

Harmonization of fibrinogen assay results: study within the framework of the Dutch project 'Calibration 2000'

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SUMMARY

In a Dutch project for harmonization of fibrinogen assays, the commutability of potential calibrators for fibrinogen was assessed by means of a twin-study design, which is, in essence, a multicentre, split-patient sample, between-field-methods protocol. The study consisted of simultaneous analysis of fresh-frozen patient plasmas and three potential calibrators for fibrinogen by 48 Dutch laboratories forming 24 couples. The state-of-the-art intralaboratory standard deviation was used to assess the commutability of the potential calibrators. The potential calibrators were commutable for the Clauss, but not for the prothrombin time (PT)-derived assays. One potential calibrator was used in an attempt to harmonize fibrinogen assay results in a Dutch field study. The interlaboratory coefficient of variation (CV) of three out of four test samples could be reduced significantly using the common calibrator. The average overall CV for the four test samples was 10.3% using the routine measurements and 7.8% using the common calibrator. Despite the reduction in the overall CV, the bias between Clauss and PT-derived assay results in two coumarin test samples could not be eliminated.

INTRODUCTION

The determination of plasma fibrinogen is important for the assessment of both bleeding disorders and

thrombotic risk factors. Many prospective epidemiological studies have reported positive associations between the risk of coronary heart disease and plasma fibrinogen levels. There is interest in the possibility that measure-

ment of fibrinogen may help in disease prediction or prevention (Fibrinogen Studies Collaboration, 2005).

Many studies have shown considerable interlaboratory variations in plasma fibrinogen assay results. Several attempts have been made to improve interlaboratory variation in fibrinogen measurements (Chantarangkul, Tripodi & Mannucci, 1994; Takamiya *et al.*, 2005). Fibrinogen should be considered as an analyte, which is rather heterogeneous in human samples and is not directly traceable to SI units. Reference measurement procedures, independent of routinely employed analytical principles, are currently lacking. Thus, the value assignment of candidate secondary reference materials may be problematic (Panteghini, 2007).

The Dutch project 'Calibration 2000' aims at harmonizing laboratory results via calibration by development of commutable, matrix-based, secondary reference materials (Baadenhuijsen *et al.*, 2002). Commutability is defined as the degree to which a material yields the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships for the same procedures applied to those types of material for which the procedures are intended (Miller, 2003). As far as we know, the commutability of lyophilized materials for fibrinogen assays has been investigated in one study only (Kallner *et al.*, 2003).

The purpose of this study was to assess the commutability of three potential calibrators for fibrinogen assays. One of these was selected as a common calibrator for the Dutch laboratories in an attempt to harmonize fibrinogen assay results. A flowchart of the study is shown in Figure 1.

MATERIALS AND METHODS

The second international standard for fibrinogen in plasma (code: 98/612) was obtained from the National Institute for Biological Standards and Control (Potters Bar, UK). This standard had an established potency of 2.2 mg fibrinogen per ml (Whitton *et al.*, 2000).

One potential calibrator was prepared from three human plasma units. These plasma units were obtained from healthy blood donors. The blood sample (500 ml) was collected in standard collection bags containing

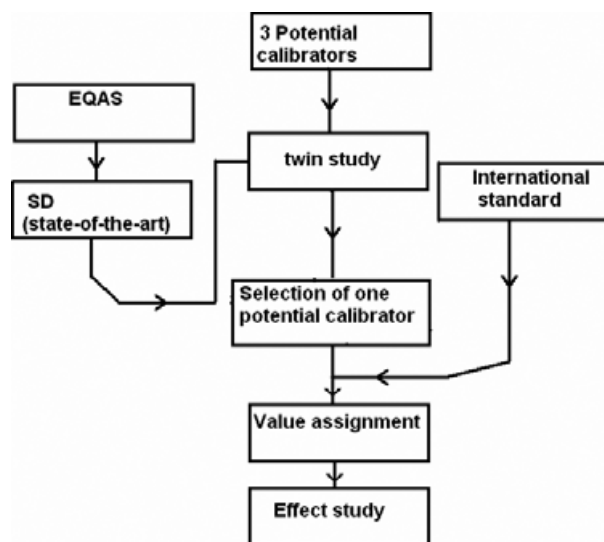


Figure 1. Flowchart of the 'Calibration 2000' fibrinogen study.

70 ml of citrate-phosphate-dextrose (CPD). After centrifugation for 20 min at 4000 *g*, the plasma units were pooled. One part of the pooled plasma was filled in 1.0 ml aliquots in plastic tubes and was deep-frozen at -70°C (potential calibrator number 1). The second part of the pooled plasma was buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES, final concentration: 29 mM), freeze-dried in siliconized glass vials (1 ml per vial) which is referred to as potential calibrator number 2. A commercial control plasma, STA Preci-clot Plus I, was purchased from Roche Diagnostics Nederland BV (Almere, the Netherlands). This material was collected using trisodium citrate as anticoagulant and is referred to as potential calibrator number 3. Lyophilized test plasmas were prepared from pooled normal plasmas and pooled patient plasmas, and were also buffered with HEPES (Van den Besselaar, Haas & Kuypers, 2006).

Assessment of the state-of-the-art standard deviation

In the Netherlands, approximately 120 clinical laboratories participate in the national external quality assessment scheme (EQAS) for fibrinogen assays. Of these, approximately 100 use assays according to Clauss (1957) and approximately 10–20 use prothrombin time (PT)-derived assays (Rossi *et al.*, 1988).

The state-of-the-art standard deviation (SD_{SA}) was defined as the median intralaboratory SD of laboratories participating in the Netherlands EQAS. Six lyophilized test plasmas were included in 4–12 surveys. Two of these were prepared by pooling plasmas from patients treated with oral anticoagulants ('Coumarin' plasmas). Two other plasmas were derived from patients with mild haemophilia (factor VIII deficient). Another was prepared by pooling plasmas from healthy donors ('Normal' plasma). Another was made deficient in antithrombin (AT-deficient). The test plasmas were coded differently in each survey. For each participant, the SD was calculated for each test plasma, if results from four or more surveys were available. The SD_{SA} was determined for each test plasma and could be fitted to the empirical formula $\ln(SD_{SA}) = a + b \times F$, in which F is the fibrinogen assay value in g/l.

Value assignment

Six laboratories were invited to participate in the value assignment of the selected potential calibrator for fibrinogen. Each laboratory used the same design for the fibrinogen assay by the Clauss method, but used their local thrombin reagent and instrument. In each laboratory, five vials of the selected potential calibrator were analysed. Three dilutions of the selected calibrator and five dilutions of the second international standard for fibrinogen (coded 98/612) were tested in parallel. Calibration curves were prepared by plotting clotting times vs. fibrinogen concentrations on double-logarithmic scales. The second international standard for fibrinogen was collected in the same anticoagulant (CPD) as the potential calibrators number 1 and 2 (Whitton *et al.*, 2000). The dilutions of the international standard and the selected calibrator were similar and therefore the citrate concentrations were also similar.

Twin study

The twin study consisted of the simultaneous analysis of patient plasmas and potential calibrator materials for fibrinogen. Forty-eight laboratories were included and twenty-four couples were formed. The laboratories acting as partners were selected on the basis of a modest geographical distance between them. In 17 couples, both partners used the Clauss method. In each of the other couples, one partner used the Clauss method

and the other partner used either PT-derived fibrinogen assay (six laboratories) or an ammonium sulphate precipitation assay (one laboratory). The principle of the latter assay method has been described (Bakker *et al.*, 1992). A single PT reagent (i.e. PT-Fibrinogen Recombinant, HemosIL; Instrumentation Laboratory, Milano, Italy) but different types of instrument were used by the six laboratories for the PT-derived fibrinogen assay. Each laboratory couple was asked to select 30 fresh patient plasmas, preferably spanning the relevant concentration interval for fibrinogen. After these samples were split into two portions and frozen at $-20\text{ }^{\circ}\text{C}$, one portion from each sample was transported to the partner laboratory. The three potential calibrators were sent beforehand to each participant. The interchanged fresh-frozen samples and the three potential calibrators were then analysed by both partner laboratories in three runs.

The statistical analysis of the data was performed essentially as described by Baadenhuijsen *et al.* (2002). The regression residuals of the potential calibrators were expressed as the absolute values of the perpendicular distances of each potential calibrator to the respective patient regression line and were normalized by expressing them as multiples of the state-of-the-art intralaboratory SD (SD_{SA}). A fibrinogen concentration-dependent correction of the SD_{SA} was carried out by use of a log-linear approximation of the precision profile of the intralaboratory variation. The decision limit for accepting a potential calibrator as commutable was set at three SD_{SA} according to previous studies (Baadenhuijsen *et al.*, 2002).

Effect of harmonization

One of the potential calibrators was used to determine the effect of harmonization on the fibrinogen assay. The selected calibrator and four lyophilized test plasmas were mailed to 69 participants of the Dutch EQAS. Two test plasmas were pooled plasmas obtained from patients treated with oral anticoagulants (coded A and D), one test plasma was obtained from a patient with mild haemophilia A (coded B) and one test plasma was a pooled plasma obtained from healthy donors (coded C). Each participant analysed the four test plasmas using the routine fibrinogen assay system. In addition, each participant prepared a new calibration curve using the selected calibrator. The fibrinogen concentrations of

Table 1. Median fibrinogen concentration and median intralaboratory standard deviation (SD) in lyophilized plasma samples obtained from regular external quality assessment scheme in the Netherlands

Type of plasma sample	Claus methods (Dade Behring)			Claus methods (Roche)			PT-derived methods (IL)		
	<i>n</i>	Median fibrinogen (g/l)	Median SD (g/l)	<i>n</i>	Median fibrinogen (g/l)	Median SD (g/l)	<i>n</i>	Median fibrinogen (g/l)	Median SD (g/l)
AT-deficient	46	2.21	0.13	23	2.53*	0.11	17	2.56*	0.14
Normal	47	2.55	0.16	25	2.84*	0.13	17	2.80*	0.24
Factor VIII deficient	30	2.84	0.13	21	3.13*	0.13	13	3.18*	0.17
Factor VIII deficient	44	3.42	0.18	25	3.65*	0.21	17	3.73*	0.21
Coumarin	32	3.30	0.19	26	3.60*	0.19	21	3.92**	0.33
Coumarin	45	2.99	0.17	23	3.37*	0.16	17	3.63**	0.23

n, number of laboratories.

*Significant difference with Claus methods (Dade Behring); **significant difference with Claus methods (Dade Behring and Roche).

the four test plasmas were determined by each participant using the new calibration curve. The participants were requested to report their routine and new calibration curves.

Student's *t*-test was used to assess differences in fibrinogen levels between methods. A significance level of 5% was used. Differences between fibrinogen concentrations determined with the routine and new calibration curves were tested with Student's *t*-test on paired observations. Differences in coefficient of variation (CV) were tested with Snedecor's variance ratio test (*F*-test), as described by Moroney (1968).

RESULTS

Assessment of the state-of-the-art SD

Six lyophilized plasmas were analysed by participants of the Dutch EQAS, in multiple surveys comprising a period of approximately 2 years. Some participants provided measurements from 12 surveys but others from only four. Three major groups of assay methods were selected, i.e. participants using reagents from Roche Diagnostics for the assay according to Claus, participants using reagents from Dade Behring (Marburg, Germany) for the assay according to Claus and participants using reagents from Instrumentation Laboratory for the PT-derived fibrinogen assay. These three groups represented approximately 75% of all

Dutch participants in the study period. The results are shown in Table 1. For the Claus methods, the mean fibrinogen levels obtained with Roche reagents were significantly ($P < 0.05$) higher than those obtained with Dade Behring reagents. For four lyophilized plasma samples (i.e. AT-deficient, Normal and two factor VIII-deficient), the differences between the Claus method using Roche reagents and the PT-derived methods were not significant. The mean fibrinogen levels determined with PT-derived methods were significantly higher than those determined with Claus methods using Dade Behring reagents. For the two coumarin plasmas, the mean fibrinogen levels determined with PT-derived methods were higher than those with all Claus methods.

The median intralaboratory variation determined for the PT-derived methods was greater than that for the Claus methods (Table 1). For each lyophilized plasma and each technical group, the logarithm of the median of the intralaboratory SD was plotted as a function of the median fibrinogen measurement (Figure 2). A linear regression line was calculated and the resulting formula $\ln(\text{SD}_{\text{SA}}) = -3.076 + 0.418 \times F$ was used for analysis of the twin study.

Twin study

Results were obtained from 48 laboratories forming 24 pairs. The squared correlation coefficients of the

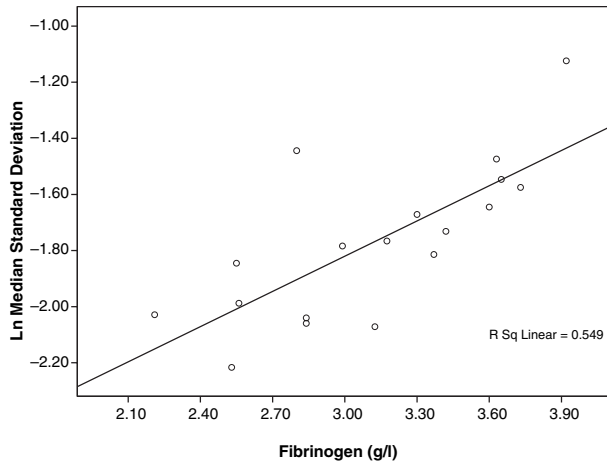


Figure 2. Natural logarithm (ln) of the median intra-laboratory standard deviation of the fibrinogen concentration as a function of the median fibrinogen concentration (g/l) for lyophilized test plasmas in the Dutch external quality assessment scheme.

Table 2. Mean fibrinogen levels in potential calibrators determined by the participants of the twin study

Potential calibrator	Claus methods (n = 42)		PT-derived methods (n = 6)	
	Mean (g/l)	SD	Mean (g/l)	SD
1	2.86	0.24	2.93	0.22
2	2.72	0.22	2.64	0.14
3	2.60	0.24	2.58	0.13

n, number of laboratories; SD, between-laboratory standard deviation.

fibrinogen concentrations of the fresh-frozen patients' samples ranged between 0.837 and 0.997 with a mean value of 0.957. The mean fibrinogen concentrations of the three potential calibrators determined with Claus methods were similar to those with the PT-derived methods (Table 2). The normalized regression residuals of the three potential calibrators are shown in Figure 3. Potential calibrator number 1 had a normalized residual greater than 3.0 in only one laboratory couple. In three laboratory pairs, the normalized residuals were greater than 3.0 for potential calibrator number 2 and potential calibrator number 3. The normalized residuals greater than 3.0 were observed in laboratory pairs in which one laboratory used the

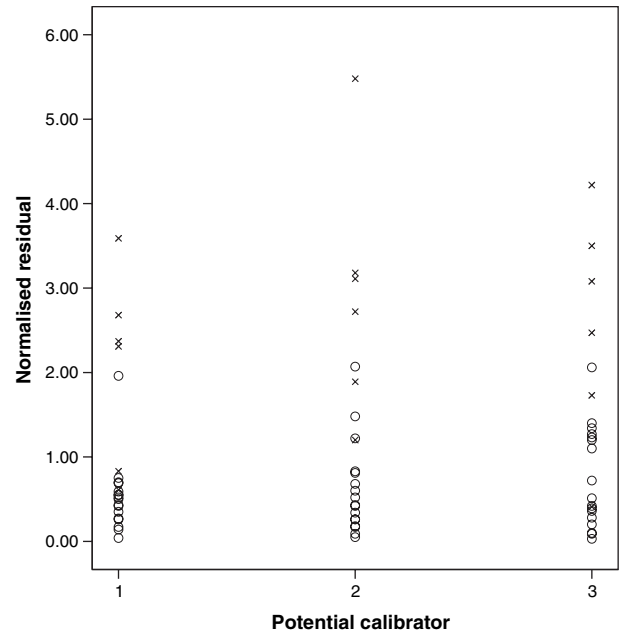


Figure 3. Normalized regression residuals for the potential calibrators to the patients' regression line, as derived from the twin study. Normalized residuals were calculated as multiples of SD_{SA} . Laboratory pairs using Claus methods only or Claus/precipitation methods are indicated by open circles (O). Mixed pairs using Claus and PT-derived methods are indicated by crosses (X).

Claus method and the other a PT-derived fibrinogen assay. The normalized residuals in the one laboratory couple using an ammonium sulphate precipitation assay were 0.69, 0.68 and 1.10 for the three potential calibrators, respectively. The pattern of the normalized residuals was similar for the three potential calibrators. It was decided to select the lyophilized potential calibrator number 2 for the value assignment and the study of the effect of harmonization, because the shipment of lyophilized material is easier and less expensive than that of deep-frozen plasma samples.

Value assignment

For each laboratory, the logarithms of the clotting times were plotted against the logarithms of the dilutions. The lines for the standard and the selected potential calibrator number 2 were parallel in four laboratories. The results from two other laboratories were rejected because the lines for the standard and

the potential calibrator were not parallel. Probably, the latter two laboratories made errors in the dilutions of the international standard or the potential calibrator. The fibrinogen concentration of potential calibrator number 2 was calculated from the regression line of the standard. The calculated fibrinogen concentrations by the four laboratories were: 2.49, 2.58, 2.60 and 2.52 g/l, respectively. The mean value (2.55 g/l) was used for the assessment of harmonization.

Effect of harmonization

Results were obtained from 60 laboratories participating in the Dutch EQAS. Of these, 54 used Clauss methods and five used PT-derived fibrinogen assays. One laboratory used a turbidimetric method. The mean fibrinogen concentrations obtained by laboratories using Roche reagents were greater than the mean levels obtained by the laboratories using Dade Behring reagents (Table 3). After calibration with calibrator number 2, the differences between these groups were reduced. The overall mean fibrinogen concentrations obtained with the routine methods were slightly greater than the mean concentrations obtained after calibration with the common calibrator (Table 3).

For coumarin samples A and D, the PT-derived fibrinogen concentrations read from the routine calibration curves were 13% and 15% greater than the levels obtained with the Clauss methods. Using potential calibrator 2 for the new calibration line, the laboratories with the PT-derived methods still obtained

levels for samples A and D that were 13% greater than the levels reported by the laboratories using Clauss methods.

The interlaboratory variation of the fibrinogen concentrations is reported in Table 3. There was a trend to lower interlaboratory variation following calibration with the common calibrator number 2. Over all methods, there was a significant ($P < 0.05$) reduction in the interlaboratory CV for samples A, B and D. For some samples (e.g. sample C, Dade Behring Clauss methods) the interlaboratory CV was higher after harmonization. This could be explained by the observation that some laboratories made errors in the reading of the calibration curve prepared with the common calibrator. We did not correct these errors or exclude the data from these laboratories because they represent 'real life'.

DISCUSSION

In the Dutch EQAS for plasma fibrinogen, significant differences were observed between the major technical groups (Table 1). The origin of the differences was not clear but might be because of calibration errors or lack of commutability of the lyophilized plasma samples or a combination of these. Thus, part of the overall interlaboratory variation could be explained by systematic differences between methods.

The state-of-the-art intralaboratory SD of the fibrinogen assay was estimated from repeated measurements in lyophilized plasmas by approximately 75% of Dutch

Table 3. Mean fibrinogen concentrations (g/l) and interlaboratory coefficient of variation (CV in %) in four lyophilized test plasma samples obtained by laboratories using their routine and the common calibrator calibration curves

Plasma	Clauss, Dade Behring (<i>n</i> = 29)				Clauss, Roche (<i>n</i> = 16)				PT-derived (<i>n</i> = 5)				All methods (<i>n</i> = 60)			
	Routine		Common		Routine		Common		Routine		Common		Routine		Common	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
A	3.16	9.2	3.11	7.6	3.50	6.1	3.27	8.0	3.86	9.1	3.62	5.2	3.29	10.8	3.23	8.2*
B	3.61	8.3	3.55	5.6	3.77	5.0	3.55	5.4	3.53	12.2	3.37	5.6	3.61	8.4	3.57	6.0*
C	2.67	8.9	2.66	11.2	2.82	6.6	2.65	4.4	2.53	9.0	2.44	7.7	2.65	10.7	2.63	9.2
D	3.47	8.0	3.42	5.7	3.84	7.7	3.60	7.5	4.26	17.3	3.94	8.3	3.63	11.4	3.56	7.9*

**F*-test: $P < 0.05$.

clinical laboratories. We assumed that the lyophilized plasmas were appropriate for this purpose. The SD increased with increasing fibrinogen level. Although two lyophilized test plasmas had a relatively high median intralaboratory SD for the PT-derived fibrinogen assays in comparison with the Clauss assays (see Table 1), the results from PT-derived assays and Clauss methods were taken together for the estimation of the average relationship between the logarithm of the SD and the mean fibrinogen concentration (Figure 2). The regression line represented the SD_{SA} as a function of the fibrinogen level. SD_{SA} was used to calculate the normalized residuals for three potential calibrators in the twin study.

In the twin study, all couples using only Clauss methods had low normalized residuals for the three potential calibrators. Furthermore, one couple using Clauss and ammonium sulphate precipitation methods had low normalized residuals as well. There were six couples in which one laboratory used a Clauss method and the other a PT-derived method. Three of these six couples had normalized residuals greater than 3 for one or more potential calibrators (Figure 3). This suggested that the potential calibrators were commutable for the laboratories using only Clauss methods but not for all laboratories using a PT-derived method. There are a few possible explanations for the limited commutability of the potential calibrators. Calibrators number 2 and 3 were lyophilized and the properties of fibrinogen may be altered by the lyophilization (Jensen *et al.*, 2002). Secondly, calibrators number 1 and 2 were prepared from CPD-blood, while routine plasmas are collected in plain citrate. It cannot be excluded that the different composition of the potential calibrators influenced the commutability using the PT-derived method.

For practical reasons, potential calibrator number 2 was chosen to study the effect of local calibration on the harmonization of the assay. Potential calibrator number 2 and the four test plasmas were freeze-dried materials and could be mailed to the participants at ambient temperatures. The mean fibrinogen levels measured by the Clauss methods were slightly reduced by calibration with calibrator number 2 (Table 3). After calibration with calibrator number 2, the differences in the mean fibrinogen levels (Clauss method) between Dade Behring and Roche reagents were reduced (Table 3). This suggests that the differ-

ences between Dade Behring and Roche systems were because of the calibrators provided by these manufacturers. There was a trend to lower interlaboratory variation of the Clauss methods after calibration with the common calibrator number 2. This trend was also observed for the PT-derived methods. When all methods were taken together, there was a modest, significant reduction of the interlaboratory variation for three of four test plasmas. The average overall CV for the four test plasmas was 10.3% using the routine fibrinogen measurements and 7.8% after harmonization using calibrator number 2 (Table 3). Using the formula $\ln(SD_{SA}) = -3.076 + 0.418 \times F$ we calculated the CV(state of the art) for the four test plasmas (all methods, $n = 60$). The CV(state of the art) ranged from 5.3% (sample C), 5.5% (sample A) to 5.8% (samples B and D). Thus, the interlaboratory CV after harmonization was only slightly higher than the average intralaboratory CV.

For the two test plasmas derived from patients treated with oral anticoagulants (A and D), the mean fibrinogen levels obtained with Clauss methods were lower than the mean levels obtained with the PT-derived methods. For test plasmas A and D, the difference in the mean fibrinogen levels between the Clauss methods and the PT-derived methods was reduced slightly after local calibration with potential calibrator number 2.

Our study confirms the conclusion of a previous report that the use of a common calibrator improved interlaboratory agreement (Chantarangkul, Tripodi & Mannucci, 1994). Furthermore, our study confirms previous observations of systematic overestimation of PT-derived fibrinogen levels in plasmas from patients on oral anticoagulant therapy (De Cristofaro & Landolfi, 1998; Mackie *et al.*, 2002). Coumarin plasma, by producing a lower thrombin amount, can generate higher amounts of thick fibrin fibres responsible for higher turbidity values, leading to overestimation of the PT-derived fibrinogen levels (De Cristofaro & Landolfi, 1998). The relationship between Clauss methods and PT-derived methods for coumarin plasmas is not the same as the relationship for plasmas in the normal range. The bias observed for coumarin plasmas cannot be eliminated by using a common calibrator. Therefore, we recommend that fibrinogen assays of coumarin plasmas should be performed with the Clauss method.

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REFERENCES

- Baadenhuijsen H., Steigstra H., Cobbaert C., Kuypers A., Weykamp C. & Jansen R. (2002) Commutability assessment of potential reference materials using a multicenter split-patient-sample between-field-methods (twin-study) design: study within the framework of the Dutch project 'Calibration 2000'. *Clinical Chemistry* 48, 1520–1525.
- Bakker A.J., Gorgels J.P.M.C., Draaisma J., Jongendijk M., Altena L., Hamersma A. & Weiland A. (1992) Simple method for correcting total protein in plasma for actual fibrinogen content. *Clinical Chemistry* 38, 2221–2223.
- Chantarangkul V., Tripodi A. & Mannucci P.M. (1994) Results of a collaborative study for fibrinogen measurement. Evidence that the use of a common calibrator improves interlaboratory agreement. *Blood Coagulation and Fibrinolysis* 5, 761–766.
- Clauss A. (1957) Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. *Acta haematologica* 17, 237–246.
- De Cristofaro R. & Landolfi R. (1998) Measurement of plasma fibrinogen concentration by the prothrombin-time-derived method: applicability and limitations. *Blood Coagulation and Fibrinolysis* 9, 251–259.
- Fibrinogen Studies Collaboration (2005) Plasma fibrinogen level and the risk of major cardiovascular diseases and non-vascular mortality: an individual participant meta-analysis. *JAMA* 294, 1799–1809.
- Jensen T., Halvorsen S., Godal H.C. & Skjøsberg O.H. (2002) Influence of freeze-drying on the clotting properties of fibrinogen in plasma. *Thrombosis Research* 105, 499–502.
- Kallner A., Egberg N., Yu A., Merzoug-Djilali R., Blomberg I., Jörneskog G. & Blombäck M. (2003) Are results of fibrinogen measurements transferable? *Clinical Chemistry and Laboratory Medicine* 41, 804–808.
- Mackie I.J., Lawrie A.S., Kitchen S., Gaffney P.J., Howarth D., Lowe G.D.O., Martin J., Purdy G., Bigsby P. & Rumley A. (2002) A performance evaluation of commercial fibrinogen reference preparations and assays for Clauss and PT-derived fibrinogen. *Thrombosis and Haemostasis* 87, 997–1005.
- Miller W.G. (2003) Specimen materials, target values and commutability for external quality assessment (proficiency testing) schemes. *Clinica Chimica Acta* 327, 25–37.
- Moroney M.J. (1968) *Facts from Figures*. Penguin Books, Harmondsworth, UK.
- Panteghini M. (2007) Traceability, reference systems and result comparability. *The Clinical Biochemist. Reviews* 28, 97–104.
- Rossi E., Mondonico P., Lombardi A. & Preda L. (1988) Method for the determination of functional (clottable) fibrinogen by the new family of ACL coagulometers. *Thrombosis Research* 52, 453–468.
- Takamiya O., Hando S., Tekondo M., Bando S., Ohkura M., Negoro T., Yoshida T., Higashi K., Ohnuma O., Kubota K. & Tatsumi N. (2005) Japanese collaborative study for fibrinogen assay: variability of the fibrinogen assay between different laboratories does not improve when a common calibrator is used. *Clinical and Laboratory Haematology* 27, 177–183.
- Van den Besselaar A.M.H.P., Haas F.J.L.M. & Kuypers A.W.H.M. (2006) Harmonisation of factor VIII:C assay results: study within the framework of the Dutch project 'Calibration 2000'. *British Journal of Haematology* 132, 75–79.
- Whitton C.M., Sands D., Hubbard A.R. & Gaffney P.J. (2000) A collaborative study to establish the 2nd international standard for fibrinogen, plasma. *Thrombosis and Haemostasis* 84, 258–262.