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Fibrinogen determination according to Clauss: commutability assessment of International and commercial standards and quality control samples

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Abstract

Background: Many clinical laboratories use a clotting rate assay according to Clauss for the determination of fibrinogen in citrated plasma. The aim of the present study was to assess the commutability of the current International Standard for fibrinogen (coded 09/264), three commercial fibrinogen standards, and 10 freeze-dried plasma quality control samples from various sources.
Methods: Clotting rate assays according to Clauss were performed on three automated instruments (Sysmex CA1500, STA-Rack Evolution and ACL-Top 700), using three commercial thrombin reagents (Siemens, Stago, and Instrumentation Laboratory). Relationships between the results obtained with the three instruments were determined with 25 fresh-frozen plasma samples obtained from patients. The deviations of the assay results obtained with the freeze-dried samples were compared with the deviations obtained with the fresh-frozen samples, according to approved CLSI guideline C53A.
Results: Freezing and thawing had no influence on the assay results. There were significant differences in the mean assay results (fibrinogen, g/L) for the fresh-frozen plasma samples between the three automated instruments: 2.51 (STA-Rack Evolution), 2.25 (ACL-Top 700) and 2.20 (Sysmex CA1500). Similar differences were observed for several freeze-dried plasma samples. Some freeze-dried plasma samples, including the International Standard, were out of the 95% confidence interval for the relationship between STA-Rack Evolution and Sysmex CA1500.
Conclusions: Some freeze-dried plasmas including the international standard for fibrinogen are not commutable among automated instruments for fibrinogen clotting rate assays according to Clauss. Our results have consequences for all interested parties in the traceability chain (WHO, industry, external quality assessment schemes, clinical laboratories).

Keywords: Clauss assay; commutability; external quality assessment; fibrinogen.

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Introduction

Many methods have been published for the determination of fibrinogen in plasma [1, 2]. The majority of clinical laboratories in the USA used the clotting rate assay according to Clauss [3, 4]. Also in other countries, e.g. the Netherlands, the clotting rate assays have been used [5] and are still the most used assays in clinical practice

(data from the Dutch Foundation for Quality Assurance in Medical Laboratories [SKML]). Most laboratories now use commercial reagents and an automated method, and a multiplicity of products are available, varying in thrombin strength, buffer composition, calibration method and dilution range [6]. Several studies have shown that there can be systematic differences between the fibrinogen results obtained with various commercial kits [7]. A comparison of the various fibrinogen standards used in commercial assay kits has generated concern in that the claimed levels differed more than 30% from the measurement with a reference method [8, 9]. These reports prompted the National Institute for Biological Standards and Control, in cooperation with the Fibrinogen Subcommittee of the International Society on Thrombosis and Haemostasis, to organize a study with a view to establish a standard for plasma fibrinogen [10]. This preparation was established by the World Health Organization (WHO) as the first international standard Fibrinogen Plasma. In later years, it has been replaced by the second and third international standards [11, 12].

It is now generally accepted in laboratory medicine that reference materials should be commutable. The concept of commutability was originated and first applied to enzyme activity measurements to emphasize that the materials for both internal and external quality control programs must exhibit properties comparable with those of clinical specimens. It implies that the relationship between any two analytical procedures for patient specimens would also apply to a commutable reference material. To better clarify these issues, the Clinical and Laboratory Standards Institute (CLSI) recommends the use of the following definition for the term commutability: the equivalence of the mathematical relationships among the results of different measurement procedures for a reference material and for representative samples of the type intended to be measured [13].

Data from a series of the Dutch Foundation for Quality Assurance in Medical Laboratories (SKML) proficiency testing exercises between 2012 and 2015 demonstrated a significant difference in Clauss fibrinogen results obtained by users of various commercial assay kits. The origin of the observed differences may be due to either a calibration error or lack of commutability of the freeze-dried standards or control samples or both.

The purpose of the present study was to assess the commutability of three commercial standards, the international standard and various freeze-dried quality control materials for fibrinogen determination in citrate plasma.

Materials and methods

The WHO international standard for fibrinogen plasma (coded 09/264) was obtained from the National Institute for Biological Standards and Control (Potters Bar, UK). Standard human plasma and Dade Thrombin Reagent were obtained from Siemens Healthcare Diagnostics Products GmbH (Marburg, Germany). STA-Unicalibrator and STA-Fib reagent were obtained from Diagnostica Stago (Asnières, France). HemosIL Calibration Plasma and HemosIL Fibrinogen-C reagent were obtained from Instrumentation Laboratory (Werfen, Breda, The Netherlands). Fibrinogen from human plasma was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands).

Fibrinogen determinations according to Clauss were performed with Dade Thrombin Reagent on a Sysmex CA-1500 instrument (Sysmex Corporation, Kobe, Japan), with STA-Fib reagent on a STA-Rack Evolution instrument (Diagnostica Stago, Asnières, France) and with HemosIL Fibrinogen-C reagent using ACL Top 700 instrument (Instrumentation Laboratory, Bedford, MA, USA). Dilutions of plasma samples were made with Dade Owren's Veronal Buffer, STA Owren Koller buffer and with HemosIL Factor Diluent, respectively. Multiple dilutions were used for each plasma sample. For the standards, five different dilutions were used, and for the other samples, at least three different dilutions.

Venous blood was collected in Vacutainer tubes containing 0.105 mol/L buffered sodium citrate (Becton Dickinson, Plymouth, UK). Citrated blood samples were centrifuged for 10 min at 2700 *g* at 20 °C. Residual sodium citrate plasma prepared from samples of patients that was collected as part of their routine care was frozen in plastic tubes at −80 °C. In a preliminary experiment, fibrinogen was determined in fresh samples before freezing and after storage at −80 °C. Frozen samples were thawed in a water bath at 37 °C for 5 min. Fibrinogen was determined with STA-Fib reagent on a STA-Rack Evolution instrument.

Various freeze-dried quality control (QC) plasma samples prepared for external quality assessment of coagulation tests were obtained from the ECAT Foundation (Voorschoten, The Netherlands) and from the Section Coagulation of the Dutch Foundation for Quality Assurance in Medical Laboratories (SKML; Nijmegen, The Netherlands). The freeze-dried QC plasma samples were stored in siliconized glass vials and capped with rubber stoppers. The QC samples were reconstituted with 1 mL of purified water per vial. The freeze-dried QC samples provided by SKML were buffered with 20 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). One QC sample was prepared by pooling plasma samples of healthy individuals (coded HNP-21). A second QC sample consists of a pool of plasma samples of patients on vitamin K antagonists (coded Cou-32). Three QC samples, coded Fib-1, Fib-2 and Fib-3, were prepared by artificial depletion of plasma fibrinogen using salting-out procedures [14]. Two QC samples, coded Fib-5 and Fib-6, were prepared by spiking pooled normal plasma with human fibrinogen. Fib-5 and Fib-6 were prepared from the same batch but 10% cryolyoprotectant (Streekziekenhuis Koningin Beatrix, Winterswijk, The Netherlands) was added to Fib-6 before freeze-drying.

Statistical analysis

Fibrinogen concentrations in test samples were calculated using the parallel line model of log-transformed clotting times against log-transformed fibrinogen concentrations of the reference material,

using the program CombiStats (European Directorate for the Quality of Medicines and Healthcare, Strasbourg, France). In this program, the statistical significance of deviations from parallelism or linearity is assessed by analysis of variance [15]. In addition, CombiStats generated 95% confidence limits for each fibrinogen assessment.

Fibrinogen concentrations determined with the three above-mentioned Clauss assays were plotted against each other. Orthogonal regression lines ($Y = a + b \cdot X$, in which Y represents the fibrinogen level measured with the assay method plotted along the vertical axis and X the level measured with the method along the horizontal axis) and the standard deviation about the line (SD_L) were calculated as described previously [16]. The perpendicular distance of a point (x, y) to the line was calculated with the formula $d = |y - b \cdot x - a| / \sqrt{(b^2 + 1)}$. The residual of each point to the regression line, i.e. its perpendicular distance to the line, was normalized by calculating the ratio of the distance to the standard deviation, i.e. d/SD_L.

Student's t-test on paired observations was used for comparison of fibrinogen levels determined with different assays (SPSS Statistics version 23, IBM Corporation, Armonk, NY, USA). A significance level of 0.05 was used.

Results

Fibrinogen levels reported by participants of the Dutch External Quality Assessment Scheme (SKML) are shown in Table 1. One QC sample (HNP-21) was used in 14 consecutive surveys. The differences between the mean levels obtained with three reagents were statistically significant. The differences between the mean levels of another QC sample (Cou-32) were significant when STA-Fib was compared to Fibrinogen-C and when STA-Fib was compared to Dade Thrombin reagent.

Fibrinogen levels determined with STA-Fib in 10 fresh samples were compared to those measured after freezing and thawing (Table 2). There was no significant difference demonstrating that frozen samples could be used for commutability studies.

Fibrinogen levels in 25 fresh frozen samples determined with three systems are shown in Table 3. In all assays the international standard (coded 09/264) was used for calibration of the assays and calculation of the fibrinogen levels. The 95% confidence intervals generated by the CombiStats program increased in the order STA-Fib < Dade Thrombin < Fibrinogen-C. For several samples a significant deviation from parallel lines was observed. The differences in fibrinogen levels between STA-Fib and Fibrinogen-C and the differences between STA-Fib and Dade Thrombin were significant ($p < 0.001$).

Table 1: Fibrinogen levels reported by participants of the Dutch External Quality Assessment Scheme (SKML).

QC Sample	Survey	Dade Thrombin			STA-Fib			Fibrinogen-C		
		n	Mean	SD	n	Mean	SD	n	Mean	SD
HNP-21	2010-2	58	2.68	0.15	38	2.80	0.14	13	2.71	0.16
	2010-3	59	2.64	0.20	41	2.82	0.14	17	2.85	0.15
	2010-4	60	2.67	0.18	40	2.85	0.17	18	2.91	0.19
	2010-5	61	2.74	0.18	39	2.83	0.14	17	2.83	0.13
	2010-6	54	2.64	0.15	40	2.79	0.15	13	2.68	0.22
	2011-1	58	2.71	0.15	43	2.80	0.14	15	2.86	0.14
	2011-2	54	2.71	0.14	43	2.80	0.14	15	2.71	0.19
	2011-3	59	2.72	0.17	44	2.83	0.13	16	2.82	0.24
	2011-4	61	2.74	0.18	45	2.86	0.15	12	2.81	0.18
	2011-5	62	2.75	0.14	45	2.87	0.13	12	2.83	0.12
	2011-6	60	2.73	0.12	44	2.88	0.12	11	2.78	0.19
	2012-1	57	2.68	0.14	44	2.88	0.16	14	2.70	0.18
	2012-2	55	2.74	0.17	43	2.89	0.16	13	2.65	0.12
	2012-3	57	2.72	0.15	46	2.89	0.11	15	2.63	0.12
	Weighted mean		2.71			2.84			2.77	
	Significance		$p < 0.001$[a]			$p = 0.019$[b]			$p = 0.025$[c]	
Cou-32	2012-5	58	3.45	0.17	46	3.68	0.25	13	3.42	0.12
	2013-1	60	3.45	0.20	43	3.75	0.17	14	3.34	0.21
	2013-3	56	3.49	0.16	48	3.74	0.21	14	3.25	0.21
	2013-5	57	3.45	0.19	44	3.83	0.17	13	3.32	0.20
	Weighted mean		3.46			3.75			3.33	
	Significance		$p = 0.003$[a]			$p = 0.005$[b]			$p = 0.06$[c]	

Mean fibrinogen levels (g/L) obtained with three reagents (Dade Thrombin, STA-Fib, and HemosIL Fibrinogen-C) and the between-laboratory standard deviation (SD) are shown. n is the number of reported results. [a]Dade Thrombin vs. STA-Fib. [b]STA-Fib vs. Fibrinogen-C. [c]Fibrinogen-C vs. Dade Thrombin.

Table 2: Fibrinogen levels (g/L) in fresh and frozen/thawed samples.

Sample	Before freezing	After freezing/ thawing
A	3.2	3.1
B	3.3	3.2
C	2.4	2.3
D	3.8	3.8
E	2.7	2.8
F	6.9	7.0
G	4.7	4.6
H	7.3	7.2
I	2.5	2.6
J	2.0	2.1
Average (n = 10)	3.88	3.86
Significance	p = 0.399	

Fibrinogen was determined with STA-Fib reagent on a STA-Rack Evolution instrument.

The differences between Dade Thrombin and Fibrinogen-C were not significant.

Fibrinogen levels in the three commercial standards and 10 QC samples determined with three systems are shown in Table 4. In all assays the international standard (coded 09/264) was used for the calibration of the assays and calculation of the fibrinogen levels. For several samples, a significant deviation from parallel lines was observed. The differences in fibrinogen levels between STA-Fib, Fibrinogen-C and Dade Thrombin were significant. There was a trend of lower fibrinogen levels obtained with Dade Thrombin as compared with the manufacturers' stated fibrinogen values for their respective standards.

Fibrinogen levels were also determined using the manufacturers' stated values for their respective standards for construction of the reference lines. Table 5 shows the results for the 25 frozen samples. The highest dilution of the standard human plasma for the assay with Dade Thrombin induced non-parallelism for many test samples and was excluded for the calculations. The differences between STA-Fib and Fibrinogen-C and the differences between STA-Fib and Dade Thrombin were significant. Table 6 shows the results for the freeze-dried standards and QC samples. The differences for the QC samples between STA-Fib and Fibrinogen-C were significant.

Table 3: Fibrinogen levels (g/L) in frozen samples determined with three assay systems, using the international standard for the calibration line.

Sample number	Dade Thrombin	STA-Fib	Fibrinogen-C
1	2.63[a] (2.47–2.80)	2.97 (2.90–3.05)	2.59 (2.37–2.82)
2	2.12[a] (1.99–2.25)	2.28 (2.22–2.34)	2.15 (1.98–2.33)
3	2.38[a] (2.23–2.54)	2.56[a] (2.49–2.64)	2.39[a] (2.21–2.59)
4	2.95 (2.77–3.14)	3.54 (3.45–3.63)	3.38 (3.11–3.67)
5	1.77 (1.66–1.88)	2.01 (1.96–2.07)	1.78 (1.63–1.94)
6	2.70 (2.48–2.96)	3.18 (3.09–3.27)	3.03 (2.79–3.30)
7	2.10[a] (1.96–2.24)	2.37 (2.31–2.44)	2.14[a] (1.97–2.31)
8	2.32 (2.16–2.49)	2.79 (2.72–2.87)	2.58 (2.38–2.80)
9	2.56[a] (2.40–2.74)	2.79 (2.72–2.86)	2.72[a] (2.51–2.95)
10	2.57 (2.41–2.74)	2.96 (2.87–3.05)	2.85 (2.60–3.13)
11	2.20[a] (2.07–2.34)	2.47 (2.41–2.53)	2.18 (2.02–2.36)
12	2.89 (2.71–3.08)	3.43 (3.34–3.52)	3.10 (2.86–3.37)
13	0.89[a] (0.84–0.93)	1.08[a] (1.05–1.10)	0.89 (0.73–1.07)
14	1.91 (1.79–2.05)	2.18 (2.12–2.24)	1.95 (1.80–2.11)
15	1.68 (1.59–1.77)	1.92[a] (1.87–1.97)	1.71[a] (1.56–1.87)
16	2.71 (2.51–2.92)	3.22 (3.14–3.30)	2.83 (2.60–3.07)
17	0.61 (0.58–0.65)	0.74 (0.73–0.76)	0.66 (0.58–0.76)
18	1.61 (1.53–1.69)	1.89[a] (1.84–1.93)	1.60[a] (1.44–1.76)
19	2.44[a] (2.29–2.60)	2.65 (2.59–2.72)	2.36 (2.18–2.55)
20	2.93 (2.75–3.12)	3.38 (3.29–3.48)	2.92 (2.69–3.18)
21	2.23[a] (2.10–2.38)	2.50 (2.44–2.56)	1.85[a] (1.71–2.01)
22	2.68 (2.51–2.86)	3.19 (3.09–3.28)	2.83 (2.60–3.09)
23	2.19[a] (2.06–2.34)	2.46 (2.40–2.52)	2.14[a] (1.96–2.33)
24	2.43[a] (2.29–2.59)	2.64 (2.56–2.71)	2.25 (2.07–2.46)
25	1.43 (1.35–1.50)	1.65[a] (1.60–1.69)	1.37[a] (1.22–1.52)
Average (n = 25)	2.20	2.51	2.25
Significance	p < 0.001 (Dade Thrombin vs. STA-Fib)	p < 0.001 (STA-Fib vs. Fibrinogen-C)	p = 0.129 (Fibrinogen-C vs. Dade Thrombin)

In parentheses: 95% confidence limits. [a]Non-parallelism.

Table 4: Fibrinogen levels (g/L) in freeze-dried commercial standards and control samples determined with three assay systems, using the international standard for the calibration line.

	Stated value	Dade Thrombin	STA-Fib	Fibrinogen-C
Standard human plasma	2.44	2.31[a] (2.19–2.44)	2.62 (2.57–2.68)	2.50[a] (2.35–2.66)
STA-Unicalibrator	3.10	2.65[a] (2.50–2.80)[b]	3.05 (2.98–3.12)	2.81[a] (2.62–3.01)
HemosIL Calibration Plasma	3.15	2.92[a] (2.74–3.11)[b]	3.10 (3.04–3.16)	3.15 (2.87–3.45)
QC sample: ECAT normal	–	3.05[b] (2.89–3.22)	3.25[a] (3.18–3.31)	3.19 (2.93–3.48)
QC sample: ECAT abnormal	–	1.07 (0.98–1.16)	1.11 (1.07–1.14)	1.08 (0.99–1.19)
QC sample: ECAT INR = 2.5	–	2.57[a] (2.34–2.83)	2.58 (2.51–2.65)	2.29 (2.08–2.53)
QC sample: SKML Fib-1	–	0.86 (0.78–0.93)	0.92 (0.88–0.96)	0.91 (0.83–0.99)
QC sample: SKML Fib-2	–	1.30[a] (1.22–1.39)	1.33 (1.29–1.37)	1.32 (1.20–1.45)
QC sample: SKML Fib-3	–	2.20 (2.03–2.38)	2.49 (2.41–2.57)	2.47 (2.26–2.70)
QC sample: SKML Fib-5	–	3.93[a] (3.67–4.23)	4.67 (4.53–4.82)	4.63 (4.21–5.12)
QC sample: SKML Fib-6	–	4.19[a] (3.92–4.51)	4.79 (4.63–4.95)	4.70 (4.27–5.21)
QC sample: SKML HNP-21	–	2.79[a] (2.61–2.99)	2.96 (2.88–3.03)	2.75 (2.52–2.99)
QC sample: SKML Cou-32	–	3.40[a] (3.19–3.63)	3.87 (3.75–3.99)	3.67 (3.36–4.03)
Average (n = 13)	–	2.56	2.83	2.73
Significance		p = 0.001 (Dade Thrombin vs. STA-Fib)	p = 0.006 (STA-Fib vs. Fibrinogen-C)	p = 0.028 (Fibrinogen-C vs. Dade Thrombin)

In parentheses: 95% confidence limits. [a]Non-parallelism. [b]Non-linearity.

Table 5: Fibrinogen levels (g/L) in frozen samples determined with three assay systems.

Sample number	Reagent: Dade Thrombin Standard: SHP (Siemens)	Reagent: STA-Fib Standard: Unicalibrator	Reagent: Fibrinogen-C Standard: HemosIL Calibration Plasma
1	2.73[b] (2.69–2.78)	3.02 (2.96–3.08)	2.58 (2.34–2.85)
2	2.16[b] (2.13–2.19)	2.33 (2.27–2.38)	2.15 (1.96–2.36)
3	2.45[a] (2.39–2.50)[b]	2.61[a] (2.55–2.68)	2.39[a] (2.18–2.62)
4	3.13[a] (3.09–3.18)[b]	3.59[b] (3.51–3.66)	3.35 (3.04–3.72)
5	1.78[a] (1.75–1.80)[b]	2.06 (2.01–2.11)	1.79 (1.62–1.98)
6	2.85 (2.62–3.10)	3.23 (3.15–3.30)	3.01 (2.73–3.33)
7	2.13 (2.06–2.20)	2.42 (2.36–2.48)	2.14 (1.95–2.34)
8	2.43[a] (2.30–2.56)	2.84 (2.78–2.90)	2.57 (2.34–2.83)
9	2.66 (2.57–2.74)	2.84 (2.78–2.90)	2.70 (2.46–2.98)
10	2.69[a] (2.62–2.75)	3.01 (2.93–3.09)	2.84 (2.56–3.17)
11	2.25[a] (2.23–2.28)[b]	2.52[b] (2.46–2.57)	2.19 (1.99–2.40)
12	3.08[a] (2.98–3.17)	3.47 (3.40–3.55)	3.07 (2.79–3.40)
13	0.93[a] (0.92–0.94)[b]	1.10[a] (1.08–1.12)	0.91 (0.73–1.09)
14	1.94[a] (1.86–2.02)	2.22 (2.17–2.28)	1.96 (1.79–2.15)
15	1.76[a] (1.73–1.80)[b]	1.96[a] (1.92–2.01)	1.73 (1.56–1.91)
16	2.86[a] (2.70–3.04)	3.27[b] (3.20–3.34)	2.81 (2.56–3.11)
17	0.66[a] (0.65–0.67)[b]	0.76 (0.74–0.78)	0.67 (0.57–0.77)
18	1.68[a] (1.66–1.70)[b]	1.93 (1.89–1.97)	1.62[a] (1.44–1.80)
19	2.52[b] (2.47–2.57)	2.70 (2.64–2.76)	2.35 (2.15–2.58)
20	3.10[a] (3.06–3.14)[b]	3.43 (3.35–3.51)	2.91 (2.64–3.23)
21	2.29 (2.23–2.34)	2.54[b] (2.49–2.59)	1.87[a] (1.70–2.05)
22	2.83[a] (2.73–2.94)	3.23 (3.15–3.32)	2.82 (2.55–3.13)
23	2.25 (2.19–2.31)	2.50 (2.45–2.56)	2.14 (1.95–2.36)
24	2.50[a] (2.47–2.53)[b]	2.68 (2.62–2.75)	2.26 (2.04–2.49)
25	1.48[a] (1.47–1.50)[b]	1.69 (1.65–1.73)	1.39 (1.22–1.56)
Average (n = 25)	2.29	2.56	2.25
Significance	p < 0.001 (Dade Thrombin vs. STA-Fib)	p < 0.001 (STA-Fib vs. Fibrinogen-C)	p = 0.187 (Fibrinogen-C vs. Dade Thrombin)

For each assay system, the corresponding commercial standard was used to construct the calibration line. In parentheses: 95% confidence limits. [a]Non-parallelism. [b]Non-linearity. SHP, standard human plasma.

Table 6: Fibrinogen levels (g/L) in freeze-dried standards and control samples determined with three assay systems.

	Stated value	Dade Thrombin Standard: SHP (Siemens)	STA-Fib Standard: Unicalibrator	Fibrinogen-C Standard: HemosIL Calibration Plasma
Standard human plasma	2.44	–	2.67[b] (2.61–2.72)	2.49 (2.32–2.68)
STA-Unicalibrator	3.10	2.89[a] (2.81–2.97)[b]	–	2.82 (2.61–3.04)
HemosIL Calibration Plasma	3.15	3.09[a] (2.96–3.23)[b]	3.15[b] (3.10–3.20)	–
International Standard	2.7	2.85[a] (2.70–3.01)	2.75 (2.68–2.81)	2.70 (2.47–2.97)
QC sample: ECAT normal	–	3.33[a] (3.26–3.40)[b]	3.31[a] (3.25–3.36)	3.17 (2.87–3.53)
QC sample: ECAT abnormal	–	1.12[a] (1.11–1.14)[b]	1.14 (1.10–1.17)	1.08 (0.98–1.20)
QC sample: ECAT INR = 2.5	–	2.65 (2.42–2.91)	2.63 (2.57–2.69)	2.29 (2.06–2.56)
QC sample: SKML Fib-1	–	0.86 (0.79–0.93)	0.95 (0.91–0.99)	0.90 (0.82–1.00)
QC sample: SKML Fib-2	–	1.34[a] (1.31–1.36)[b]	1.36 (1.32–1.41)	1.33 (1.19–1.47)
QC sample: SKML Fib-3	–	2.27 (2.11–2.43)	2.53 (2.46–2.61)	2.46 (2.23–2.73)
QC sample: SKML Fib-5	–	4.28 (4.14–4.42)	4.71 (4.60–4.84)	4.60 (4.08–5.28)
QC sample: SKML Fib-6	–	4.55[a] (4.49–4.61)[b]	4.82 (4.69–4.96)	4.70 (4.16–5.42)
QC sample: SKML HNP-21	–	2.92 (2.83–3.02)	3.01 (2.94–3.07)	2.73 (2.48–3.02)
QC sample: SKML Cou-32	–	3.65[b] (3.60–3.71)	3.91 (3.81–4.02)	3.64 (3.28–4.10)
Average (n = 10: QC samples only)	–	2.70	2.84	2.69
Significance		p = 0.019 (Dade Thrombin vs. STA-Fib)	p = 0.002 (STA-Fib vs. Fibrinogen-C)	p = 0.914 (Fibrinogen-C vs. Dade Thrombin)

For each assay system, the corresponding commercial standard was used to construct the calibration line. SHP, standard human plasma. In parentheses: 95% confidence limits. [a]Non-parallelism. [b]Non-linearity.

To assess the commutability of the freeze-dried standards and the QC samples, the results with the assays shown in Tables 5 and 6 were plotted against each other. Three plots could be made. An example of one of these plots is shown in Figure 1. Linear orthogonal regression lines with 95% confidence limits were calculated for the frozen samples. The position of the freeze-dried samples relative to the confidence interval was assessed. Several samples were very near to the limits of the confidence interval. In the plot of Dade Thrombin against STA-Fib (Figure 1) the standard HemosIL, the international standard (09/264) and several QC-samples were out of the 95% confidence interval. The normalized residuals for the freeze-dried samples are shown in Table 7. In all three plots, there were several cases where the normalized residual was greater than 2. We did not calculate the normalized residuals for the commercial standards if they were used as references in any plot.

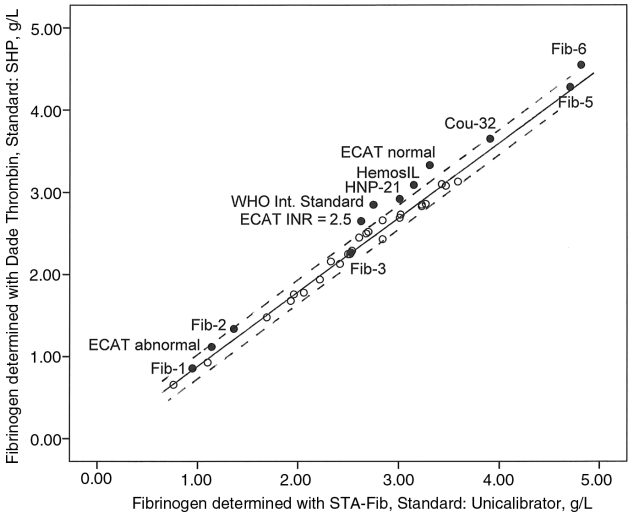

Figure 1: Fibrinogen levels for frozen plasmas (open symbols) and freeze-dried standards and QC samples (closed symbols).
On the horizontal axis: the levels determined with STA-Fib reagents on a STA-Rack Evolution analyzer, using STA-unicalibrator as standard. On the vertical axis: the levels determined with Dade Thrombin, Sysmex CA1500, using standard human plasma (SHP) as standard. The continuous line represents the regression line and the dashed lines the 95% confidence limits for the 25 frozen samples.

Discussion

The purpose of the present study was to assess the commutability of freeze-dried materials used as standards or as QC materials for the fibrinogen determination in plasma. Our study was limited to the kinetic fibrinogen determination according to Clauss, which is the most used fibrinogen assay in clinical laboratories. The fibrinogen determination according to Clauss is performed with various commercial reagents, standards and instruments. In the Netherlands, three assay systems are mostly used for the determination according to Clauss. Significant differences between these assay systems were observed in

Table 7: Normalized residuals of each lyophilized plasma sample to various patient samples regression lines.

Sample	Normalized residual		
	Dade Thrombin (y-axis) vs. Fibrinogen-C (x-axis)	Dade Thrombin (y-axis) vs. STA-Fib (x-axis)	Fibrinogen-C (y-axis) vs. STA-Fib (x-axis)
International Standard	0.96	5.95	2.24
STA-Unicalibrator	0.40	–	–
Standard human plasma	–	–	1.13
HemosIL Calibration Plasma	–	4.10	–
QC sample: ECAT normal	1.16	5.55	1.80
QC sample: ECAT abnormal	0.28	1.77	1.31
QC sample: ECAT INR = 2.5	2.42	4.55	0.21
QC sample: SKML Fib-1	0.93	0.43	1.29
QC sample: SKML Fib-2	0.44	2.09	1.67
QC sample: SKML Fib-3	1.64	0.15	1.96
QC sample: SKML Fib-5	2.04	0.75	2.79
QC sample: SKML Fib-6	0.75	3.34	2.76
QC sample: SKML HNP-21	1.27	3.44	0.48
QC sample: SKML Cou-32	0.17	2.16	1.04

For each assay system, the corresponding commercial standard was used to construct the calibration line.

the Dutch External Quality Assessment Scheme (Table 1). There are two possible explanations for the differences between the assays systems. The first is an error in the stated fibrinogen level of one or more standards provided by the manufacturers. The second is a lack of commutability of the standards or the QC materials or both. Before we could address these questions, we verified that frozen plasma samples and fresh samples give identical results in the fibrinogen assay (Table 2). Alesci et al. concluded that freezing and storage of plasma had little effect on results of fibrinogen assays [17]. We showed that no significant effect was observed (Table 2).

The between-method differences observed in our study were less than 10% in many instances. However, there were also instances in which the bias was in excess of 10% (see Tables 3 and 5). According to Fraser et al., allowable bias may be assessed from biological variation [18]. In a recent study, the biological variation of fibrinogen was assessed [19]. Using biological variation results, De Maat et al. estimated the allowable bias for fibrinogen and obtained a value of 5.2% [19].

In previous studies, commutability of potential reference materials was assessed using a multicenter split-patient-sample between-field-method (twin-study) design [20, 21]. In those studies, regression line residuals for the potential reference materials were normalized by expressing them as multiples of the state-of-the-art within-laboratory SD (SD_{SA}). The twin-study design has been used in a previous study on the assessment of the commutability of potential reference materials for fibrinogen assays [5]. In that study, normalized residuals were calculated as multiples of SD_{SA}, and the results suggested that three potential reference materials were commutable for laboratories using Clauss assays [5]. In the present study performed by a single laboratory, the residuals were normalized by expressing them as multiples of the within-laboratory SD about the regression line (SD_L). We are of the opinion that in a single-center study, SD_L is more appropriate than SD_{SA} for normalization of residuals.

One limitation of our study was that several results were compromised by non-parallelism or non-linearity of the dilution lines of standards and test samples (Tables 3–6). Despite this limitation, 95% confidence intervals were provided by the CombiStats program. It is not known whether non-parallelism or non-linearity can be avoided completely in any assay method. When frozen samples were analysed with three different assay systems with the same standard for calibration (i.e. the international standard), a significant difference between the assay systems was observed (Table 3). This finding already suggested that the standard was not commutable, because no difference should have been observed between the assay systems if the standard was commutable. Similar differences between the assay systems were observed for the freeze-dried QC materials (Table 4). It is reassuring to note that the manufacturers' stated levels for STA-Unicalibrator, standard human plasma and HemosIL fibrinogen standards were within the confidence interval determined with the corresponding manufacturers' assay systems in our laboratory and the international standard used for calibration (Table 4). However, it is not possible

to assess the commutability of the QC materials from the levels shown in Table 4. To assess the commutability, we plotted the results of frozen and freeze-dried samples in the same graph and determined the 95% confidence interval for the frozen samples only. When a freeze-dried sample is out of the 95% confidence interval, it is regarded as not commutable [13]. This is equivalent to a normalized residual greater than 2. Several freeze-dried samples were out of the 95% confidence interval (i.e. normalized residual >2), among which is the international standard (see Figure 1 and Table 7). It should be noted that most of the freeze-dried plasmas in Figure 1 were on the same side of the regression line determined for frozen plasmas, suggesting that there is a common feature in freeze-dried plasmas inducing non-commutability. One of the two assay systems compared in Figure 1 was photo-optical (Sysmex CA1500) and the other was mechanical (STA-Rack Evolution). Clot detection in photo-optical instruments is different from that in mechanical instruments. If the international standard for fibrinogen is not commutable, it is not possible to assign true values to secondary or commercial standards. To avoid non-commutability in standard preparations, frozen samples could be used rather than freeze-dried. A disadvantage of frozen standards is the need for dry ice during transportation and the risk of thawing during delay that may occur at customs in international shipments. Another option is to try and change the plasma lyophilization procedure aiming at improved commutability of the fibrinogen standards. Although commutability of calibrators is mandatory for achieving equivalence of measurement results, it does not guarantee the absence of bias. Elimination of bias may require the identification and control of all relevant influence parameters and quantities and eventually multiparametric traceability [22].

The lack of frozen samples with fibrinogen levels similar to those of three QC samples with high levels (Cou-32, Fib-5, and Fib-6) and the lack of paired comparison of non-lyophilized plasma versus lyophilized plasmas are other limitations of our study. The assessment of the commutability of the high fibrinogen QC samples is based on extrapolation of the orthogonal regression line and is therefore less reliable than that of the other QC samples and standard samples.

In conclusion, we have shown that differences in fibrinogen results obtained with various Clauss assay systems can be explained by non-commutability of the freeze-dried international standard used in these assays. Our findings have consequences for all stakeholders (i.e. WHO, industry, external quality assessment schemes, and clinical laboratories) in the traceability chain.

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