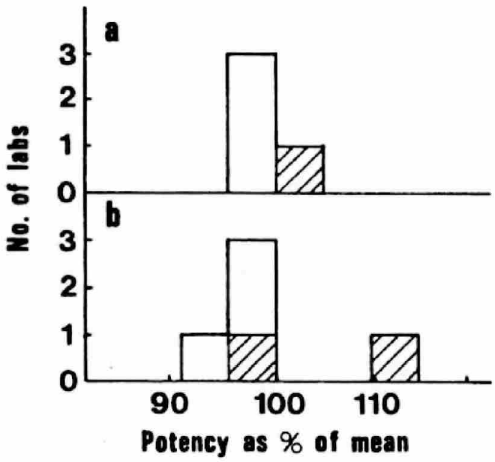

FIG 2. As in Fig 1, the histograms show the distribution of laboratory mean potency estimates for: (a) 1st, and (b) 2nd British Working Standard Concentrate by assay against the 2nd International Standard.

also re-assayed 2·5 years after its original calibration. The results of these re-assays showed a marked reduction (−15%) in potency for the 4th B.S., no significant difference for the 7th B.S., and a slightly higher potency (+6%) for the 1st B.W.S. Concentrate.

Long-Term Reproducibility of Laboratories

Although the set of participating laboratories was different in each study, a small 'core' took part in most of the collaborative studies. A reasonable degree of consistency was maintained, with all the one-stage assay methods giving results higher than the mean, and the two-stage methods giving potencies lower than the mean.

Standardization of Reagents

A comparison of the results with laboratories' own reagents and the standardized reagents is shown in Fig 3. Standardization of either the phospholipid or haemophilic plasma separately had little effect on the discrepancies among the one-stage assays, but agreement among the two-stage assays was substantially improved in Studies Nos. 3 and 4 by use of the standardized reagents. However, in spite of improved agreement between laboratories, the mean potencies given by the one-stage and two-stage assays remained the same and hence standardization of reagents did not alter the discrepancy between the two assay methods, which remained at about 20%.

TABLE II. Potencies of British Working Standards for Factor VIII (Concentrate)

Standard	*Code No.*	*Mean potency (iu/ampoule)*	*95% Confidence limits*
1st	76/540	2·92	2·86–2·98
2nd	79/506	3·82	3·50–4·17

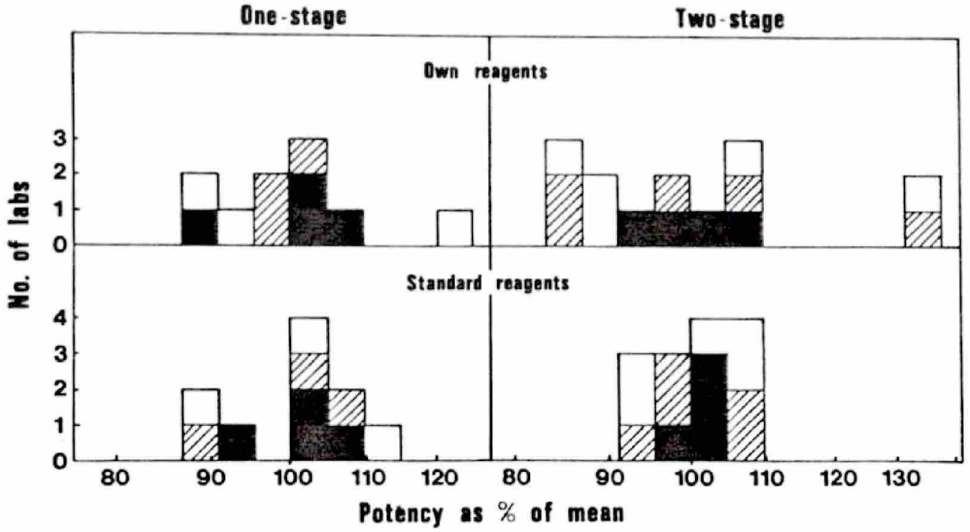

FIG 3. The histograms show the effect of standardization of assay reagents for the 6th (unshaded), 7th (diagonally shaded) and 8th (filled) British Standards. In the one-stage assays, phospholipid was standardized for the 6th and 7th B.S., and haemophilic plasma was standardized for the 8th B.S.; joint standardization of these reagents was not carried out. In the two-stage assays, serum, phospholipid and factor V were standardized for the 6th and 7th B.S., and a standard freeze-dried combined reagent was used for the 8th B.S.

Effect of $Al(OH)_3$ Adsorption

An obvious technical difference between the one-stage and two-stage assays is the $Al(OH)_3$ adsorption of plasma samples, normally regarded as an essential feature of two-stage assays but not carried out in the one-stage method. To investigate the contribution of this procedure to the discrepancy between the methods, parts 2 and 3 of Study No. 4 were carried out with and without $Al(OH)_3$ adsorption respectively, all other reagents and techniques being identical. In order to perform the two-stage assay without adsorption, it was necessary to modify the assay by use of a combined reagent and a non-subsampling system, as originally described by Denson (1967), and by increasing the dilutions of factor VIII and the reagent (Barrowcliffe & Kemball-Cook, 1980); these modifications were therefore used in parts 2 and 3 of this study.

Table III shows that the presence or absence of the $Al(OH)_3$ adsorption step had a substantial effect on the overall potency in both assay systems. Omission of the adsorption step in two-stage assays increased the potency of the plasma by 13%, and its inclusion in the one-stage

TABLE III. Effect of $Al(OH)_3$ adsorption on potencies of the 8th British Standard (Plasma)

	Mean potency (iu/ampoule)	
	One-stage	*Two-stage*
With adsorption	0·53	0·49*
No adsorption	0·58*	0·55

* Normal procedure.

TABLE IV. Stability of plasma standards

	Predicted potency loss (%/year)		
Standard	*−20°C*	*4°C*	*20°C*
6th	4·5	30·0	68·4
7th	0·02	1·0	9·3
8th	0·17	2·8	13·5

assays decreased the potency by 8%. When compared to the normal methods of assay (i.e. with adsorption for two-stage and without for one-stage assays), standardization of the adsorption step (either omission or inclusion for both assay systems) reduced the discrepancy between the two methods to less than 10%. Thus it appears that, in this study at least, $Al(OH)_3$ adsorption accounts for at least half of the overall 20% discrepancy between one-stage and two-stage assays, as previously reported in brief (Barrowcliffe *et al*, 1980).

Stability of Plasma Standards

The potency losses of the 6th, 7th and 8th Standards, as predicted from the accelerated degradation data, are shown in Table IV.

DISCUSSION

The system of standardization of factor VIII involving calibration of plasma standards against the International Standard (concentrate) has highlighted substantial discrepancies in assay results between laboratories. The two types of discrepancy which have been consistently observed are a basic 20% difference between one-stage and two-stage assays (Kirkwood & Barrowcliffe, 1978) and differences within each method, which have been shown to be due to the reagents used (Kirkwood *et al*, 1977). It is clear therefore that in any collaborative study selection of laboratories and their reagent systems and, in particular, the balance between one-stage and two-stage assays, may considerably influence the potency assigned to the standard. This is illustrated by the results on the 5th B.S., which was assigned a potency of 0·58 i.u. per ampoule in the U.K. study (five two-stage and two one-stage laboratories), but in an international collaborative study (Barrowcliffe & Kirkwood, 1978) involving 10 one-stage and five two-stage laboratories, the same plasma was assayed at 0·68 i.u./ampoule.

Since it has been shown (Kirkwood *et al*, 1977) that laboratories can obtain very good agreement when using the same reagents, we investigated the effect of standardizing some of the reagents in the two most recent studies. In the two-stage method, use of standardized reagents, particularly in the form of a single freeze-dried combined reagent, did substantially improve the agreement between laboratories (Fig 3). However, for the one-stage assays, standardization of either the phospholipid or haemophilic plasma alone had relatively little effect. It may be that both these reagents need to be standardized or, alternatively, other aspects of the method, such as activation procedure, may need to be more rigidly specified.

In spite of the improved agreement between laboratories, standardization did not alter the basic difference between one-stage and two-stage assays, which remained at about 20%. In the 8th B.S. study we investigated one aspect of the two-stage method which, it has been suggested (Seghatchian *et al*, 1979), may contribute towards this discrepancy, i.e. the $Al(OH)_3$ adsorption step. Table III shows clearly that the $Al(OH)_3$ adsorption step had a significant effect on the potencies obtained, and this has both theoretical and practical implications. It has been shown (Seghatchian *et al*, 1979) that $Al(OH)_3$ does not remove significant amounts of factor VIII related antigen (VIII R: Ag) but recent studies (Peake, 1980) indicate that substantial amounts of the factor VIII clotting antigen (VIII C: Ag) may be adsorbed from plasma by $Al(OH)_3$. Since in these assays both standard and test samples were treated with $Al(OH)_3$, the results would suggest that a larger proportion of VIII: C molecules has been removed from the plasma than from the concentrate standard. This is in keeping with the fact that the concentrate standard is an intermediate purity material, which has already been adsorbed with $Al(OH)_3$ during its production. At the practical level, it should now be possible to obtain much better agreement between laboratories in future collaborative studies, by the use of standardized reagents and omission of the $Al(OH)_3$ adsorption step, although a discrepancy of about 5–10% remains between the one-stage and two-stage methods.

The series of concentrate standards was developed for use as working standards for control of therapeutic concentrates within the U.K. Although there have been some discrepancies between laboratories in their calibration (Fig 2), the differences between laboratories are much smaller than in the calibration of the plasma standards. Another advantage of concentrate standards is the high stability of factor VIII in this form as compared to that in freeze-dried plasma (Bangham *et al*, 1971). However, the stability of factor VIII in plasma does depend on the methods of collection, centrifuging and freeze-drying. A study by Godfrey *et al* (1975) showed that factor VIII stability in plasma was much improved by buffering the anticoagulant, especially when the plasma was subsequently freeze-dried. HEPES or MOPS was therefore added to the plasma after removal of cells for all standards except the 6th. In spite of this, re-assays on the 4th Standard indicated a considerable potency loss. However, the time of addition of buffer may be critical; for the 4th Standard HEPES was added on receipt of the pooled plasma at the Standards Processing Laboratory, i.e. at least 3 h after collection, whereas for subsequent standards the buffer was added after the first spin, i.e. within 45 min of collection. It is not possible to assess whether the poor stability of the 6th British Standard was due to the absence of buffer, since the centrifugation and freeze-drying conditions were also different from those of the other standards.

At the time of establishment of the 1st International Standard, there was a clear preference for a concentrate standard because of both greater stability and similarity to therapeutic materials. However, it is now apparent that comparison of plasmas against concentrates is subject to considerable inter-laboratory variation. With improved stability of the freeze-dried plasma standards, the accelerated degradation data indicate that it would now be feasible to establish a plasma reference preparation with a life of at least 5 years. The use of such a reference plasma for calibrating successive batches of working plasma standards would circumvent most of the inter-laboratory and inter-method variation, as can be seen from Table V. This plasma could also serve as a long-term reference preparation for the other factor VIII related activities. This suggestion has now been adopted by the Factor VIII Subcommittee of the International

TABLE V. Mean potencies of 8th British Standard (Plasma) when assayed against a previous plasma standard (7th B.S.) as a reference

	Mean potency (iu/ampoule)		
Part No.*	One-stage	Two-stage	Overall
1	0·57	0·57	0·57
2	0·56	0·58	0·57
3	0·56	0·57	0·57

* See Methods section, Collaborative study No. 4.

Committee on Thrombosis and Haemostasis, and work on preparation and calibration of a plasma reference preparation is proceeding.

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REFERENCES

AUSTEN, D.E.G. & RHYMES, I.L. (1975) *A Laboratory Manual of Blood Coagulation*. Blackwell Scientific Publications, Oxford.

BANGHAM, D.R., BIGGS, R., BROZOVIĆ, M., DENSON, K.W.E. & SKEGG, J.L. (1971) A biological standard for measurement of blood coagulation factor VIII

activity. *Bulletin of the World Health Organization*, **45**, 337–351.

BANGHAM, D.R. & BROZOVIĆ, M. (1974) Factor VIII International Units and reference materials. *Thrombosis et Diathesis Haemorrhagica*, **31**, 3–11.

BARROWCLIFFE, T.W. & KEMBALL-COOK, G. (1980) Modifications to the two-stage assay of factor VIII. (To be published.)

BARROWCLIFFE, T.W. & KIRKWOOD, T.B.L. (1978) An international collaborative assay of factor VIII clotting activity. *Thrombosis and Haemostasis*, **40**, 260–271.

BARROWCLIFFE, T.W., KIRKWOOD, T.B.L. & RIZZA, C.R. (1980) Aluminium hydroxide adsorption and factor VIII clotting assays. *Lancet*, **i**, 820.

BIGGS, R., EVELING, J. & RICHARDS, G. (1955) The assay of anti-haemophilic globulin activity. *British Journal of Haematology*, **1**, 20–34.

CAMPBELL, P.J. (1974) International biological standards and reference preparations. II. Procedures used for the production of biological standards and reference preparations. *Journal of Biological Standardisation*, **2**, 259–267.

DENSON, K.W.E. (1967) The simplified two-stage assay of factor VIII using a combined reagent. In: *Transactions of the International Committee on Thrombosis and Haemostasis, Chapel Hill, North Carolina, 1966.*

GODFREY, R., RHYMES, I.L., BIDWELL, E. & BARROWCLIFFE, T.W. (1975) The buffering of anticoagulants for blood collection. *Thrombosis et Diathesis Haemorrhagica*, **34**, 879–882.

HARDISTY, R.M. & MACPHERSON, J.C. (1962) A one-stage factor VIII (antihaemophilic globulin) assay and its use on venous and capillary plasma. *Thrombosis et Diathesis Haemorrhagica*, **7**, 215–219.

KIRKWOOD, T.B.L. (1977) Predicting the stability of biological standards and products. *Biometrics*, **33**, 736–742.

KIRKWOOD, T.B.L. & BARROWCLIFFE, T.W. (1978) Discrepancy between one-stage and two-stage assay of factor VIII:C. *British Journal of Haematology*, **40**, 333–338.

KIRKWOOD, T.B.L., RIZZA, C.R., SNAPE, T.J., RHYMES, I.L. & AUSTEN, D.E.G. (1977) Identification of sources of inter-laboratory variation in factor VIII assay. *British Journal of Haematology*, **37**, 559–568.

NEWMAN, J., JOHNSON, A.J., KARPATKIN, M.H. & PUSZKIN, S. (1971) Methods for the production of clinically effective intermediate- and high-purity factor VIII concentrates. *British Journal of Haematology*, **21**, 1–41.

PEAKE, I.R. (1980) Unpublished data.

SEGHATCHIAN, M.J., KEMBALL-COOK, G. & BARROWCLIFFE, T.W. (1979) Absorption of factor VIII by aluminium hydroxide. *Haemostasis*, **8**, 106–116.