External Quality Assurance of Antithrombin, Protein C, and Protein S Assays

Results of the College of American Pathologists Proficiency Testing Program in Thrombophilia

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• ***Context.*—Hereditary and acquired deficiencies of antithrombin (AT), protein C (PC), and protein S (PS) are risk factors for venous thromboembolism. Proper diagnosis requires high-quality assays for these proteins.**

***Objective.*—To determine the accuracy and interlaboratory precision of AT, PC, and PS assays used by laboratories participating in the United States College of American Pathologists proficiency testing program in thrombophilia and to grade the performance of laboratories.**

***Design.*—Standardized normal plasma with assigned analyte values was sent in 2 separate challenges to participating laboratories. Participants measured AT, PC, and PS levels using local methods.**

***Results.*—When compared with the assigned values for the international standard, the order of assay accuracy from highest to lowest was AT activity, PC antigen, AT antigen, total PS antigen, PC activity, PS activity, and free PS antigen (range of assay bias, 2.6%–8.8%). The order of assay precision from highest to lowest was PC activity, AT activity, AT antigen, total PS antigen, PS activity, free PS antigen, and PC antigen (range of assay coefficient of variation, 6.1%–20.0%). Most testing events (87.8%) could be graded as pass or fail using a target range of ±3 standard deviations from the method-specific mean. The pass rate was 98.2% for all AT, PC, and PS testing events combined.**

***Conclusions.*—Accuracy and precision were higher for AT assays and lower for PC and PS assays. It was feasible to grade individual laboratory performance.**

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Venous thromboembolism (VTE) (deep vein thrombosis and/or pulmonary embolism) is an important cause of morbidity and mortality. The annual incidence of VTE is 0.1%, which translates into 307 000 people in the United States per year.[1–3] The rate varies with age ranging from 0.01% in early adulthood to 1% in those older than 60 years. Pulmonary embolism has a frequency of 32% among those with a first episode of VTE, and an in-hospital mortality rate of 23%.[4]

Inherited thrombophilia is a well-known risk factor for VTE. Hereditary deficiencies of the anticoagulant proteins antithrombin (AT), protein C (PC), and protein S (PS) have a prevalence of 1% to 2%, 2% to 5%, and 1% to 3% in patients with a history of VTE, respectively.[5] The relative risk of developing a recurrent VTE is increased in these disorders with an annual incidence of 10.0%, 6.0%, and 8.4% for heterozygous deficiencies of AT, PC, and PS, respectively.[6]

The diagnosis of AT, PC, and PS deficiency is based on the quantitative assay of these proteins in plasma. Proper diagnosis is highly dependent on the accuracy and precision of these tests. False-positive and false-negative classification can occur when using assays with poor performance characteristics.

The first goal of this study was to determine the accuracy and interlaboratory precision of AT, PC, and PS assays used by laboratories in the United States. This was done using external quality assurance data obtained from participants of the 2008 and 2009 College of American Pathologists proficiency testing program in thrombophilia. The secondary coagulation standard Lot No. 3 from the Scientific and Standardization Committee/International Society on Thrombosis and Haemostasis (SSC/ISTH) was

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Table 1. Method-Specific Performance of Antithrombin, Protein C, and Protein S Assays on Scientific and Standardization Committee/International Society on Thrombosis and Haemostasis (SSC/ISTH) Secondary Coagulation Standard Lot No. 3 Among Participants of the College of American Pathologists Thrombophilia Survey

Analyte	SSC/ISTH[a] Value, IU/mL	Survey	Test Principle	Method[b]
Antithrombin				
Activity	0.93	2008-CGS2-A	Chromogenic end point	Beckman Coulter HemosIL Dade Behring Berichrom Diagnostica Stago Stachrom
	0.93	2009-CGS2-A	Chromogenic end point	Beckman Coulter HemosIL Diagnostica Stago Stachrom Siemens Berichrom
Antigen	0.95	2008-CGS2-A	Immunoturbidimetric	Diagnostica Stago Liatest
	0.95	2009-CGS2-A	Immunoturbidimetric	Diagnostica Stago Liatest
Protein C				
Activity	0.89	2008-CGS2-A	Chromogenic end point	Diagnostica Stago Stachrom Beckman Coulter HemosIL Protein C All chromogenic methods
	0.89	2008-CGS2-A	Clot end point	Dade Behring Berichrom Diagnostica Stago Staclot Beckman Coulter HemosIL ProClot All clot methods
	0.89	2009-CGS2-A	Chromogenic end point	Diagnostica Stago Stachrom Beckman Coulter HemosIL Protein C Siemens Berichrom Protein C All chromogenic methods
	0.89	2009-CGS2-A	Clot end point	Diagnostica Stago Staclot Beckman Coulter HemosIL ProClot Siemens Protein C Coag All clot methods
Antigen	0.89	2008-CGS2-A	ELISA	Corgenix Reaads Diagnostica Stago Asserachrom
	0.89	2009-CGS2-A	ELISA	Corgenix Reaads Diagnostica Stago Asserachrom
Protein S				
Activity	0.78	2008-CGS2-A	Clot end point	Diagnostica Stago Staclot Beckman Coulter HemosIL
	0.78	2009-CGS2-A	Clot end point	Diagnostica Stago Staclot Beckman Coulter HemosIL Siemens
Total Antigen	0.85	2008-CGS2-A	ELISA	Corgenix Reaads Diagnostica Stago Asserachrom All ELISA methods
	0.85	2008-CGS2-A	Immunoturbidimetric	Diagnostica Stago Liatest
	0.85	2009-CGS2-A	ELISA	Corgenix Reaads Diagnostica Stago Asserachrom All ELISA methods
	0.85	2009-CGS2-A	Immunoturbidimetric	Diagnostica Stago Liatest
Protein S Free Antigen	0.88	2008-CGS2-A	ELISA	Corgenix Reaads Diagnostica Stago Asserachrom All ELISA methods
	0.88	2008-CGS2-A	Immunoturbidimetric	Diagnostica Stago Liatest Beckman Coulter HemosIL All immunoturbidimetric methods
	0.88	2009-CGS2-A	ELISA	Corgenix Reaads Diagnostica Stago Asserachrom All ELISA methods
	0.88	2009-CGS2-A	Immunoturbidimetric	Diagnostica Stago Liatest Beckman Coulter HemosIL All immunoturbidimetric methods

Abbreviations: CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay.

[a] Analyte values were assigned by standard calibration exercises.

[b] Beckman Coulter, Inc, Brea, California; Corgenix, Westminster, Colorado; Dade Behring Corp, Deerfield, Illinois; Diagnostica Stago, Inc, Parsippany, New Jersey; Siemens, Newark, Delaware.

[c] The *t* test (2-tailed) was used to compare the means of all chromogenic methods with the means of all clot methods (protein C activity) and the means of all ELISA methods with the means of all immunoturbidimetric methods (total protein S antigen, free protein S antigen) for each year. *P* values $< .05$ were significant.

Table 1. Extended

Laboratories, No.	Mean, IU/mL	*P* Value[c]	Bias, %	CV, %
22	0.941		+1.2	7.5
41	0.896		−3.6	4.9
53	0.944		+1.5	6.1
24	0.950		+2.2	6.4
58	0.961		+3.3	8.6
25	0.863		−3.6	4.5
13	0.988		+4.0	7.6
14	0.984		+3.6	7.5
20	0.932		+4.7	3.9
16	0.859		−3.5	6.2
36	0.898		+0.9	6.4
15	0.968		+8.8	4.2
39	1.077		+21.0	8.7
4	1.020		+14.6	8.6
58	1.046	<.001	+17.5	9.1
17	0.920		+3.4	4.6
17	0.888		−0.2	3.2
7	0.944		+6.1	6.0
41	0.911		+2.4	4.9
38	1.078		+21.1	9.2
4	0.945		+6.2	6.7
7	0.923		+3.7	6.3
49	1.043	<.001	+17.2	10.6
5	0.902		+1.3	14.8
7	0.877		−1.5	12.5
10	0.874		−1.8	18.0
8	0.969		+8.9	34.6
57	0.724		−7.2	13.6
12	0.868		+11.2	14.9
60	0.783		+0.4	18.7
13	0.876		+12.3	18.5
10	0.681		−12.7	13.2
7	0.913		+7.4	18.2
11	0.806		−5.1	18.4
18	0.848		−0.3	18.9
17	0.931	.08	+9.5	10.8
6	0.805		−5.3	22.5
4	0.815		−4.1	9.2
10	0.809		−4.8	17.5
17	0.899	.06	+5.8	11.0
6	0.772		−12.3	23.4
12	0.820		−6.8	25.3
18	0.804		−8.6	24.2
23	0.964		+9.5	13.0
7	0.991		+12.6	6.7
30	0.970	<.001	+10.2	11.6
5	0.822		−6.6	44.1
8	0.875		−0.6	14.4
13	0.855		−2.9	27.1
20	0.941		+6.9	8.0
9	1.012		+15.0	3.4
29	0.963	.03	+9.4	7.6

used as the standardized plasma to evaluate performance. This plasma is ideal for evaluating accuracy because it has assigned concentration values for a variety of coagulation proteins and is commonly used by reagent kit manufacturers to assign calibrator values. The second goal of this study was to determine the feasibility of grading the performance of individual laboratories as pass or fail using method-specific peer group data.

METHODS

Proficiency Testing Specimens

SSC/ISTH secondary coagulation standard Lot No. 3 was prepared under the auspices of the SSC/ISTH Working Group on Coagulation Standards. Fifty-eight liters of normal human plasma from multiple donors were collected by plasmapheresis using 4% (weight/volume) trisodium citrate anticoagulant. The pool was distributed into 1.0-mL glass vials and freeze dried for 26 hours to produce a mean residual moisture of 0.101%. Lot No. 3 has assigned values for a variety of coagulation proteins. These value assignments were based on calibration exercises performed according to World Health Organization international standards in multicenter international collaborative studies involving both manufacturers and clinical laboratories. A summary of assigned values can be found on the Web at http://www.nibsc.ac.uk.

Lot No. 3 was mailed to participants of the College of American Pathologists proficiency testing program in thrombophilia in 2 separate mailings: 2008 (2008-CGS2-A) and 2009 (2009-CGS2-A). This plasma was supplied in 1.0-mL lyophilized vials and participants were blinded to its identity.

AT, PC, and PS Assays

Participating laboratories were asked to perform AT, PC, and PS assays (activity and antigen) according to their local methods. Participants reported the assayed values and methods used to the College of American Pathologists.

Determination of Accuracy and Interlaboratory Precision of Assays

All assayed AT, PC, and PS values from each participant were stratified by the method used. Outliers were defined as values that exceeded 3 standard deviations (SDs) from the method-specific mean. 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 method-specific mean and method-specific coefficient of variation (CV).

Method-specific bias was calculated by comparing the method-specific mean to the assigned value for SSC/ISTH Lot No. 3 (Table 1). The all-method bias was calculated by taking the average (for the 2 mailings) of the method-specific biases for each assay category. The all-method CV was calculated by taking the average (for the 2 mailings) of the method-specific CVs for each assay category. The assay categories were AT activity, AT antigen, PC activity, PC antigen, PS activity, total PS antigen, and free PS antigen.

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

Grading of Laboratory Performance

Participant results were graded as pass or fail based on whether the result fell inside (pass) or outside (fail) the ±3 SD range of the method-specific mean (after outlier exclusion). Only those method-specific peer groups that had 10 or more participant results were considered valid

Table 2. All-Method Accuracy and Precision of Antithrombin, Protein C, and Protein S Assays on Scientific and Standardization Committee/International Society on Thrombosis and Haemostasis Secondary Coagulation Standard Lot No. 3 Among Participants of the College of American Pathologists Thrombophilia Survey

Analyte	All-Method Accuracy		All-Method Precision		Final Ranking[b]
	Bias, %	Ranking[a]	CV, %	Ranking[a]	
Antithrombin					
Activity	2.6	1	6.3	2	1
Antigen	3.8	3	7.6	3	2
Protein C					
Activity	8.5	5	6.1	1	2
Antigen	3.4	2	20.0	7	4
Protein S					
Activity	8.76	6	15.8	5	5
Total Antigen	6.2	4	15.0	4	3
Free Antigen	8.79	7	17.3	6	6

Abbreviation: CV, coefficient of variation.

[a] All-method accuracy and all-method precision were ranked for each assay according to the numerical value of the biases and CVs, respectively.
[b] Final ranking for each assay was determined from the sum of the accuracy rankings and precision rankings.

for estimating a reliable peer group mean; therefore, only those peer groups were considered gradeable. The standard deviation index was determined for all failed testing events to determine the degree of variation from the method-specific mean. Standard deviation index was calculated by subtracting the assayed value from the method-specific mean and dividing that difference by the method-specific standard deviation.

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 values from which normal curve areas were obtained, followed by calculation of P values that were equal to twice the difference between 0.05 and the derived normal curve areas. Values of $P < .05$ were considered significant.

RESULTS

The method-specific accuracy and precision; the all-method accuracy and precision; and the grading of AT, PC, and PS assay categories by mailing year are summarized in Table 1, Table 2, and Table 3, respectively.

Table 3. Grading of Antithrombin, Protein C, and Protein S Assays on Scientific and Standardization Committee/International Society on Thrombosis and Haemostasis Secondary Coagulation Standard Lot No. 3 Among Participants of the College of American Pathologists Thrombophilia Survey

Analyte	Survey	Method-Specific Groups		Laboratories		Grading	
		Total No.	Gradeable No.	Total No.	Gradeable No.	Pass Rate, % (No./Total No.)	Acceptable Analyte Ranges[a]
Antithrombin							
Activity	2008-CGS2-A	3	3	116	116	99.1 (115/116)	0.765–1.028; 0.771–1.117; 0.728–1.154
	2009-CGS2-A	3	3	107	107	95.3 (102/107)	0.713–1.209; 0.768–1.132; 0.609–1.149
Antigen	2008-CGS2-A	1	1	13	13	100 (13/13)	0.762–1.213
	2009-CGS2-A	1	1	14	14	100 (14/14)	0.764–1.204
Protein C							
Activity	2008-CGS2-A	5	4	94	90	97.8 (88/90)	0.846–1.090; 0.823–1.041; 0.795–1.359; 0.700–1.018
	2009-CGS2-A	6	3	90	72	95.8 (69/72)	0.793–1.047; 0.781–1.375; 0.803–0.973
Antigen	2008-CGS2-A	2	0	12	0	NA	
	2009-CGS2-A	2	1	18	10	100 (10/10)	0.401–1.347
Protein S							
Activity	2008-CGS2-A	2	2	69	69	100 (69/69)	0.430–1.019; 0.479–1.256
	2009-CGS2-A	3	3	83	83	100 (83/83)	0.340–1.223; 0.390–1.362; 0.411–0.951
Total antigen	2008-CGS2-A	3	2	35	28	100 (28/28)	0.360–1.252; 0.630–1.232
	2009-CGS2-A	3	1	27	17	100 (17/17)	0.602–1.196
Free antigen	2008-CGS2-A	4	2	48	35	97.1 (34/35)	0.198–1.442; 0.589–1.338
	2009-CGS2-A	4	1	42	20	100 (20/20)	0.714–1.167
	Total	**42**	**27** (64.3%)	**768**	**674** (87.8%)	**98.2 (662/674)**	

Abbreviation: NA, not applicable due to no gradeable method-specific groups.

[a] Acceptable analyte ranges were based on ±3 standard deviations from the mean for each gradeable method-specific group.

These results are outlined in narrative form in the following sections.

AT Activity Assay

Results were submitted for 3 AT activity methods in 2008 and 3 methods in 2009. The accuracy and precision among these methods was high with an all-method bias of 2.6% (range of method-specific biases, 1.2%–3.6%) and an all-method CV of 6.3% (range of method-specific CVs, 4.5%–8.6%).

All of the method-specific peer groups for the AT activity assay had 10 or more assayed values; therefore, all laboratories could be graded as pass or fail in 2008 and 2009. Using a target range of ±3 SD from the peer group mean, 95.3% to 99.1% of testing events had a passing grade. Six testing events had failing grades with extreme outlier standard deviation index values of 4.2, 4.7, 5.7, 6.2, 7.9, and 9.7.

AT Antigen Assay

Results were submitted for 1 AT antigen method in 2008 and 1 method in 2009. Overall accuracy and precision was high with an all-method bias of 3.8% (range of method-specific biases, 3.6%–4.0%) and an all-method CV of 7.6% (range of method-specific CVs, 7.5%–7.6%).

The single AT antigen method could be graded in each mailing and all testing events (100%) had passing grades.

PC Activity Assay

Results were submitted for 5 PC activity methods in 2008 and 6 methods in 2009. Overall accuracy was intermediate with an all-method bias of 8.5% (range of method-specific biases, 0.2%–21.1%). Clot end-point methods produced 14.5% to 16.5% higher PC activity values than chromogenic end-point methods, which was statistically significant (Table 1). Overall precision of the PC activity methods was high with an all-method CV of 6.1% (range of method-specific CVs, 3.2%–9.2%).

Most but not all PC activity methods could be graded, and 95.8% to 97.8% of testing events had a passing grade. Five testing events had failing grades with extreme outlier standard deviation index values of 5.8, 6.2, 6.6, 10.8, and 28.2.

PC Antigen Assay

Results were submitted for 2 PC antigen methods in 2008 and 2 methods in 2009. Overall accuracy was high with an all-method bias of 3.4% (range of method-specific biases, 1.3%–8.9%). Overall precision was low with an all-method CV of 20.0% (range of method-specific CVs, 12.5%–34.6%).

Only 1 PC antigen method could be graded in the 2 mailings, and that method had 100% passing grades.

PS Activity Assay

Results were submitted for 2 PS activity methods in 2008 and 3 methods in 2009. Overall accuracy was intermediate with an all-method bias of 8.8% (range of method-specific biases, 0.4%–12.7%). Overall precision was intermediate with an all-method CV of 15.8% (range of method-specific CVs, 13.2%–18.7%).

Most PS activity peer groups could be graded, and 100% of testing events had a passing grade.

Total PS Antigen Assay

Results were submitted for 3 total PS antigen methods in 2008 and 3 methods in 2009. Overall accuracy was intermediate with an all-method bias of 6.2% (range of method-specific biases, 4.1%–9.5%). Immunoturbidimetric methods produced 9.8% to 11.1% higher total PS antigen values than enzyme-linked immunosorbent assay methods; however, this difference did not quite reach statistical significance (Table 1). Overall precision was intermediate with an all-method CV of 15.0% (range of method-specific CVs, 9.2%–22.5%).

Half of the total PS antigen methods could be graded, and 100% of testing events had a passing grade.

Free PS Antigen Assay

Results were submitted for 4 free PS antigen methods in 2008 and 4 methods in 2009. All methods were immunoassays that captured free PS using either solid phase C4b binding protein or a solid phase monoclonal antibody that specifically recognized free PS. No methods used polyethylene glycol to precipitate the C4b binding protein–PS complex.

Overall accuracy was intermediate with an all-method bias of 8.8% (range of method-specific biases, 0.6%–15.0%). Immunoturbidimetric methods produced 12.6% to 20.6% higher free PS antigen values than enzyme-linked immunosorbent assay methods, which was statistically significant (Table 1). Overall precision was intermediate with an all-method CV of 17.3% (range of method-specific CVs, 3.4%–44.1%).

One free PS antigen method could be graded each year in the 2 mailings, and 97.1% to 100% of testing events had a passing grade. One testing event had a failing grade with an extreme outlier standard deviation index value of 8.5.

Comparison of AT, PC, and PS Assays

To determine the relative performance of AT, PC, and PS assays, the all-method biases (accuracy) and all-method CVs (precision) were ranked for each assay category according to their numerical values. A final ranking for each assay was then determined from the sum of the accuracy ranking and precision ranking (Table 2).

The order of assay accuracy from highest to lowest was AT activity, PC antigen, AT antigen, total PS antigen, PC activity, PS activity, and free PS antigen (range of assay bias, 2.6%–8.8%). The order of assay precision from highest to lowest was PC activity, AT activity, AT antigen, total PS antigen, PS activity, free PS antigen, and PC antigen (range of assay CV, 6.1%–20.0%). The order of total assay performance based on the sum of the accuracy rank and precision rank, from highest to lowest, was AT activity, AT antigen and PC activity (tie), total PS antigen, PC antigen, PS activity, and free PS antigen.

Grading was dependent on assay precision. For example, the intermediate precision assays (PC antigen, PS activity, total PS antigen, free PS antigen) had a failing grade rate of 0.4% (1 of 263) compared with the high-precision assays (AT activity, AT antigen, and PC activity), which had a 7-fold higher failing grade rate of 2.7% (11 of 412; $P = .03$).

COMMENT

The College of American Pathologists proficiency testing program in coagulation provides a useful mecha-

nism for evaluating interlaboratory accuracy and precision, as well as for evaluating the feasibility of laboratory grading.[7–10] This approach was used in the current study to evaluate the commonly tested anticoagulant proteins in the laboratory evaluation of thrombophilia: AT, PC, and PS.

Accuracy and Precision of AT, PC, and PS Assays

The most useful tests for the diagnosis of AT, PC, and PS deficiency are AT activity, PC activity, and PS activity or free PS antigen, respectively.[11–13] The AT activity assay had optimal performance characteristics with high accuracy and high precision across all methods. It is likely that laboratories can exclude the diagnosis of AT deficiency with a high degree of reliability.

The scenario was quite different for PC activity, PS activity, and free PS antigen. These assays had suboptimal test characteristics with either intermediate accuracy (PC activity, PS activity, and free PS antigen) or intermediate precision (PS activity and free PS antigen). Some PC activity, PS activity, and free PS antigen methods had high accuracy, and some free PS antigen methods had high precision, indicating that the problems were restricted and not global. Nonetheless, because many laboratories used poorer performing assays it is likely that some false-positive and false-negative diagnoses of PC and PS deficiency are being made.

The causes of lower accuracy and precision among the PC and PS assays evaluated in this study are unknown. Many variables can contribute to systematic and random error. A source of systematic error that should be considered due to its ease of correction is improper calibration of the assay. One possible remedy for low accuracy may be to encourage manufacturers to uniformly calibrate their reagent kits against a common standard such as SSC/ISTH Lot No. 3.

Assay temperature, incubation time, and plasma dilution factor might be particularly important sources of error for free PS antigen because they influence the dissociation of PS from C4b binding protein.[14,15] In addition, wash steps could theoretically produce lower values for enzyme-linked immunosorbent assay–based methods compared with immunoturbidimetric methods (which typically do not use wash steps). This might explain our results for total and free PS antigen.

Grading of AT, PC, and PS Assays

All AT assay methods, and consequently all laboratories using these methods, could be graded by a conservative grading protocol based on a target range of ±3 SD from the method-specific mean. Each of these failed grades (6 total) were extreme outliers, which suggested that the assignment of a failed grade was accurate and not due to false classification (assignment of a fail grade when the true assignment should have been a pass grade).

Grading of PC and PS assays was limited in that some methods could not be graded due to an inadequate number of participant results to establish a reliable mean for the method-specific group (ie, less than 10 participants). Most laboratories, however, fell into gradeable peer groups resulting in greater than 80% gradeable laboratories for PC and PS assays. All failed grades for PC and PS assays were extreme outliers, which was similar to the AT assay and suggested accurate grading.

An interesting observation was that assays with lower precision also had the lowest proportion of failing grades (eg, PC antigen, PS activity, total PS antigen, and free PS antigen). This can be partially explained by the limitations of external quality assurance for poorer precision assays. Methods with poorer precision tend to have wide acceptable ranges for a passing grade, making it difficult to detect outliers unless they are very extreme. For example, 1 of the free PS antigen methods with intermediate precision (CV, 25.3%; see Table 1) had an acceptable range of 0.198 to 1.442 IU/mL (mean, 0.820 IU/mL). The lowest result was 0.29 IU/mL and appeared to be a failing grade on visual inspection because the next lowest value was 0.76 IU/mL (2.6-fold higher). Nonetheless the 0.29 IU/mL value received a passing grade because of the wide acceptable range. Highly effective external quality assurance through a laboratory grading approach will require high-precision assays.

Summary

Accuracy and precision were higher for AT assays and lower for PC and PS assays. Grading of individual laboratories was feasible. Further improvement in test performance is warranted for certain PC and PS assay methods to improve the grading of laboratories and to increase the reliability of thrombophilia diagnosis.

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