

Local Calibration of International Normalised Ratio Improves between Laboratory Agreement: Results from the UK National External Quality Assessment Scheme

S. Kitchen, I. Jennings, T. A. L. Woods, F. E. Preston
on behalf of UK NEQAS (Blood Coagulation) Steering Committee

From the UK NEQAS for Blood Coagulation, Sheffield, UK

Summary

In the present study we have performed local calibration of International Normalised Ratio (INR) measurement systems in a large series of laboratories. We assigned INRs to five lyophilised plasma calibrants, one prepared from normal plasma and four using plasma from warfarinised patients, using different International Reference Preparations for Thromboplastin. These five calibrants, and two lyophilised test plasmas were analysed by 349 centres using 60 different thromboplastin instrument combinations.

Plasma calibrants were assigned INRs using the WHO reference thromboplastin RBT-90 or the European reference thromboplastin CRM 149R. Each participating centre determined PTs of the calibrants with their local system. These PTs were then used to construct a local calibration graph relating PT to INR. The PTs of test plasmas were converted directly into INR using the local calibration model and into INR using the conventional method. The overall medians of conventionally derived INRs of two test plasmas analysed in 349 centres were 2.50 and 3.10, compared to 2.47 and 3.04 after local calibration where RBT-90 was employed to assign INRs to calibrants. Use of CRM 149R to assign INRs to calibrants led to a significant ($p < 0.0001$) increase in INR to 2.7 and 3.36 respectively. When results were grouped according to the thromboplastin employed, agreement between results with different reagents was improved by local calibration. There was a significant reduction ($p < 0.01$) in the spread of results in different centres as indicated by a reduction in coefficient of variation.

Introduction

The International Normalised Ratio (INR) is a widely adopted reporting system which standardises the variability in the responsiveness of thromboplastin reagents to the defect induced by administration of coumarin drugs (1). The system recommended by WHO (2) employs the International Sensitivity Index (ISI), which defines the sensitivity of a reagent in relation to the first primary International Reference Preparation (IRP) for thromboplastin coded 67/40 (3, 4). The advantage of this system is that the same INR should be obtained irrespective of the reagent used, although thromboplastin-related differences between INRs have been reported (5, 6). The WHO system was originally based on Prothrombin Times (PT) determined using manual tilt tube technique. Coagulometers are increasingly used to determine PTs and derive INRs and in some cases have been shown to influence the ISI of

thromboplastins (7-9). For this reason some thromboplastins have instrument-specific ISI values.

Variation between individual instruments of the same type may occur (10) to such an extent that assignment of a single ISI for that type of instrument may not be appropriate. An alternative approach to the problem of instrument effects on ISI, is to perform a local calibration using a series of plasmas which have been assigned INR or PT values prior to local testing. A calibration curve can then be constructed which relates local PT results to assigned INRs, and which can subsequently be used to convert local PT of test plasmas into INR. This approach has been applied in the French External Quality Assessment (EQA) scheme and resulted in a substantial decrease in the interlaboratory variation between INRs of the same test plasma analysed in different centres with the coefficient of variation (CV) reduced from 18.5% to 12.5% (11). Other groups have demonstrated improvements in relation to other reagents and instruments (12-15).

The purpose of the present study was to assess local calibration of a wide range of instrument/thromboplastin combinations, employed at the time of study by participants of the UK National External Quality Assessment Scheme (NEQAS) for Blood Coagulation, using five lyophilised plasma calibrants.

Materials and Methods

Plasma Calibrants

Venous blood samples were obtained from patients receiving warfarin with a range of INR values and from normal subjects. In each case 4.5 ml of blood was mixed with 0.5 ml of 0.105 M buffered Trisodium citrate (Vacutainer, Becton Dickinson Ltd., Oxford, UK), and centrifuged at $2500 \times g$ for 10 min.

A pool of normal plasmas, and four pools of warfarinised plasmas were prepared (grouped according to whether the INR result of the plasma was low, intermediate or upper therapeutic range). Each pool was stored deep frozen at -70°C prior to lyophilisation for six days in 0.5 ml aliquots containing 16.8 mM hydroxyethylpiperazine ethane sulphonic acid (HEPES). Each pool consisted of plasmas from 150-200 subjects. The lyophilised aliquots were stored at -20°C for up to six months prior to despatch by post to participants, who reconstituted the plasma with 0.5 ml distilled water at room temperature. The five calibrants were coded S1 (pooled normal) and S2, S3, S4 and S5 (pooled warfarinised samples). The levels of factor V in the five calibrants were within normal limits by one stage assay (range 78-98 u/dl). Fibrinogen concentrations were in the range 3.5-4.1 g/l (normal range 1.5-3.7 g/l).

Test Plasmas

Two test plasmas coded 95/27 and 95/28 were prepared. Each was from a single warfarinised donor, and was collected into citrate phosphate dextrose anticoagulant. Approximately 600 ml was collected over 45 min using a Haemonetics Ultralite instrument. Processing and lyophilisation was as previ-

Correspondence to: Mr. S. Kitchen, UK NEQAS for Blood Coagulation, 305 Western Bank, Sheffield S10 2TJ, UK – Tel.: +44 114 271 2955; FAX Number: +44 114 271 2149

ously described (5). Test plasmas were buffered with 16.8 mM HEPES prior to lyophilisation.

Assignment of INRs to Calibrants (S1-S5)

Prothrombin times of samples S1-S5 were determined in triplicate on two different days, using the following four International Reference Preparations for thromboplastin and manual tilt tube technique: Rabbit, plain – CRM 149R (European Community Bureau of Reference), (16); WHO Rabbit, plain – RBT 90 (17); WHO Human, plain – BCT 253 (18); WHO Bovine, combined – OBT 79 (3). The geometric mean PT of 20 fresh normal plasmas (MNPT) was calculated for each reference thromboplastin, and used to determine INRs for calibrants S1-S5. Assigned INRs for CRM 149R were the means of results in seven different centres (each determining a local MNPT). Assigned INRs for each of the WHO reference materials were based on results in a single centre due to the limited availability of these thromboplastins.

Tests Performed by Each Participating Centre

Each participant performed triplicate PTs of the five calibrants, S1-S5, using the thromboplastin and instrument routinely used for patient INR determination.

In addition, each participant determined PTs of test plasmas 95/27 and 95/28 in the way that routine patient samples are analysed (i.e. the same number of replicate tests as for routine clinical material). The calibrants and two test plasmas were distributed in a single exercise.

Calculation of INRs of Test Samples

Two types of INR were calculated for each local PT result of 95/27 and 95/28. One was calculated in the conventional way using the MNPT in local use and reagent ISI. The second type was derived from a calibration graph-relating local PT to assigned INRs of S1-S5. For each laboratory the linear regression relationship and correlation coefficient (r) was calculated for locally determined PTs of calibrants against assigned INRs. Two local calibration graphs were constructed for each participant. The first compared local PTs to INRs assigned on the basis of CRM 149R (mean result of INRs in seven centres). The second was prepared by replacing INRs assigned on the basis of CRM 149R with those assigned using RBT-90.

Table 1 INRs of plasma calibrants as determined with different reference thromboplastins in a single centre (six replicate determinations)

	CRM 149R		RBT-90		BCT-253		OBT-79	
	mean INR	sd	mean INR	sd	mean INR	sd	mean INR	sd
S1	0.99	0.02	0.98	0.04	1.04	0.07	0.98	0.05
S2	1.87	0.07	1.85	0.05	1.83	0.10	1.81	0.07
S3	2.38	0.13	2.28	0.25	2.33	0.17	2.31	0.09
S4	2.78	0.17	2.70	0.10	2.67	0.06	2.70	0.06
S5	3.89	0.11	3.46	0.11	3.33	0.12	3.30	0.15

Table 2 INRs of lyophilised plasma calibrants analysed with CRM 149R in seven different centres

	Mean INR	Range of results	Standard deviation
S1	1.01	0.99 - 1.03	0.02
S2	1.97	1.81 - 2.13	0.13
S3	2.48	2.27 - 2.71	0.16
S4	2.93	2.58 - 3.27	0.26
S5	3.87	3.27 - 4.27	0.40

Results

INRs of Calibrants S1-S5

The INRs of S1-S5 as determined with four reference thromboplastins used in a single centre are shown in Table 1. INRs determined with CRM 149R were greater than those determined with RBT-90, BCT 253 or OBT-79 for the four calibrants prepared from pooled warfarinised plasmas. The INRs of S1-S5 determined with CRM 149R in seven different centres are shown in Table 2. In the case of S5, INRs obtained with CRM 149R were significantly greater than with each of the other three IRPs (analysis of variance, $p < 0.001$).

Local Calibration Graphs

Results were received from 349 laboratories. A total of 18 different thromboplastins and 21 different instruments were used in 60 reagent/instrument combinations. Approximately 60% of centres used a technique in which the ISI of the reagent was assigned for the instrument in use. The remainder used a manual ISI, despite employing a coagulometer to determine clotting times. When CRM 149R was used to assign INRs, the correlation coefficient was 0.985 or greater in 322 out of 349 centres and 0.990 or greater in 272 of the 349 centres, with an overall mean of 0.991. Correlation coefficients were similar when calibrant INRs determined using RBT-90 were substituted for CRM 149R values.

INRs of Test Samples (95/27 and 95/28)

Effect of Local Calibration on INR Obtained with Different Reagents

Three INRs were calculated from locally determined PTs of 95/27 and 95/28, one based on conventional calculation, and two by reference to the regression relationship, relating local PT of S1-S5 to INRs of S1-S5 assigned using CRM 149R or RBT 90 as described above. When all results were combined there was a statistically significant (ANOVA $p < 0.0001$) increase of 8% in INR, following local calibration based on CRM 149R compared to conventional INRs. Median conventional INRs increased, from 2.5 to 2.7 and 3.1 to 3.36 for 95/27 and 95/28 respectively. In contrast, overall median INRs following local calibration based on RBT 90 were practically identical to conventional INRs (2.47 and 2.5 for 95/27, 3.04 and 3.1 for 95/28, no significant difference). Results were grouped according to thromboplastin in local use, and are shown in Table 3 (95/27) and Table 4 (95/28) for reagents used by five or more centres, including coefficients of variation (CV). It should be noted that reagents are used in combination with different instruments. The effect of local calibration on results obtained by users of reagents employed by 20 or more centres were evaluated. Local calibration based on CRM 149R led to statistically significant (ANOVA $p < 0.05$ to $p < 0.001$) increase in INRs in all five groups, although the magnitude of increase varied from 18.6% (Diagen) to 2.4% (IL PT Fib HS Plus). The largest changes following local calibration based on RBT-90 were a decrease in INRs obtained by users of IL by 7.8%, and an increase in INRs obtained by Diagen users of 6.8%. A similar pattern of results was observed for 95/27 (Table 3). Local calibration based on RBT-90, was associated with a statistically significant change in median INRs for only two of the five reagents used by 20 or more centres, a reduction in INRs (ANOVA $p < 0.001$) obtained with IL (HS Plus), and an increase ($p < 0.001$) in INRs obtained with Sysmex thromboplastin.

Thromboplastin	Number of users	Conventional INR		Locally Calibrated INR - 1 (CRM 149R)*		Locally Calibrated INR - 2 (RBT/90)*	
		median	CV(%)	median	CV(%)	median	CV(%)
Dade Innovin	7	2.36	5.3	2.71	9.9	2.48	9.4
Diagen Activated	20	2.16	14.8	2.49	7.8	2.28	7.2
Instrumentation Lab. PT Fib HS	11	2.52	11.4	2.74	2.9	2.50	2.7
Instrumentation Lab. PT Fib HS Plus	149	2.69	8.0	2.77	5.4	2.52	5.2
Manchester	67	2.30	9.5	2.46	10.7	2.26	10.0
Manchester Low Opacity	28	2.29	5.2	2.43	4.0	2.24	3.4
Nycomed Thrombotest	13	2.50	5.3	2.87	5.1	2.59	4.8
Organon Simplastin Excel S	10	2.53	13.1	2.81	6.3	2.54	7.9
Sysmex PT (Ca)	26	2.20	5.5	2.57	4.4	2.35	4.1
ALL	349	2.50	11.6	2.70	8.6	2.47	8.04

* locally calibrated INRs calculated first on the basis of plasma calibrant INRs assigned using CRM 149R, and secondly recalculated from the same local PT but on the basis of plasma calibrant INRs assigned using RBT-90.

Table 3 INR results analysed according to Thromboplastin Reagent (reagents used by five or more centres are shown): Sample 95/27

Thromboplastin	Number of users	Conventional INR		Locally Calibrated INR - 1 (CRM 149R)*		Locally Calibrated INR - 2 (RBT/90)*	
		median	CV(%)	median	CV(%)	median	CV(%)
Dade Innovin	7	2.96	25.1	3.47	29.0	3.14	27.8
Diagen Activated	20	2.80	19.0	3.32	10.7	2.99	10.1
Instrumentation Lab. PT Fib HS	11	3.22	11.7	3.40	1.9	3.08	2.1
Instrumentation Lab. PT Fib HS Plus	149	3.30	7.2	3.38	4.2	3.06	2.1
Manchester	67	3.00	11.9	3.31	11.6	3.00	11.0
Manchester Low Opacity	27	2.90	5.5	3.25	4.3	2.95	4.1
Nycomed Thrombotest	13	2.90	7.6	3.43	7.4	3.11	7.1
Organon Simplastin Excel S	10	3.24	15.6	3.54	13.0	3.20	12.4
Sysmex PT (Ca)	26	2.78	7.3	3.21	3.3	2.91	3.2
ALL	348	3.10	11.8	3.36	8.3	3.04	8.0

* locally calibrated INRs calculated first on the basis of plasma calibrant INRs assigned using CRM 149R, and secondly recalculated from the same local PT but on the basis of plasma calibrant INRs assigned using RBT-90.

Table 4 INR results analysed according to Thromboplastin Reagent (reagents used by five or more centres are shown): Sample 95/28

Instrument	Thromboplastin	Number of users	Conventional INR		Locally Calibrated INR - 1 (CRM 149R)**		Locally Calibrated INR - 2 (RBT/90)**	
			median	CV(%)	median	CV(%)	median	CV(%)
Automated Coagulation	Diagen Activated	6	2.16	7.6	2.62	5.44	2.40	4.5
	IL PT Fib HS*	11	2.52	11.4	2.74	2.88	2.50	2.7
	IL PT Fib HS Plus*	146	2.69	8.0	2.77	5.4	2.50	5.2
Laboratory Series	Manchester	9	2.27	12.0	2.52	3.1	2.32	2.9
	Manchester Low Opacity*	17	2.26	4.3	2.47	2.3	2.27	2.2
KC Series	Diagen Activated	6	2.05	6.3	2.55	7.6	2.33	7.1
	Manchester	28	2.30	10.1	2.48	13.1	2.28	12.4
	Thrombotest	10	2.53	5.8	2.83	4.7	2.57	4.4
MLA Series	Innovin*	6	2.41	5.7	2.70	10.8	2.47	10.2
Sysmex Series	Manchester Low Opacity	6	2.38	8.2	2.34	4.7	2.16	3.4
	Sysmex PT (Ca)*	19	2.21	5.8	2.55	3.4	2.36	4.2

*Reagent/Instrument combinations with a manufacturer's assigned ISI specific for that coagulometer

** locally calibrated INRs calculated first on the basis of plasma calibrant INRs assigned using CRM 149R, and secondly recalculated from the same local PT but on the basis of plasma calibrant INRs assigned using RBT-90.

Table 5 INR results according to Reagent/Instrument combinations: Sample 95/27

Relationship between INRs with Different Reagents

When conventionally calculated INRs were compared, there were highly significant differences (of up to 19%) between results obtained with different reagents used by 10 or more centres for both test samples. (Kruskal-Wallis non-parametric ANOVA, p <00001). After local calibration, when INRs were assigned to calibrants with CRM 194R or with RBT 90, significant difference between results with different

reagents (of up to 10%) were still present (Kruskal-Wallis non-parametric ANOVA, p <0001) but the ranking of results with different reagents was different.

Results Obtained with Particular Reagent/instrument Combinations

Eleven reagent/instrument combinations were used in five or more centres. The median INRs and CV for these combinations are shown in

Table 6 INR results according to Reagent/
Instrument combinations: Sample 95/28

Instrument	Thromboplastin	Number of users	Conventional INR		Locally Calibrated INR - 1 (CRM 149R)**		Locally Calibrated INR - 2 (RBT/90)**	
			median	CV(%)	median	CV(%)	median	CV(%)
Automated Coagulation Laboratory Series	Diagen Activated	6	2.79	11.6	3.40	8.0	3.01	7.9
	IL PT Fib HS*	11	3.22	11.7	3.40	1.9	3.08	2.1
	IL PT Fib HS Plus*	146	3.30	7.2	3.39	4.2	3.06	4.1
Manchester Series	Manchester	9	2.88	13.7	3.35	3.7	3.03	3.6
	Manchester Low Opacity*	16	2.89	4.3	3.30	2.8	2.99	2.7
KC Series	Diagen Activated	6	2.70	9.5	3.40	9.6	3.08	9.2
	Manchester	28	3.05	12.1	3.34	11.2	3.02	10.7
	Thrombotest	10	2.95	8.2	3.47	7.0	3.17	6.8
MLA Series	Innovin*	6	2.92	27.4	3.46	32.0	3.13	30.6
Sysmex Series	Manchester Low Opacity	6	2.96	9.2	3.12	4.5	2.82	4.1
	Sysmex PT (Ca)*	19	2.82	7.0	3.20	3.1	2.91	3.2

*Reagent/Instrument combinations with a manufacturer's assigned ISI specific for that coagulometer

** locally calibrated INRs calculated first on the basis of plasma calibrant INRs assigned using CRM 149R, and secondly recalculated from the same local PT but on the basis of plasma calibrant INRs assigned using RBT-90.

Table 5 (sample 95/27) and Table 6 (sample 95/28). In five (of eleven) the reagent had a manufacturer's assigned ISI for that particular instrument. For these five groups, agreement between results with different reagents/instrument combinations was improved by local calibration. For sample 95/28 there was a 17% difference between highest and lowest conventional INRs, and only an 8% difference between highest and lowest locally calibrated INRs.

Between Laboratory Agreement

When all INR results were combined (irrespective of reagent), local calibration was associated with an improvement in between laboratory agreement as indicated by a lower coefficient of variation (CV). Based on conventional INRs the CVs were 11.6% and 11.8% for 95/27 and 95/28. There was a statistically significant ($p < 0.01$, F test), (19) reduction in standard deviation following local calibration and leading to CVs of 8.0-8.6% (Tables 3 and 4). There were highly significant ($p < 0.05$ - $p < 0.001$, F test) reduction in standard deviation and CV within some reagent groups (Tables 3 and 4). Improvements (reductions) were similar whether INRs were assigned to calibrants using CRM 149R or RBT-90. The elevated CV for users of Innovin in the analysis of sample 95/28 were influenced by an outlying result of INR 1.2. Exclusion of this result would reduce the CVs for this group to less than 9%.

Discussion

It is well established that the ISI of some thromboplastins are influenced by the use of coagulometers and for this reason some thromboplastins have instrument specific ISI values assigned. An alternative approach is to perform a local calibration of the reagent/instrument combination.

In the present study, we have assessed local calibration of a wide range of instrument/reagent combinations for INR determination in the UK NEQAS for Blood Coagulation, and compared the use of different reference thromboplastins for assignment of INRs to plasma calibrants.

The number of calibrants required for a successful calibration is not known, but was thought to be 12 in the system described by Duffy and co-workers (20), which employed calibrants prepared from single

donations. Other studies have employed calibrants prepared by pooling plasmas from different patients receiving warfarin, and have demonstrated that as few as five calibrants can be used (13). The present study employed five calibrants, and in general, the five data points on the graph relating local PT to assigned INR were close to the regression line, as indicated by correlation coefficients of greater than 0.985 in 322 of 349 participating centres. Linear regression rather than orthogonal regression was employed, since this is simpler, available in a wider range of centres, and has been shown to be suitable (20, 21).

In the present study local PT and assigned INRs were plotted on a linear scale as used by van den Besselaar (13). Other studies here employed logarithmic scale. We compared the two methods using data from 10 unselected centres employing the two most commonly used thromboplastins. Locally calibrated INRs by the two methods were within 1.5% of each other, irrespective of the IRP used to assign INRs to calibrants (data not shown).

One calibrant used in the present study was prepared from normal plasmas, and the remainder from patients receiving warfarin therapy. Whilst calibrants composed of artificially depleted normal plasmas have been used successfully (15), Stevenson and co-workers (22) concluded that a universally applicable scheme will only be possible, when plasma calibrants prepared from warfarinised patients are employed.

The assignment of reference values to the calibrants is an important source of potential variation between different calibrant sources. In the present study, the effect of local calibration was influenced by the use of different reference thromboplastins to assign INRs. The INR of the five calibrants were similar if RBT-90, BCT-253 or OBT-79 were used. However, the INRs of the calibrants prepared from warfarinised patient plasmas were greater with CRM-149R. In contrast to our data the INRs of some other lyophilised plasma were greater with RBT/90 than with CRM 149R (23). The INR value of calibrants obtained with the reference material (CRM 149R) varied in different centres. This suggests that assignment of INRs to calibrants for routine use should not be done in a single centre.

In our study, if CRM-149R assigned INRs were used for calibrants, the locally calibrated INRs of two test plasmas increased (relative to conventionally calculated INRs) for all widely used reagents, including reagents with instrument specific ISI values. This suggests either that all manufacturers have under-estimated the ISI of their reagent, or that CRM 149R is not appropriate for assigning INRs to this type of calibrant.

The selection of RBT-90 to determine INRs of calibrants is particularly appropriate, since its ISI was the consensus of calibrations against each of the other WHO IRPs available at the time, removing the bias which had been present between some previous IRPs (17), although it may not be possible to assign an INR value to lyophilised plasmas which are valid for all thromboplastins.

The test plasmas in the present study were collected into citrated phosphate dextrose anticoagulant and it has been shown that the citrate concentration can influence the INR of lyophilised plasmas (23). In two previous studies the relationship between results with different reagents was similar for fresh and lyophilised plasmas, prepared and lyophilised in the same way as the present study (5, 6). Nevertheless, the possibility of effects related to use of lyophilised test plasmas cannot be completely excluded.

The underlying principle of the INR system is that same results should be obtained irrespective of the laboratory method employed. This was not the case for conventionally calculated INRs of two test plasmas. For example, 149 users of PT-Fib HS Plus obtained a median INR of 3.30 for one sample, compared to a median of 2.78 obtained by users of Sysmex PT reagent. Agreement between results with different reagents was improved by local calibration, and ranged from 2.91 to 3.20 for the same plasma when RBT/90 was used to assign INRs to calibrants. The median of all 348 centres results, including all reagents used, was practically unchanged. Local calibration (using RBT-90) therefore reduced the spread of results without an alteration in the median value. This reduction in the spread of results is illustrated by the statistically significant reduction in the CV of INR results. When all centres results were grouped together, the CV of conventionally derived INRs was 11.6 and 11.8% for the two test plasmas. This was reduced to 8% in both cases following local calibration (based on RBT-90).

The most marked improvements in between laboratory agreement, indicated by reductions in CV, occurred within some groups of centres using the same thromboplastin reagent and coagulometer. For example, the CV of INRs determined by 11 centres using one reagent/instrument combination could be reduced from 11.7% to 2.1% by local calibration. For some reagent/instrument combinations there was no improvement in CV.

In addition to the improvements in between laboratory agreement there are a number of other potential advantages of local calibration. In the model employed in the present study there is no requirement for a reagent ISI. This removes a source of variation and potential error related to the calibration of the reagent which may vary according to the selection of reference thromboplastin, number of centres involved in calibration and sample collection system. This type of local calibration does not require a locally determined MNPT. This may be an advantage particularly in smaller centres where the requirement to test 20 or more normal subjects can be inconvenient. However, an alternative source of variation is introduced by the assignment of INRs to calibrants. This may be influenced by the selection of reference thromboplastin and the design of the calibration process.

We have clearly demonstrated that local calibration of instrument/reagent combinations for INR determinations can be successfully applied to a wide range of different coagulometers and thromboplastins at least using the type of calibrants employed here. When RBT-90 was used to assign INRs to calibrants derived from normal plasmas or warfarinised patient plasmas, local calibration was associated with no change in the overall median INR of two test plasmas. Importantly, there was an improvement in agreement between results obtained with different thromboplastins, and a reduction in interlaboratory variation,

as indicated by a reduced CV amongst more than 340 centres analysing the same test plasmas. In routine practice several different sources of calibrant may be employed and further study of the effects of different sources of calibrants is required.

Acknowledgements

The assistance of UK NEQAS participants is gratefully acknowledged, together with the help of the seven centres who assisted in the assignment of INRs to plasma calibrants. We also wish to thank Dr. Fieller of Sheffield University for statistical advice and Mrs. M. Thorpe for preparation of this manuscript.

References

- Loeliger EA, van den Besselaar AMHP, Broekmans AW. Intensity of oral anticoagulation in patients monitored with various thromboplastins. *New E J Med* 1983; 308: 1228-9.
- WHO expert committee on biological standardisation. Thirty-third report. WHO Tech Report Series 1983; 687: 81-105.
- Hermans J, van den Besselaar AMHP, Loeliger EA, van der Velde EA. A collaborative study of reference materials for thromboplastins. *Thromb Haemost* 1983; 50: 712-7.
- Kirkwood TBL. Calibration of reference thromboplastins and standardisation of the prothrombin time ratio. *Thromb Haemost* 1983; 49: 238-44.
- Kitchen S, Walker ID, Woods TAL, Preston FE. Thromboplastin related differences in the determination of International Normalised Ratio. *Thromb Haemost* 1994; 72: 426-9.
- Kitchen S, Jennings I, Woods TAL, Walker ID, Preston FE. Two recombinant tissue factor reagents compared to conventional thromboplastins for determination of International Normalised Ratio: a 33-laboratory collaborative study. *Thromb Haemost* 1996; 76: 372-6.
- Poggio M, van den Besselaar AMHP, van der Velde EA, Bertina RM. The effects of some instruments for PT testing on the ISI of two rabbit brain thromboplastin reagents. *Thromb Haemost* 1988; 62: 868-74.
- D'Angelo A, Seveso MP, D'Angelo SV et al. Comparison of two automated coagulometers and the manual tilt tube method for the determination of the prothrombin time. *Am J Clin Path* 1989; 92: 321-8.
- Ray MJ, Smith IR. The dependence of the ISI on the coagulometer used to perform the prothrombin time. *Thromb Haemost* 1990; 63: 424-9.
- Thomson JM, Taberner DA, Poller L. Automation and prothrombin time: a UK field study of two widely used coagulometers. *J Clin Path* 1990; 43: 679-84.
- Houbouyan LL, Goguel Af. Long-term French experience in INR standardisation by a procedure using plasma calibrants. *Am J Clin Pathol* 1997; 108: 83-9.
- Clarke K, Taberner DA, Thomson JM, Morris JS, Poller L. Assessment of value of calibrated lyophilised plasma to determine International Sensitivity Index for coagulometers. *J Clin Path* 1992; 42: 58-60.
- Van den Besselaar AMHP. Use of lyophilised calibration plasmas and control blood for INR calculation in external quality assessment of the PT. *Am J Clin Path* 1994; 102: 123-7.
- Poller L, Thomson JM, Taberner D, Clarke K. The correction of coagulometer effects on INRs: a multicentre evaluation. *Br J Haem* 1994; 86: 112-7.
- Poller L, Triplett DA, Hirsh J, Carroll J, Clarke K. The value of plasma calibrants in correcting for coagulometer effects on INR; An international multicentre study. *Am J Clin Path* 1995; 103: 358-65.
- Van den Besselaar AMHP, Bertina RM. Multicentre calibration of the secondary reference material for thromboplastin, rabbit plain, coded CRM 149R. *Thromb Haemost* 1991; 65: 263-7.
- Van den Besselaar AMHP. Multicentre study of replacement of the international reference preparation for thromboplastin, rabbit plain. *Thromb Haemost* 1993; 70: 794-9.

18. Thomson JM, Tomenson JA, Poller L. The calibration of the second primary International reference preparation for thromboplastin (Thromboplastin, Human, Plain, coded BCT/253). *Thromb Haemost* 1984; 52: 336-42.
19. Haber A, Runyon RP. *General Statistics* (2nd Edition), Addison-Wesley London 1973.
20. Dufty JMK, Craig S, Stevenson KJ, Taberner DA. Calculation of System International Sensitivity Index: How many calibrants are required? *J Clin Path* 1997; 50: 40-4.
21. Poller L, Barrowcliffe TW, van den Besselaar AMHP, Jespersen J, Tripodi A, Houghton D. A simplified statistical method for local INR using linear regression. *B J Haem* 1997; 98: 640-7.
22. Stevenson KJ, Craig S, Dufty JMK, Taberner DA. System ISI calibration: A universally applicable scheme is possible only when coumarin plasma calibrants are used. *Br J Haem* 1997; 96: 435-41.
23. Van den Besselaar AMHP. Comparison of lyophilised plasmas with fresh plasma for calibration of thromboplastin reagents in oral anticoagulant control. *Br J Haem* 1996; 93: 437-44.

Received March 12, 1998 Accepted after resubmission October 1, 1998

**Ordering has never been
so easy:**

<http://www.schattauer.com>