External Quality Assurance of Fibrinogen Assays Using Normal Plasma

Results of the 2008 College of American Pathologists Proficiency Testing Program in Coagulation

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• ***Context.*—Proper diagnosis and therapy of fibrinogen deficiency requires high-quality fibrinogen assays.**

***Objective.*—To assess the interlaboratory bias, precision, and grading of fibrinogen assays used by laboratories participating in the United States College of American Pathologists proficiency testing program in coagulation.**

***Design.*—Two identical vials of normal plasma were sent to more than 3500 laboratories. Participants measured fibrinogen levels using local methods.**

***Results.*—Fifty different fibrinogen methods were evaluated. All-method bias was 8.3% (range of method-specific biases, 0.0%–27.0%) and all-method coefficient of variation was 7.7% (range of method-specific coefficients of variation, 0.7%–25.8%). After controlling for reagent/instrument type, mean fibrinogen levels were 11.6% higher for prothrombin time–based reagents compared to Clauss ($P < .001$), and coefficient of variation was 46% lower for mechanical endpoint instruments compared to photo-optical. Most testing events (97.4%) could be reliably graded as pass or fail using a target range of ±20% from the method mean (total pass rate, 98.8%). Total fail rate was 3.0-fold lower for mechanical instruments compared to photo-optical (0.5% versus 1.5%, $P = .001$). Nonetheless many photo-optical methods had very high precision and very low fail rates.**

***Conclusions.*—Fibrinogen assays showed highly variable methodology and performance characteristics. Bias, precision, and grading were affected by the type of reagent or instrument used.**

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Fibrinogen deficiency is an important risk factor for bleeding,[1–3] and can be acquired or inherited. Acquired deficiency is most common and causes include disseminated intravascular coagulation,[4] liver disease,[5] hemodilution,[6] and acquired dysfibrinogenemia.[7] Inherited deficiency is caused by mutations of the fibrinogen Aα, Bβ, or γ gene that result in quantitative or qualitative defects.[8] Fibrinogen activity levels are important in the diagnosis or management of these conditions, particularly in guiding transfusion therapy with cryoprecipitate or fibrinogen concentrate.[2,3,9]

The first goal of this study was to determine the interlaboratory bias and precision of contemporary fibrinogen activity assays used by a large number of laboratories, and to correlate these parameters with assay variables (reagent type, instrument type, reagent/instrument type). The second goal was to grade the performance of individual laboratories as pass or fail using method-specific peer group data, and to correlate grading with bias, precision, and assay variables. This was done using external quality assurance data obtained from participants of the 2008 College of American Pathologists proficiency testing program in coagulation.

METHODS

Proficiency Testing Specimens

Two identical vials of normal plasma named CGL-07 and CGL-09 were mailed to more than 3500 participants of the College of American Pathologists proficiency testing program in coagulation in 2008 (2008-CGL-B survey). These plasmas were obtained from a commercial vendor, prepared by pooling plasmapheresis collections obtained

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from normal donors, aliquoted into 1.0-mL vials, and lyophilized. The laboratory was instructed to reconstitute the plasma by the addition of 1.0 mL of water. Participants were blinded to the identity of the plasmas.

Fibrinogen Assays

Each participating laboratory was asked to perform fibrinogen activity assays according to its local method and to report the assayed values and method used to the College of American Pathologists. A *method* was defined as a specific reagent and a specific instrument that were combined to create a fibrinogen assay. Each method was classified according to reagent type, instrument type, and reagent/instrument type. There were 2 reagent types (Clauss-based and prothrombin time [PT]–based), 2 instrument types (mechanical endpoint and photo-optical endpoint), and 3 reagent/instrument types (Clauss/mechanical, Clauss/photo-optical, PT-based/photo-optical). Each method was also classified according to peer group size. There were 2 peer group sizes, including a large peer group (if data were reported for 10 or more laboratories) and a small peer group (if data were reported for fewer than 10 laboratories).

Determination of Interlaboratory Bias and Precision of Fibrinogen Assays

All fibrinogen values were assigned to 5 different stratification groups as defined in ''Fibrinogen Assays'': method, reagent type, instrument type, reagent/instrument type, and peer group size. Summary statistics were calculated for each stratification group, including number of laboratories, mean, and coefficient of variation (CV). Outliers were defined as values that exceeded 3 standard deviations from the mean of each stratification group. The mean ± 3 SD was recalculated after this initial outlier exclusion for no more than 2 iterations to exclude any remaining outliers. The final data set, after all outliers were excluded, was used to calculate the final mean and CV. For instrument type and reagent/instrument type calculations, the AMAX 190, 200, and 400 instruments (Trinity Biotech, Bray, Ireland) were excluded from analysis because it was uncertain if participants were reporting fibrinogen values using the mechanical or photo-optical endpoint mode of operation.

Method-specific bias was calculated by comparing the method-specific mean to the all-method mean. The all-method mean was calculated by taking the average of the method-specific means. The all-method bias was calculated by taking the average of the method-specific biases. The all-method CV was calculated by taking the average of the method-specific CVs.

Test bias was classified into 1 of 3 categories based on the magnitude of the method-specific bias (low bias, bias ≤5%; intermediate bias, bias 6%–10%; high bias, bias >10%). Test precision was classified into 1 of 4 categories based on the magnitude of the method-specific CV (low precision, CV >20%; intermediate precision, CV 11%–20%; high precision, CV 6%–10%; very high precision, CV ≤5%).

Grading of Laboratory Performance

Participant results were graded as pass or fail based on the result falling inside (pass) or outside (fail) the ±20% target range from the method-specific mean (after outlier exclusion). Only those methods that had 10 or more participant results (ie, large peer groups) were considered valid for estimating a reliable peer group mean; therefore only those methods were graded.[10]

Statistical Analysis

Differences between 2 independent means were tested for significance using the 2-tailed t test. Differences between 2 proportions were tested for significance by calculation of z scores. Linear correlation coefficients were tested for significance using analysis of variance. Values of $P < .05$ were considered significant.

RESULTS

Fibrinogen Assays

Study participants used 18 different reagents supplied by 5 manufacturers (Table 1). These were classified into Clauss-based (n = 9) and PT-based (n = 9) reagent types. Participants used 20 different instruments supplied by 5 manufacturers (Table 2). These were classified into me-

Table 1. Characteristics of Fibrinogen Reagents

Manufacturer	Reagent Brand Name	Reagent Type	Reagent No.
Dade Behring Corporation, Deerfield, Illinois	Fibrinogen Determination	Clauss (bovine thrombin)	1
	Innovin	PT-derived (human recombinant thromboplastin)	2
	Multifibren	Clauss (bovine thrombin)	3
	Thromboplastic C Plus	PT-derived (rabbit brain thromboplastin)	4
	Thromborel S	PT-derived (human placenta thromboplastin)	5
Diagnostica Stago Incorporated, Parsippany, New Jersey	Diagnostica Stago	Clauss (human thrombin)	6
Fisher HealthCare, Houston, Texas	Fibrinogen Assay	Clauss (bovine thrombin)	7
Instrumentation Laboratory, Bedford, Massachusetts	Fibrinogen C	Clauss (bovine thrombin)	8
	HemosIL PT-Fib	PT-derived (rabbit brain thromboplastin)	9
	HemosIL PT-Fib HS	PT-derived (rabbit brain thromboplastin)	10
	HemosIL PT-Fib HS Plus	PT-derived (rabbit brain thromboplastin)	11
	HemosIL PT-Fib Recombinant	PT-derived (rabbit recombinant thromboplastin)	12
	HemosIL Recombiplastin	PT-derived (human recombinant thromboplastin)	13
	HemosIL Recombiplastin 2G	PT-derived (human recombinant thromboplastin)	14
	QFA Thrombin	Clauss (bovine thrombin)	15
Trinity Biotech, Bray, Ireland	Fibrinogen Kit	Clauss (bovine thrombin)	16
	Fibriquick	Clauss (bovine thrombin)	17
	MDA Fibriquick	Clauss (bovine thrombin)	18

Abbreviation: PT, prothrombin time.

Table 2. Characteristics of Fibrinogen Instruments			
Manufacturer	Instrument Brand Name	Instrument Type	Instrument No.
Behnk Electronik, Norderstedt, Germany	Thrombolyzer CPT	Photo-optical	1
	Thrombolyzer CPT X, XR, RR	Photo-optical	2
Dade Behring Corporation, Deerfield, Illinois	BCS, BCS XP	Photo-optical	3
	BCT	Photo-optical	4
	Sysmex CA Series	Photo-optical	5
Diagnostica Stago Incorporated, Parsippany, New Jersey	ST4, STRT4, STRT8	Mechanical	6
	STA	Mechanical	7
	STA Compact	Mechanical	8
	STA-R, Evolution	Mechanical	9
Instrumentation Laboratory, Bedford, Massachusetts	ACL 7000 and lower	Photo-optical	10
	ACL 8000, 9000, 10000, Elite, Pro	Photo-optical	11
	ACL Futura, Advance	Photo-optical	12
	ACL Top	Photo-optical	13
	Electra 1400, 1600, 1800C	Photo-optical	14
Trinity Biotech, Bray, Ireland	AMAX 190, 200, 400	Mechanical or photo-optical	15
	AMAX Destiny	Mechanical	16
	Coagamate MAX	Photo-optical	17
	Coagamate MTX	Photo-optical	18
	Coagamate XM	Photo-optical	19
	MDA Series	Photo-optical	20

chanical endpoint (n = 5) and photo-optical endpoint (n = 14) instrument types. One instrument was capable of both mechanical and photo-optical endpoint modes depending on user discretion. There were a total of 50 distinct fibrinogen methods based on various combinations of reagent and instrument (Table 3).

Bias of Fibrinogen Assays

Method-specific bias was highly variable, ranging from 0.0% to 27.0%, with an all-method bias of 8.3% (Table 3). Method-specific means were highly reproducible between the 2 identical plasmas CGL-07 and CGL-09; the average percentage deviation in method-specific mean was 1.5% (range 0.03%–5.7%). A sizeable number of methods (28%–32%) and laboratories (43.7%–44.1%) were classified as having high bias (bias >10%) (Table 4).

Reagent type had the greatest effect on bias. PT-based reagents had 11.7% higher fibrinogen values than Clauss-based reagents after controlling for reagent/instrument type (Table 5).

Instrument type had a small effect on bias. Photo-optical endpoint instruments had 2.8% lower fibrinogen levels than mechanical endpoint instruments after controlling for reagent/instrument type (Table 5). Although this effect was statistically significant, it was not considered clinically significant.

Peer group size did not have a consistently significant effect on bias.

Precision of Fibrinogen Assays

Method-specific precision was highly variable, with CVs ranging from 0.7% to 25.8% and an all-method CV of 7.7% (Table 3). Most methods (82%–88%) and laboratories (98.0%–98.9%) were classified as having high precision or better (CV ≤10%), and some methods (26%–30%) and laboratories (11.7%–13.4%) had very high precision (CV ≤5%) (Table 4).

Instrument type had the greatest effect on precision. Mechanical endpoint instruments had on average 46% lower CV (higher precision) than photo-optical endpoint instruments after controlling for reagent/instrument type (Table 5). Although most photo-optical instruments showed overall lower precision, there were a few with precision similar to mechanical endpoint instruments.

Peer group size had a small effect on precision. Large peer groups had 24% lower CV than small peer groups.

Reagent type had the smallest effect on precision. Clauss-based reagents had 10% lower CV than PT-based reagents after controlling for reagent/instrument type.

Grading of Fibrinogen Assays

Most laboratories (n = 3513–3515; 97.4%) used methods that were classified into large peer groups (n = 29 methods; 58%) (Table 6). Among these gradable laboratories, 98.7% to 98.9% received passing grades using a target range of ±20% from the method-specific mean. A very low proportion of laboratories (2.6%) couldn't be graded using this grading scheme because they fell into small peer groups; however, these groups represented a sizeable number of fibrinogen methods (n = 21 methods; 42%).

Method-specific failure rates were variable, ranging from 0.0% to 12.5%. Failure rates were significantly affected by instrument type. Mechanical endpoint instruments gave 2.5- to 3.6-fold lower failure rates than photo-optical endpoint instruments (Table 7). If the grades for specimens CGL-07 and CGL-09 were combined, then the overall failure rate was 3.0-fold lower for mechanical instruments compared to photo-optical ($P = .001$). Many methods using photo-optical instruments had very low fail rates (n = 12–13 methods with fail rate of 0.0%) despite the overall higher fail rate for this instrument type. Failure rates were not significantly affected by reagent type.

There was a significant linear correlation between failure rate and method-specific CV (for CGL-07, $r^2 = 0.343$, $P < .001$; for CGL-09, $r^2 = 0.440$, $P < .001$). This suggested that the lower failure rate of mechanical endpoint instruments was due to their higher precision. There was a lower and inconsistent linear correlation between failure rate and bias (for CGL-07, $r^2 = 0.107$, $P = .08$; for CGL-09, $r^2 = 0.152$, $P = .04$).

Table 3. Method-Specific Bias and Precision of Fibrinogen Assays on Normal Plasma Among Participants of the 2008 College of American Pathologists Coagulation Survey

Reagent[a]	Instrument[b]	Specimen[c]	No. of Laboratories	Mean, mg/dL	Bias, %	CV, %
1	3	CGL-07	89	304.4	+2.9	7.5
		CGL-09	89	306.4	+3.4	7.8
1	5	CGL-07	1161	258.2	−12.7	5.7
		CGL-09	1162	259.1	−12.5	6.0
1	***8***	CGL-07	2	269.0	−9.0	7.9
		CGL-09	2	273.5	−7.7	5.4
1	14	CGL-07	6	277.8	−6.0	4.0
		CGL-09	6	272.3	−8.1	4.8
2	5	CGL-07	9	252.9	−14.5	7.7
		CGL-09	9	249.9	−15.6	8.4
3	3	CGL-07	142	349.9	+18.3	6.4
		CGL-09	142	351.2	+18.6	6.1
3	4	CGL-07	5	311.3	+5.3	10.4
		CGL-09	5	299.6	+11.5	11.4
3	5	CGL-07	8	319.9	+8.2	25.8
		CGL-09	8	311.6	+5.2	25.2
4	5	CGL-07	10	251.8	−14.8	10.0
		CGL-09	10	257.3	−13.1	8.0
5	3	CGL-07	2	226.2	−23.5	1.3
		CGL-09	2	228.7	−22.8	1.7
6	***6***	CGL-07	6	278.4	−5.8	1.8
		CGL-09	6	288.9	−2.5	4.2
6	***7***	CGL-07	7	286.3	−3.2	7.8
		CGL-09	7	290.8	−1.8	5.8
6	***8***	CGL-07	800	280.6	−5.1	5.8
		CGL-09	801	281.6	−4.9	5.5
6	***9***	CGL-07	213	275.2	−6.9	5.0
		CGL-09	213	276.5	−6.6	5.0
7	15	CGL-07	2	301.0	+1.8	6.1
		CGL-09	2	306.5	+3.5	0.7
8	11	CGL-07	39	291.4	−1.4	7.6
		CGL-09	39	290.6	−1.9	7.6
8	12	CGL-07	58	274.4	−7.2	6.7
		CGL-09	58	276.9	−6.5	6.4
8	13	CGL-07	96	292.4	−1.1	5.8
		CGL-09	96	294.7	−0.5	7.0
9	10	CGL-07	90	319.0	+7.9	8.4
		CGL-09	90	319.5	+7.9	7.6
9	11	CGL-07	79	329.8	+11.5	8.6
		CGL-09	79	333.6	+12.6	7.2
9	12	CGL-07	76	304.1	+2.8	7.0
		CGL-09	76	305.1	+3.0	7.4
10	10	CGL-07	24	317.0	+7.2	7.4
		CGL-09	24	322.4	+8.8	6.5
10	11	CGL-07	15	301.3	+1.9	9.0
		CGL-09	15	309.9	+4.6	9.0
10	12	CGL-07	16	301.1	+1.8	5.2
		CGL-09	16	296.2	+0.0	6.9
11	10	CGL-07	6	332.7	+12.5	14.2
		CGL-09	6	336.2	+13.5	15.7
11	11	CGL-07	3	375.7	+27.0	17.6
		CGL-09	3	356.7	+20.4	15.7
11	12	CGL-07	2	290.5	−1.8	5.1
		CGL-09	2	282.5	−4.6	1.2
12	10	CGL-07	22	302.1	+2.2	8.5
		CGL-09	22	305.8	+3.2	9.3
12	11	CGL-07	50	309.4	+4.6	8.2
		CGL-09	50	310.8	+4.9	9.0
12	12	CGL-07	97	294.1	−0.5	5.7
		CGL-09	97	290.9	−1.8	5.4
13	10	CGL-07	2	356.5	+20.6	18.0
		CGL-09	2	350.0	+18.2	19.8
13	11	CGL-07	20	360.6	+22.0	7.1
		CGL-09	20	365.2	+23.3	8.4
13	12	CGL-07	57	246.7	−16.6	5.2
		CGL-09	57	245.0	−17.3	5.8
13	13	CGL-07	29	304.3	+2.9	3.1
		CGL-09	29	304.5	+2.8	3.6
14	10	CGL-07	4	330.8	+11.9	4.2
		CGL-09	4	335.2	+13.2	3.7
14	11	CGL-07	16	364.1	+23.1	12.4

Table 3. Continued

Reagent[a]	Instrument[b]	Specimen[c]	No. of Laboratories	Mean, mg/dL	Bias, %	CV, %
		CGL-09	16	367.5	+24.1	11.5
14	12	CGL-07	24	255.8	−13.5	10.4
		CGL-09	24	260.9	−11.9	11.4
14	13	CGL-07	16	296.0	+0.1	4.4
		CGL-09	16	296.8	+0.2	4.6
15	14	CGL-07	54	290.7	−1.7	5.2
		CGL-09	54	290.8	−1.8	5.2
16	15	CGL-07	8	280.8	−5.0	8.0
		CGL-09	8	264.9	−10.6	4.3
16	***16***	CGL-07	25	270.3	−8.6	7.6
		CGL-09	25	270.4	−8.7	8.0
17	1	CGL-07	3	292.3	−1.1	5.4
		CGL-09	3	304.7	+2.9	13.5
17	2	CGL-07	3	277.3	−6.2	12.6
		CGL-09	3	273.7	−7.6	12.2
17	***16***	CGL-07	2	267.5	−9.5	6.6
		CGL-09	2	268.5	−9.4	8.2
17	17	CGL-07	4	298.9	+1.1	7.2
		CGL-09	4	303.2	+2.4	5.5
17	18	CGL-07	43	275.4	−6.9	6.2
		CGL-09	43	272.9	−7.9	7.4
17	19	CGL-07	2	316.2	+6.9	4.3
		CGL-09	2	319.7	+7.9	0.5
17	20	CGL-07	30	278.7	−5.8	5.7
		CGL-09	30	277.2	−6.4	5.2
18	18	CGL-07	5	266.4	−9.9	8.2
		CGL-09	5	275.0	−7.2	8.4
18	20	CGL-07	31	277.2	−6.3	8.9
		CGL-09	31	277.8	−6.2	6.7

Abbreviation: CV, coefficient of variation.

[a] Each reagent is numbered according to Table 1.
[b] Each instrument is numbered according to Table 2. Mechanical endpoint instruments are noted in bold italicized font.
[c] Specimens CGL-07 and CGL-09 were different vials of the same normal plasma.

COMMENT

Fibrinogen assays play an important role in the evaluation of bleeding patients. Very low levels of fibrinogen are associated with an increased risk of bleeding often requiring treatment with cryoprecipitate or other fibrinogen concentrates.[1–3,9] It is therefore critical to have a reliable fibrinogen assay.

Two kinds of errors could lead to falsely decreased or falsely elevated fibrinogen results and consequently to overtreatment or undertreatment. The first kind of error is imprecision (high CV), which may randomly produce clinically significant errors. Although our study did not assess what level of imprecision is clinically unacceptable, only one reagent-instrument combination had a CV >20% (Table 3), which we estimated may be a clinically unacceptable level of imprecision.

The second type of error is inaccuracy (high bias), which may produce clinically significant errors even if the assay is precise. Prothrombin time–based fibrinogen assays can generate falsely high fibrinogen results depending on the type of thromboplastin used and the patient's underlying condition.[11,12] For example, PT-based assays can overestimate fibrinogen results in patients with and without dysfibrinogenemia,[13,14] and can disagree with Clauss-based assays in patients with low fibrinogen.[13,15] One study even concluded that some PT-based fibrinogen assays were clinically unsafe.[11]

Our results supported these findings. Although our study did not assess what level of bias is unacceptable for clinical use, we estimated that >20% bias may be clinically unacceptable. Five reagent-instrument combinations had >20% bias, and all 5 involved PT-based reagents (Table 3). Laboratory directors are cautioned to carefully evaluate the combination of reagent and instrument, along with the intended use of the assay, before selecting a fibrinogen assay. Although some PT-based assays with high bias may

Table 4. Summary of Bias and Precision of Fibrinogen Assays on Normal Plasma Among Participants of the 2008 College of American Pathologists Coagulation Survey

Performance Characteristic	Category[a]	Specimen[b]	Methods, No. (%)	Laboratories, No. (%)
Bias	Low	CGL-07	20 (40)	1430 (40.7)
		CGL-09	20 (40)	1432 (40.7)
	Intermediate	CGL-07	16 (32)	548 (15.6)
		CGL-09	14 (28)	534 (15.2)
	High	CGL-07	14 (28)	1535 (43.7)
		CGL-09	16 (32)	1549 (44.1)
Precision	Low	CGL-07	1 (2)	8 (0.2)
		CGL-09	1 (2)	8 (0.2)
	Intermediate	CGL-07	5 (10)	30 (0.8)
		CGL-09	8 (16)	62 (1.8)
	High	CGL-07	31 (62)	3065 (87.2)
		CGL-09	26 (52)	2972 (84.6)
	Very high	CGL-07	13 (26)	410 (11.7)
		CGL-09	15 (30)	473 (13.4)

[a] Bias was classified into 3 categories based on the magnitude of the method-specific bias: low bias, bias ≤5%; intermediate bias, bias 6% to 10%; high bias, bias >10%. Precision was classified into 4 categories based on the magnitude of the method-specific coefficient of variation (CV): low precision, CV >20%; intermediate precision, CV 11% to 20%; high precision, CV 6% to 10%; very high precision, CV ≤5%.
[b] Specimens CGL-07 and CGL-09 were different vials of the same normal plasma.

Table 5. Effect of Assay Variables on the Bias and Precision of Fibrinogen Assays Among Participants of the 2008 College of American Pathologists Coagulation Survey

Assay Variable	Specimen[a]	Methods, No.	Laboratories, No.	Mean, mg/dL	*P* Value[b]	CV, %
Reagent type						
Clauss	CGL-07	27	2844	273.3		8.4
	CGL-09	27	2846	274.6		8.6
PT-derived	CGL-07	23	669	303.3	<.001[c]	11.7
	CGL-09	23	669	304.1	<.001[c]	12.0
Instrument type						
Mechanical	CGL-07	7	1055	279.1		5.8
	CGL-09	7	1056	280.3		5.6
Photo-optical	CGL-07	41	2448	281.2	.07[d]	12.5
	CGL-09	41	2449	281.9	.16[d]	12.5
Reagent/instrument type						
Clauss/mechanical	CGL-07	7	1055	279.1		5.8
	CGL-09	7	1056	280.3		5.6
Clauss/photo-optical	CGL-07	18	1779	271.4	<.001[e]	10.5
	CGL-09	18	1780	272.5	<.001[e]	10.8
PT-derived/photo-optical	CGL-07	23	669	303.3	<.001,[f] <.001[g]	11.7
	CGL-09	23	669	304.1	<.001,[f] <.001[g]	12.0
Peer group size						
Large	CGL-07	29	3422	279.2		10.2
	CGL-09	29	3424	280.5		10.4
Small	CGL-07	21	91	290.4	<.001[h]	13.7
	CGL-09	21	91	289.0	.007[h]	13.5

Abbreviations: CV, coefficient of variation; PT, prothrombin time.

[a] Specimens CGL-07 and CGL-09 were different vials of the same normal plasma.
[b] Differences between 2 independent means were tested for significance using the 2-tailed *t* test. *P* values < .05 were significant.
[c] PT-derived was compared to Clauss.
[d] Photo-optical was compared to mechanical.
[e] Clauss/photo-optical was compared to Clauss/mechanical.
[f] PT-derived/photo-optical was compared to Clauss/photo-optical.
[g] PT-derived/photo-optical was compared to Clauss/mechanical.
[h] Small peer group size was compared to large peer group size.

be acceptable for research applications, they should probably not be used clinically.

In addition to the above conclusions about the appropriateness of specific reagent-instrument combinations, we can also draw some general conclusions about contemporary fibrinogen assays. First, there were a high number of fibrinogen methods used by clinical laboratories. This was due to a competitive marketplace (ie, 5 reagent kit manufacturers) and multiple reagent/instrument combinations (ie, Clauss versus PT-based reagents, and mechanical versus photo-optical instruments).

Table 6. Grading of Fibrinogen Assays on Normal Plasma Among Participants in the 2008 College of American Pathologists Coagulation Survey

Variable	Plasma Specimen[a]	
	CGL-07	CGL-09
Method-specific groups		
Total, No.	50	50
Gradeable, No.	29	29
Gradeable, %	58	58
Laboratories		
Total, No.	3513	3515
Gradeable, No.	3422	3424
Gradeable, %	97.4	97.4
Grading		
Failed events, No.	37	44
Passed events, No.	3385	3380
Pass rate, %	98.9	98.7

[a] Specimens CGL-07 and CGL-09 were different vials of the same normal plasma.

Second, there was wide disparity in bias and precision among fibrinogen methods. This was consistent with College of American Pathologists data published 20 years ago showing method-specific biases as high as 77 mg/dL on normal plasma and method-specific CVs ranging from 7.4% to 21.6%.[16,17] Bias was still high for some methods in the current study, up to 27%, which was equivalent to about 80 mg/dL. Other external quality assurance organizations also observe high interlaboratory bias, and some groups suggest that this can be reduced by using a common fibrinogen calibrator, although this is controversial.[18–20] Precision was improved in our study, as shown by some methods and laboratories with very high precision (CV ≤5%).

Third, bias and precision were differentially affected by reagent and instrument type. PT-based reagents gave higher fibrinogen levels, suggesting that the mechanism of fibrin clot generation (direct addition of thromboplastin versus thrombin) was an important source of systematic error. Mechanical endpoint instruments gave (on average) higher precision, implying that random error was dependant on fibrin clot properties. Mechanical properties such as clot tensile strength may have greater reproducibility than photo-optical properties such as clot turbidity or light scattering. The observation that some photo-optical instruments had very high precision indicated that this technology could also be optimized for high performance.

Fourth, the performance of laboratories could be graded using method-specific peer group data. The high overall

Table 7. Effect of Assay Variables on Grading Fibrinogen Assays Among Participants in the 2008 College of American Pathologists Coagulation Survey

Assay Variable	Specimen[a]	Methods, No.	Laboratories, No.	Fail Rate[b], % (No./Total No.)	*P* Value[c]
Reagent type					
Clauss	CGL-07	13	2781	1.01 (28/2781)	
	CGL-09	13	2783	1.11 (31/2783)	
PT-derived	CGL-07	16	641	1.40 (9/641)	.39[d]
	CGL-09	16	641	2.03 (13/641)	.06[d]
Instrument type					
Mechanical	CGL-07	3	1038	0.38 (4/1038)	
	CGL-09	3	1039	0.58 (6/1039)	
Photo-optical	CGL-07	26	2384	1.38 (33/2384)	.009[e]
	CGL-09	26	2385	1.59 (38/2385)	.02[e]
Reagent/instrument type					
Clauss/mechanical	CGL-07	3	1038	0.38 (4/1038)	
	CGL-09	3	1039	0.58 (6/1039)	
Clauss/photo-optical	CGL-07	10	1743	1.38 (24/1743)	.003[f]
	CGL-09	10	1744	1.43 (25/1744)	.04[f]
PT-derived/photo-optical	CGL-07	16	641	1.40 (9/641)	.97[g], .02[h]
	CGL-09	16	641	2.03 (13/641)	.30[g], .006[h]

Abbreviation: PT, prothrombin time.

[a] Specimens CGL-07 and CGL-09 were different vials of the same normal plasma.
[b] Participant results were graded as pass or fail based on the result falling inside (pass) or outside (fail) the ±20% target range from the method-specific mean.
[c] Differences between 2 fail rates were tested for significance by calculation of *z* values. *P* values < .05 were significant.
[d] PT-derived was compared to Clauss.
[e] Photo-optical was compared to mechanical.
[f] Clauss/photo-optical was compared to Clauss/mechanical.
[g] PT-derived/photo-optical was compared to Clauss/photo-optical.
[h] PT-derived/photo-optical was compared to Clauss/mechanical.

pass rate suggested that local laboratories were applying good quality control practices. The dependence of fail rate on method precision may have implications for laboratories experiencing high fail rates on proficiency testing. For example, a laboratory may be able to decrease its fail rate by using a higher-precision method. Reagent kit vendors can play a role in this process by assisting laboratories in choosing methods with optimal performance characteristics (high precision and low bias) and adequate peer group size for grading.

One limitation of our study was that we did not assess test performance at fibrinogen concentrations near the clinical decision threshold of 100 mg/dL.[2,3] Although the preparation of a hypofibrinogenemic plasma specimen in multi-liter quantities would be technically challenging, this type of study should be encouraged. Our study of normal plasma should provide a foundation for interpreting results obtained on abnormal plasma.

In summary, fibrinogen assays showed highly variable performance characteristics. Bias, precision, and grading were differentially affected by reagent and instrument type. These variables should be considered in the selection process for a clinical fibrinogen assay.

References

1. Ciavarella D, Reed RL, Counts RB, et al. Clotting factor levels and the risk of diffuse microvascular bleeding in the massively transfused patient. *Br J Haematol*. 1987;67(3):365–368.
2. Practice parameter for the use of fresh-frozen plasma, cryoprecipitate, and platelets: Fresh-Frozen Plasma, Cryoprecipitate, and Platelets Administration Practice Guideline Development Task Force of the College of American Pathologists. *JAMA*. 1994;271(10):777–781.
3. O'Shaughnessy DF, Atterbury C, Bolton Maggs P, et al. Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. *Br J Haematol*. 2004;126(1):11–28.
4. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation: British Committee for Standards in Haematology. *Br J Haematol*. 2009;145(1):24–33.
5. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. *N Engl J Med*. 2011;365(2):147–156.
6. Bolliger D, Gorlinger K, Tanaka KA. Pathophysiology and treatment of coagulopathy in massive hemorrhage and hemodilution. *Anesthesiology*. 2010; 113(5):1205–1219.
7. Cunningham MT, Brandt JT, Laposata M, Olson JD. Laboratory diagnosis of dysfibrinogenemia. *Arch Pathol Lab Med*. 2002;126(4):499–505.
8. de Moerloose P, Neerman-Arbez M. Congenital fibrinogen disorders. *Semin Thromb Hemost*. 2009;35(4):356–366.
9. Fenger-Eriksen C, Ingerslev J, Sorensen B. Fibrinogen concentrate—a potential universal hemostatic agent. *Expert Opin Biol Ther*. 2009;9(10):1325–1333.
10. Cunningham MT, Praestgaard J, Styer PE, et al. A method for proficiency testing of small peer groups in the College of American Pathologists coagulation surveys. *Arch Pathol Lab Med*. 1999;123(3):199–205.
11. Lawrie AS, McDonald SJ, Purdy G, Mackie IJ, Machin SJ. Prothrombin time derived fibrinogen determination on Sysmex CA-6000. *J Clin Pathol*. 1998;51(6): 462–466.
12. Chitolie A, Mackie IJ, Grant D, Hamilton JL, Machin SM. Inaccuracy of the ''derived'' fibrinogen measurement. *Blood Coagul Fibrinolysis*. 1994;5(6):955–957.
13. Mackie J, Lawrie AS, Kitchen S, et al. A performance evaluation of commercial fibrinogen reference preparations and assays for Clauss and PT-derived fibrinogen. *Thromb Haemost*. 2002;87(6):997–1005.
14. Miesbach W, Schenk J, Alesci S, Lindhoff-Last E. Comparison of the fibrinogen Clauss assay and the fibrinogen PT derived method in patients with dysfibrinogenemia. *Thromb Res*. 2010;126(6):e428–e433.
15. Rumley A, Woodward M, Hoffmeister A, Koenig W, Lowe GD. Comparison of plasma fibrinogen by Clauss, prothrombin time-derived, and immunonephelometric assays in a general population: implications for risk stratification by thirds of fibrinogen. *Blood Coagul Fibrinolysis*. 2003;14(2):197–201.
16. Bovill EG, McDonagh J, Triplett DA, et al. Performance characteristics of fibrinogen assays: results of the College of American Pathologists Proficiency Testing Program 1988–1991. *Arch Pathol Lab Med*. 1993;117(1):58–66.
17. Cunningham MT, Brandt JT, Chandler WL, et al. Quality assurance in hemostasis: the perspective from the College of American Pathologists proficiency testing program. *Semin Thromb Hemost*. 2007;33(3):250–258.
18. Chantarangkul V, Tripodi A, Mannucci PM. Results of a collaborative study for fibrinogen measurement: evidence that the use of a common calibrator improves interlaboratory agreement. *Blood Coagul Fibrinolysis*. 1994; 5(5):761–766.
19. Takamiya O, Hando S, Tekondo M, et al. Japanese collaborative study for fibrinogen assay: variability of the fibrinogen assay between different laboratories does not improve when a common calibrator is used. *Clin Lab Haematol*. 2005; 27(3):177–183.
20. Van den Besselaar AM, Haas FJ, van der Graaf F, Kuypers AW. Harmonization of fibrinogen assay results: study within the framework of the Dutch project ''Calibration 2000.'' *Int J Lab Hematol*. 2009;31(5):513–520.