D-dimer

Simple Test, Tough Problems

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● ***Context.*—D-dimer is widely used for exclusion, or as an aid in diagnosis, of venous thromboembolism (VTE); however, the D-dimer assay methods available from manufacturers and the laboratory application of those methods vary widely.**

***Objective.*—To describe the current laboratory practice regarding the assay and reporting of D-dimer.**

***Design.*—Laboratories' D-dimer proficiency testing data were analyzed and laboratory practices regarding the performance and reporting of D-dimer were surveyed.**

***Results.*—Initial grading of D-dimer proficiency testing demonstrated high variability within and among methods. This variability continued to be present for several years after attempts to intervene. The number of laboratories using D-dimer to exclude VTE grew from 1500 in 2004 to more than 3500 in 2012. Survey and proficiency testing data demonstrated that 33% of laboratories changed the type or magnitude of units from that recommended by the manufacturer, a practice associated with as much as a 20-fold increase in the failure of proficiency testing. Many laboratories used a threshold for the exclusion of VTE that is higher than that recommended by the manufacturer. Many laboratories continue to use qualitative assays with insufficient sensitivity for exclusion of VTE.**

***Conclusions.*—There is considerable variability both within and among quantitative methods used to assay D-dimer by laboratories. Laboratory practice continues to vary widely regarding the type and magnitude of units reported and the setting of the threshold for the exclusion of VTE. Although improved, the variability continues despite initial efforts to intervene.**

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The coagulation cascade concludes as thrombin converts fibrinogen into fibrin monomer. Fibrin monomers self-assemble into fibers, with growth of fibrin via end-to-end and side-to-side association of molecules. They are then covalently cross-linked by factor XIIIa at the outer D domains of adjacent fibrin monomers and the central E domain of a third fibrin monomer molecule.[1] The structure developed within fibrin in which 2 D domains are covalently linked is referred to as D-dimer.

Since the 1960s, clinicians have been measuring the products of plasmin action on fibrin, in the form of fibrin(ogen) degradation products, as an indicator of intravascular fibrinolysis. Initial use of the test was to assist in the evaluation and monitoring of patients with disseminated intravascular coagulation.[2,3] In the early 1980s the first monoclonal antibody–based assays for D-dimer, a specific fibrin(ogen) degradation product, were described that provided an assay with greater specificity for fibrin proteolysis.[4–6] The clinical conditions associated with elevated levels of D-dimer are numerous. Some of these include thrombosis (arterial or venous), pulmonary embolism, venous thrombosis, disseminated intravascular coagulation, myocardial infarction, stroke, postoperative state, liver disease, malignancy, and pregnancy.[7–14]

Because many of the D-dimer tests are very sensitive, being elevated whenever there is acute thrombus in the vasculature, the finding of a low D-dimer level has become a tool used to exclude venous thromboembolism (VTE) in specific clinical situations. This diagnostic application has resulted in a proliferation of different commercially available D-dimer assay methods, with as many as 12 being reported in sufficient numbers for analysis during the time of this report. External quality assessment programs in Europe have shown poor comparability of results between methods, reflecting poor standardization of this test.[15–18]

D-dimer is reported in mass units and, as these assays have evolved, 2 different types of units have been used to represent D-dimer: the fibrinogen equivalent unit (FEU) at 340 kDa and the D-dimer unit (DDU) at 195 kDa.[19] These structures are depicted in Figure 1. Adding to the complexity of reporting these values is the variability in the magnitude of units reported, for example, ng/mL, μg/mL, and μg/L. Variability in the type and magnitude of units has led to confusion in some laboratories as they attempt to use the

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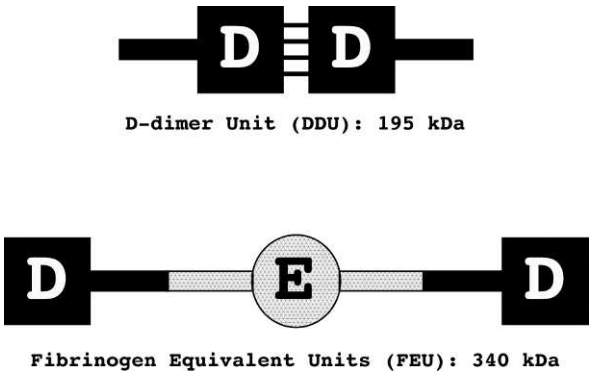

Figure 1. *Types of D-dimer units. Fibrin monomer polymerizes end to end and side to side forming a fiber, fibrin, in a process that is not enzymatically driven. The polymerization of fibrin monomer occurs with the association of the D domains of 2 fibrin monomers (referred to as D-dimer) with the E domain of a third. Soluble fragments of fibrin (fibrin degradation products) that contain variable numbers of the D-D domains (D-dimer) are produced. When assayed, the fragments are quantified in 2 different types of units, the D-dimer unit (DDU) and the fibrinogen equivalent unit (FEU). The mass of the FEU, related to the mass of fibrinogen, is about 1.7 times greater than the mass of the DDU. Because the D-dimer is reported in mass units, the type of units involved is very important in setting the threshold for the exclusion of venous thromboembolism and for interpretation of reported results.*

assay, especially as they set a threshold for the exclusion of VTE.

In 2004, the Coagulation Resource Committee of the College of American Pathologists (CAP) began to grade the performance of the quantitative D-dimer assay. The general performance of laboratories in this exercise showed remarkable variability and led to an analysis of laboratory performance for D-dimer testing by the CAP External Quality Proficiency Testing and to a survey of participants to determine laboratory practice regarding the testing, reporting, and clinical use of D-dimer. The investigation extended from 2004 to 2011 and the results are reported herein.

METHODS

Initial Evaluation of the Grading of D-dimer

The committee determined a target amount of D-dimer and a vendor prepared lyophilized specimens and verified the D-dimer concentration by using a commercial method. Aliquots were sent to 5323 participating laboratories, of which 3936 reported results for D-dimer. For this analysis, 748 laboratories enrolled in the CG2 External Proficiency Testing Program were using methods in 9 consensus groups of 10 or larger, allowing statistical comparison. In 2004, laboratories reported the type and magnitude of the units that they report and the CAP converted all data to a uniform type and magnitude (ng/mL FEU) to allow for comparison of results both within and among methods. Results of the D-dimer testing from the Participant Summary of the CAP proficiency testing, CG2-A 2004, were analyzed. The number of laboratories using a threshold for the exclusion of VTE that was higher than recommended by the manufacturer was determined. Histograms were prepared from the data reported from the 3 methods with more than 150 participants (totaling 491 of 748).

Survey of D-dimer Practice

Because of the wide, and frequently bimodal, variability in reported data and the distribution of values reported for the same specimen, a survey was prepared to determine laboratory practice related to D-dimer. The survey comprised 8 questions that addressed the type of method and manufacturer of the test used; the type and magnitude of the units generated by the test and those reported; and whether the test was used for the exclusion of VTE and, if so, the threshold value for exclusion of VTE. The survey was submitted to participating laboratories in late 2004, with the CG1-C and CG2-C proficiency testing mailings. Surveys were returned to the CAP for analysis. With the CGL-A mailing of 2011, there was a query to laboratories regarding the practice of changing type or magnitude of units used to report D-dimer. In 2004, information on the changing of units was gathered by both self-reporting and by analysis of proficiency testing data. In 2011, the only data available are the self-reported information.

Survey of Method Used to Determine Threshold for Exclusion of VTE

To clarify the method used by the laboratory to determine the threshold, a second, brief survey of 3 questions was sent to 5001 laboratories. The survey asked whether the laboratory used a quantitative assay to exclude VTE and, if so, what method was used to determine the threshold result value for exclusion of VTE; and finally, if the laboratory determined the threshold from data from its own internal study, what was the number of cases used for the study. The survey was submitted to participating laboratories in late 2006, with the CG1-C and CG2-C proficiency testing mailings. Surveys were returned to the CAP for analysis.

D-dimer Practice Following Intervention (Letter Sent to Manufacturers) D-dimer Samples and D-dimer Testing

In early 2006 a letter was sent from the CAP to manufacturers of D-dimer methods, informing them of the confusion regarding the type and magnitude of D-dimer units and the common practice by laboratories of converting the units. Manufacturers were encouraged to contact their clients to promote the use of only the type and magnitude of D-dimer units provided in the package insert.

Samples with elevated D-dimer levels (slightly elevated, target value ≈1500 ng/mL FEU; moderately elevated, target value ≈4000 ng/mL FEU) were mailed to clinical laboratories participating in the CAP 2007 CG1-B survey and 2007 CG1-C survey, respectively. Quantitative D-dimer assays were performed by using methods that were specific to the local laboratories. The variability within and among assays, using weighted mean and coefficient of variation (CV), was evaluated. The number of methods demonstrating a bimodal distribution of the results was determined.

To evaluate the persistence of these bimodal distributions, data from 2011 CGL-A were evaluated for bimodal distribution. D-dimer data from several commonly used assays were converted to ng/mL DDU, and histograms were generated

Failure Rate Related to Method of Reporting

Results from the 2007 CG1-B and CG1-C D-dimer challenges were analyzed for failure rates by comparing the laboratories that used the method of reporting recommended by the manufacturer to those who reported using a change in the type and/or the magnitude of the units. Evaluation of D-dimer in the proficiency testing survey is method specific, with acceptable performance defined as 3 standard deviations either side of the mean after exclusion of outliers.

Use of the Qualitative D-dimer Method

The CAP Coagulation Resource Committee included a questionnaire with the 2009 CGL-C challenge to learn more about participants' utilization of semiquantitative and qualitative D-dimer (Qual D-dimer) tests.

Evaluation of Package Inserts

In 2011, the package inserts from 18 methods (from 10 manufacturers) were evaluated to determine the type and magnitude of units recommended; whether the method was approved by the US Food and Drug Administration (FDA) for

Table 1. Method-Specific Interlaboratory Variance of Quantitative D-dimer Values in the College of American Pathologists 2004 CG2-A Survey

Method	No.	Mean	CV, %
1	145	3391	29.6
2	25	1262	35.0
3	13	3427	12.9
4	164	3531	26.6
5	159	2546	40.9
6	168	2728	30.6
7	45	2638	34.9
8	16	2533	29.8
9	13	1835	17.3

Abbreviation: CV, coefficient of variation.

"exclusion" or cleared for "aid in diagnosis" in the evaluation of VTE; and the threshold for the exclusion of VTE.

Data Analysis

Participants submitted their quantitative D-dimer values and the names of the D-dimer methods used in testing to the CAP for centralized data analysis. The raw data submitted to the CAP contained a wide variety of type and magnitude of units for D-dimer, reflecting the variability of D-dimer reporting units among clinical laboratories. All raw data were mathematically converted by the CAP into ng/mL FEU. Only those D-dimer methods having at least 10 participants on both the 2007 CG1-B survey and 2007 CG1-C survey were included in the analysis. The calculated all-method mean and CV was defined from the weighted average and standard deviation of the individual method-specific means.

Reproducibility was estimated by calculating method-specific CVs. The all-method CV among laboratories was defined as the weighted mean and CV of the individual method-specific mean and CVs. Reproducibility was classified into 3 categories from the overall performance of all methods compared to the magnitude of the method-specific CV (high reproducibility, CV <11%; intermediate reproducibility, CV 11% to 20%; low reproducibility, CV >20%).

RESULTS

Initial Evaluation of the Grading of D-dimer

Thirteen different methods were used by laboratories that reported D-dimer results (range of mean values: 1556 to 4371 ng/mL FEU) in the A mailing of 2004. Ten or more of the laboratories enrolled in the CG2 program used 9 of these methods. Examination of the participant summary showed remarkable variability among methods used and even within most methods. Table 1 shows the number of participants by method, the mean value reported, and the corresponding CV of the reported values. Among all methods, some laboratories used a threshold for the exclusion of VTE that was higher than recommended by the manufacturer of the method (range, 10% to 71%; average, 29.5%).

The high variation led to an evaluation of the reason for the variation. Histograms were prepared from the data for the methods. Three representative histograms are presented in Figure 2. The method numbers in the legend correspond to those in Table 1. In the case of method 5, the variability appears to be due to the distribution of results over a very broad range. In contrast, with methods 4 and 6, each method shows 2 modes of activity that are approximately 1-fold different from each other. This suggested that many participants might be confused about the type of units (DDU versus FEU) that they are reporting.

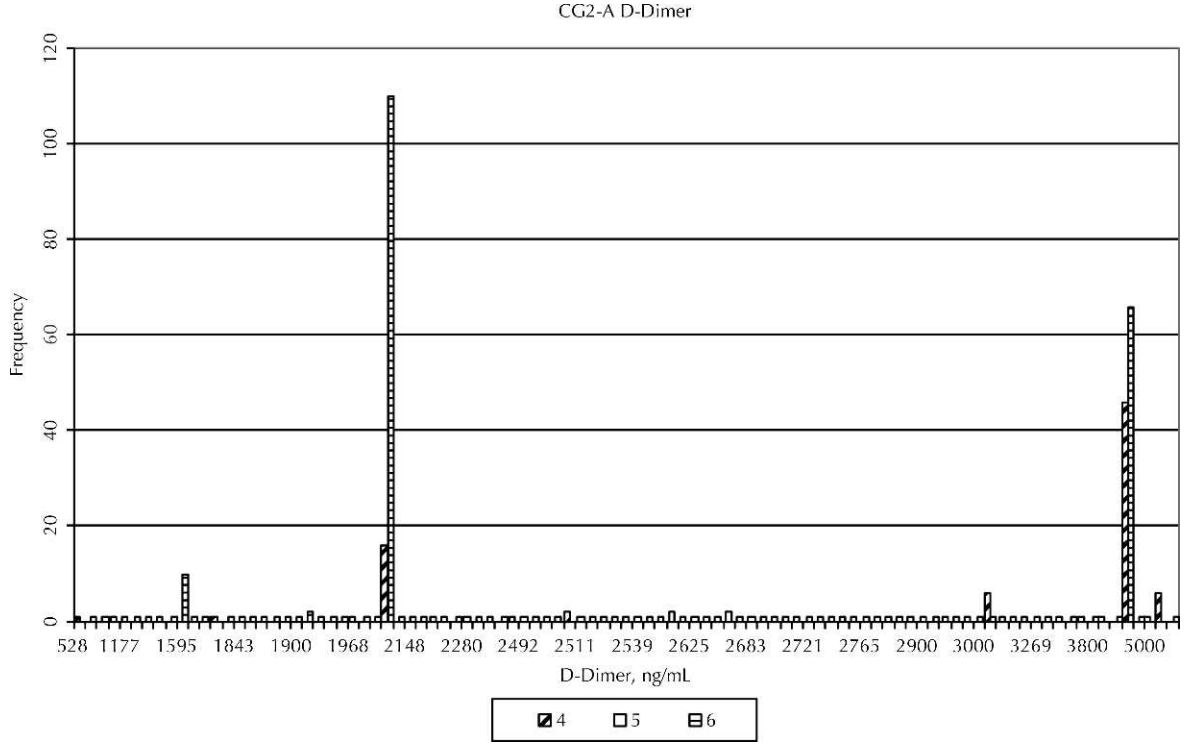

Figure 2. *Distribution of D-dimer values reported (2004). Frequency distribution of D-dimer proficiency testing data reported to the College of American Pathologists (CG2-A, 2004). Values plotted are for methods 4, 5, and 6 from Table 1. The data for method 5 demonstrate a wide variability in the data reported with no value showing a higher frequency. In contrast, methods 4 and 6 both show 2 peak frequencies at values of approximately 2000 and 4400 ng/mL. The 2-fold difference in these peaks indicates a possible erroneous assignment of the type of units (DDU or FEU) by the laboratories using the methods. Abbreviations: DDU, D-dimer unit; FEU, fibrinogen equivalent unit.*

Table 2. Reporting: All Methods[a]					
	ng/mL, No.	g/L, No.	g/mL, No.	mg/L, No.	Total
DDU	379	12	39	125	**555**
FEU	304	19	336	143	**802**
Total	**683**	**31**	**375**	**268**	**1357**

Abbreviations: DDU, D-dimer unit; FEU, fibrinogen equivalent unit.

[a] Twenty-three laboratories reported a different unit and 126 laboratories reported "don't know."

Survey of D-dimer Practice

The initial survey of 8 questions was sent to 5600 participants in the CG1 and CG2 surveys in the third mailing of 2004. Of these laboratories, 2232 reported D-dimer values in the proficiency testing challenge. The survey data were returned by a total of 2018 laboratories or 36% of the total surveys mailed; however, this represented 90% of laboratories that were reporting D-dimer results. Of the surveys returned, 1506 (75%) were from laboratories that reported using D-dimer for exclusion of VTE. For most laboratories (1460 or 97%) a quantitative assay was used for VTE exclusion; however, 29 laboratories (1.9%) used semiquantitative assays, which are not sufficiently sensitive for VTE exclusion. In addition, there were 17 (1.1%) that reported using a whole-blood assay.

Conversion of the results to different type or magnitude of units was reported by 386 laboratories (19%); however, based upon the data submitted, converting the type or magnitude of units was actually performed by 663 laboratories (33%). Table 2 is a compilation of reporting of all 9 methods. When each method was examined, there were examples for all 9 methods of the conversion of data from the type and/or magnitude of units recommended by the manufacturer to each of the type and magnitude of units shown in Table 2. Such changes in the type of units are often done by doubling a DDU result to obtain the FEU equivalent. Shifting the decimal place appropriately can change the magnitude. The units were changed by 511 of the laboratories (34%) using D-dimer for the exclusion of VTE.

In 2004, manufacturers were contacted to determine the level of D-dimer that they recommended for the exclusion of VTE. This was compared to the level adopted by the laboratories using the method. Of all 1506 laboratories, 588 (39%) reported using a D-dimer level for the exclusion of VTE that was higher than that recommended by the manufacturer. Among the 9 methods, the range of laboratories using the manufacturers' recommended threshold was 10% to 71%.

With the evaluation of failure rates in 2007 (see below) it was determined that 12.6% of laboratories were changing the type and/or magnitude of units. In 2011, the response to the CGL-A survey question about this practice indicated that 12.7% of laboratories self-reported using units that were different from those recommended by the manufacturer. This is an improvement from the initial study that showed 34% making such changes in 2004. Of interest, the data from 2007 and 2011 are self-reported and likely are an underestimate of the number making changes. In 2004, the self-reported number of laboratories making changes was 22%, while the analysis of the proficiency testing data revealed that 34% changed the type and/or magnitude of units.

Table 3. Methods Used to Determine the Threshold for Exclusion of Venous Thromboembolism	
Method	**Laboratory, No. (%)**
Manufacturer	1322 (54)
Literature	249 (10)
Local data	520 (21)
Other	116 (5)
Don't know	195 (8)
No data	28 (1)
Total	**2430**

Table 4. Number of Cases Used to Determine the Local Threshold for Exclusion of Venous Thromboembolism	
No. of Cases	**Laboratory, No. (%)**
<50	136 (26)
50–100	167 (32)
101–150	64 (12)
151–200	37 (7)
>200	42 (8)
Don't know	70 (13)
No data	4 (1)
Total	**520**

Survey Regarding the Method Used to Determine Threshold for Exclusion of VTE

Of 4112 responses to the second survey (sent in 2006), 2430 laboratories indicated that they use D-dimer to exclude VTE (1255 answered that they did not use it and 427 replied that they did not know). Of the 2430 using the test to exclude VTE, more than half (1322) used a threshold for exclusion of VTE that was recommended by the manufacturer. The methods for setting the threshold for exclusion of VTE that were reported are summarized in Table 3. Of those using a threshold other than that recommended by the manufacturer, most used a combination of the listed processes or harmonization with another method. Of interest are those who determined the threshold in their own laboratory, a total of 520 laboratories. These laboratories were asked about the number of cases that were used in the study to determine the threshold. The responses are shown in Table 4. Most (303; 58%) used 100 or fewer cases and only 42 (8%) used more than 200 cases.

D-dimer Practice Following the Letter Sent to Manufacturers

Table 5 shows the 15 different D-dimer methods included for analysis in the B and C mailings of 2007. There were 1835 and 2029 participants in the 2007 CG1-B and 2007 CG1-C survey, respectively. The number of participants per method ranged from 3 to 432 for CG1-B, and 4 to 476 for CG1-C. Among the methods, 7 had 110 to 476 samples, an adequate number to prepare frequency distributions. The results of 4 representative methods are shown in Figure 3. Despite efforts to encourage uniform reporting within methods, one can still see that a bimodal distribution is present in the CG1-B specimen (slightly elevated) in methods 2, 3, and 4, while specimen CG1-C (moderately elevated) demonstrates a bimodal pattern in all 4 methods. Furthermore, the bimodal distribution persists in the data from 6 of the 12 methods reported in the 2011 CGL-A mailing.

Table 5 also shows a comparison of the mean D-dimer values for the 12 methods. For the slightly elevated D-dimer sample (11 methods) the weighted all-method mean was 1568 ng/mL FEU with a coefficient of variation of 25.5%.

Table 5. Comparison of D-dimer Results and Imprecision Among Methods						
	Slightly Elevated Sample (2007 CG1-B)			Moderately Elevated Sample (2007 CG1-C)		
Method	No.	Mean	CV, %	No.	Mean	CV, %
bioMerieux VIDAS/Mini VIDAS[a]	261	1935	13.6	288	3912	8.4
Biosite Triage[b]	277	1048	23.0	325	2737	21.5
Dade Behring Advanced D-dimer[c]	432	1284	18.3	476	2158	8.8
Dade Behring D-dimer PLUS[c]	11	295	23.6	10	470	14.6
Dade Behring Stratus CS[c]	90	1410	6.3	110	3563	8.6
Diagnostica Stago[d]	19	2061	9.0	18	4392	9.9
Diagnostica Stago LIATEST[d]	354	2108	11.1	365	4444	8.9
Hemosil D-dimer – IL ACL 7000, 8000, 9000, 10000, Elite, Elite Pro[e]	168	1510	24.3	174	3636	27.9
Hemosil D-dimer – IL ACL Futura, Advance, TOP[e]	121	1821	19.7	144	4436	22.5
Kamiya K-Assay[f]				10	10 150	25.0
Roche COBAS Integra[g]	63	1243	6.7	78	3337	23.9
Roche Hitachi/COBAS C[g]	14	1431	30.5	14	3357	4.8
Weighted mean/CV	**1810**	1568	25.5	**2012**	3772	22.8

Abbreviation: CV, coefficient of variation.

[a] bioMerieux, Marcy l'Etoile, France.
[b] Alere San Diego, San Diego, California.
[c] Siemens Healthcare Diagnostic Products, Marburg, Germany.
[d] Diagnostica Stago, Asnières sur Seine, France.
[e] Instrumentation Laboratory Company, Bedford, Massachusetts.
[f] Kamiya Biomedical Company, Seattle, Washington.
[g] Roche Diagnostics, Mannheim, Germany.

Method-specific means varied by 7.1-fold, with a range of 295 to 2108 ng/mL FEU. For the moderately elevated D-dimer sample (12 methods), the all-method mean was 3772 ng/mL FEU (2.4-fold higher than the slightly elevated sample) with a CV of 22.8%. Method-specific means varied by 21.6-fold, with a range of 470 to 10 150 ng/mL FEU. D-dimer methods differed in the relative increase in D-dimer value of the moderately elevated sample compared to the slightly elevated sample, ranging from 1.6-fold to 3.4-fold increase in D-dimer.

The imprecision of the quantitative D-dimer assays was also examined. Table 5 shows the comparison of the CV for the 12 quantitative methods. For the slightly elevated D-dimer sample, the all-method CV was 25.5%. The method-specific CVs varied by 4.8-fold, with a range of 6.3% to 30.5%. Reproducibility was low, intermediate, and high for 27.2%, 36.4%, and 36.4% of methods, respectively. The percentage of participants using low, intermediate, and high reproducibility methods was 26.0% (n = 470 participants), 64.5% (n = 1168 participants), and 9.5% (n = 172 participants), respectively. For the moderately elevated D-dimer sample, the all-method CV was 22.8%. The method-specific CVs varied by 5.2-fold, with a range of 4.8% to 25%. Reproducibility was low, intermediate, and high for 41.7%, 8.3%, and 50.0% of methods, respectively. Several methods moved to a higher category of reproducibility for the moderately elevated sample compared to the slightly elevated sample. The percentage of participants using low, intermediate, and high reproducibility methods was 36.3% (n = 731 participants), 24.3% (n = 470 participants), and 63.2% (n = 1271 participants), respectively. D-dimer methods differed substantially in the magnitude of change in CV between the slightly and moderately elevated samples, ranging from a 3.6-fold increase in CV (lower reproducibility) to a 6.3-fold decrease in CV (higher reproducibility) for the moderately elevated sample. Six methods (54.6%) showed greater than 20% change in CV. Of these 6 methods, 4 showed higher reproducibility and 2 showed lower reproducibility for the moderately elevated sample.

Failure Rate Related to Method of Reporting

A retrospective analysis was made of the failure rate of laboratories for the D-dimer proficiency testing challenges, namely, 2007 CG1-B, 2007 CG1-C, and 2011 CGL-A. In each case, the failure rates were significantly higher among those participants that changed the reporting units (Table 6). The effect of changing reporting units on failure rates has not improved over time.

Use of the Qualitative D-dimer Method

Among 3531 respondents, 11.5% were offering a qualitative D-dimer test in 2011. A slight majority of laboratories offering a qualitative D-dimer test also offer a more sensitive quantitative D-dimer assay. The major reason for offering both types of test appears to be clinical staff preference for the qualitative method in screening for disseminated intravascular coagulation or VTE evaluation. Participants who offered only a qualitative D-dimer test indicated that the most common reason for this practice was lack of an automated instrument to perform a more sensitive quantitative D-dimer test. Of those laboratories reporting a qualitative method, 110 (27%) recommend using a qualitative D-dimer test to exclude VTE.

Evaluation of Package Inserts

The package inserts for 18 methods from 10 manufacturers were evaluated in 2011. Of 18 methods, 6 from 4 manufacturers stated that the method can be used for "exclusion" of VTE. In addition, 2 methods from 2 manufacturers stated that the method can be used for "aid in diagnosis" in VTE. Three of the inserts did not state the type of units reported and 1 method did not state the threshold for the evaluation of VTE. Table 7 summarizes the information from those methods used by 10 or more participants.

COMMENT

The use of D-dimer for the exclusion of VTE continues to grow. Among laboratories reporting D-dimer proficiency

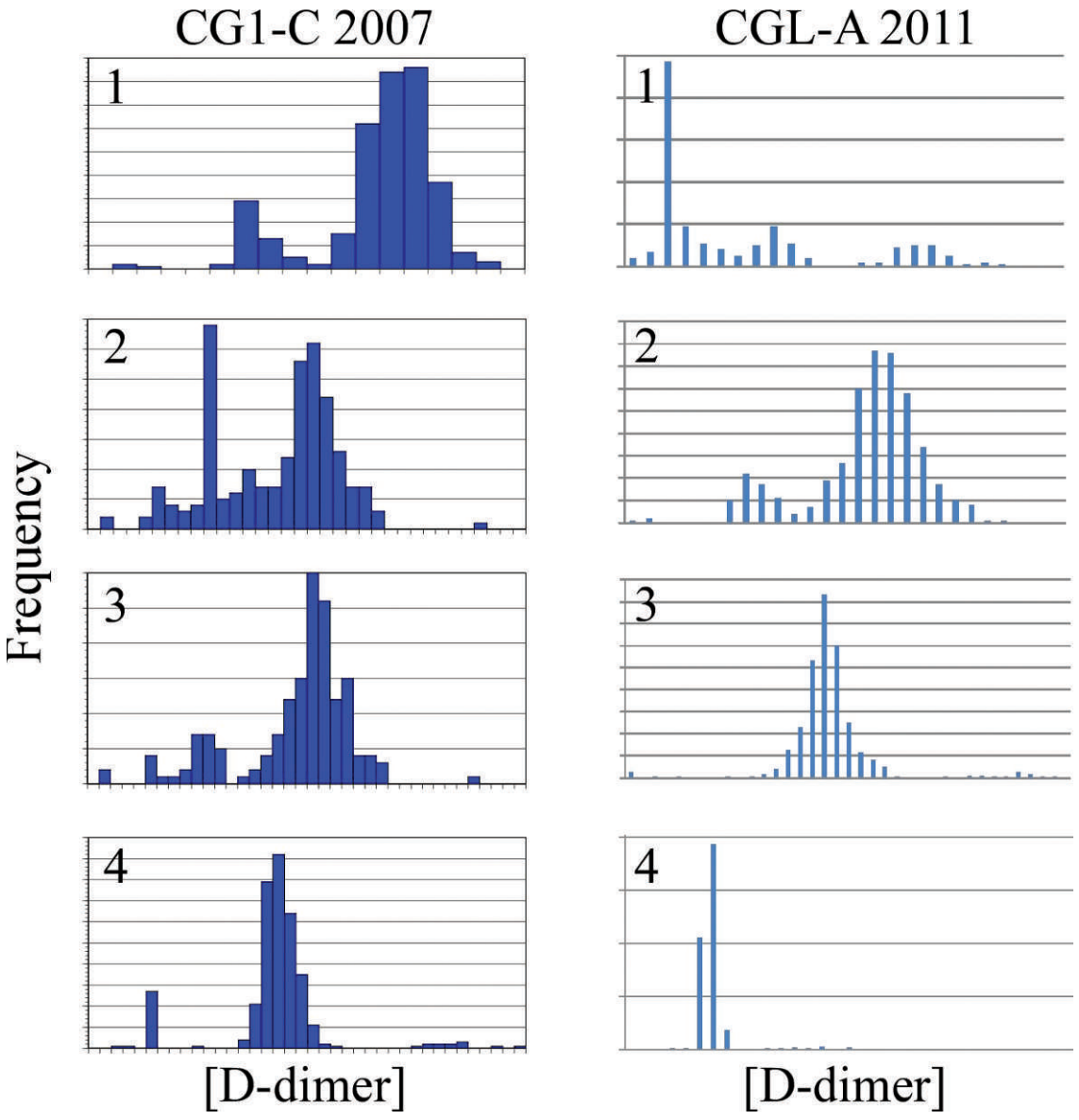

Figure 3. *Distribution of D-dimer values reported (2007 and 2011): Frequency distributions for 4 representative methods from D-dimer proficiency testing data reported to the College of American Pathologists (CG1-C 2007 and CGL-A 2011). For CG1-C 2007 mailing, all methods shown on the left side of the figure demonstrate bimodal distribution of the data (1: Biosite Triage [Alere San Diego, San Diego, California]; 2: HemosIL D-dimer [Instrumentation Laboratory Company, Bedford, Massachusetts; 3: HemosIl D-dimer [Top; Instrumentation Laboratory Company, Bedford, Massachusetts]; 4: bioMerieux VIDAS [bioMerieux, Marcy l'Etoile, France]). With CGL-A 2011 mailing (right side of figure), methods 1 and 2 show bimodal distribution (1: Siemens Diagnostics Advance [Siemens Healthcare Diagnostic Products, Marburg, Germany]; 2: Alere Triage D-dimer Test [Alere San Diego, San Diego, California]; 3: Siemens Diagnostics Innovance; 4: bioMerieux VIDAS) (see text).*

testing data, 1506 laboratories in 2004 reported that they used a quantitative assay for the exclusion of VTE, while in 2007 the number had grown to 2430 and was up to 3335 in 2011. This is a growth of more than 60% in a 3-year period and more than 121% in 7 years. With the prevalence of the assay's use, it is even more critical that laboratories, manufacturers, and regulators make a concerted effort to ensure that the D-dimer test is used and reported appropriately.

When the development of monoclonal antibodies to D-dimer, and the resulting assays, moved from the research environment to the manufacturer for the production of clinical assay, the type of units being used migrated also. As a consequence, there were, and still are, 2 different types of units with 1-fold difference in mass that continue to be used for reporting D-dimer values clinically.[8] As yet, there is no consensus internationally or in industry regarding which should be used. There are still some package inserts with approved assay kits that do not explicitly state the type of units that are being used in the assay. This has created confusion in the clinical laboratory because the type of units is not clear to the user. This problem even extends to the literature. There are examples of book chapters and peer-reviewed journal articles that address the use of D-dimer for the exclusion of VTE but do not indicate the type of units being discussed. In a review of 20 randomly chosen articles published between 2003 and 2011 in which the quantitative D-dimer value was obtained, only 5 of the articles defined the type of units used. This is an example of the general

Table 6. D-dimer Proficiency Testing Failure Rates: 2007 and 2011			
Mailing	2007 CG1-B, No. (%)	2007 CG1-C, No. (%)	2011 CGL-A, No. (%)
All participants	95/2158 (4.4)	78/2209 (3.5)	255/3853 (6.6)
Participants using recommended units	22/1864 (1.2)	32/1952 (1.6)	51/3148 (1.7)
Participants changing units	73/294 (24.8); $P < .001$	46/257 (17.9); $P < .001$	204/705 (28.9); $P < .001$
Failures (change/total)	73/95 (76.8)	46/78 (59.0)	204/255 (80.0)

confusion/ignorance regarding the 2 types of D-dimer units in use, as is the bimodal distribution and remarkably high CV detected in the initial evaluation of the 2004 D-dimer proficiency testing data, resulting in the investigation of D-dimer assay practices reported in this study. In a CAP letter to manufacturers in 2006, this problem was pointed out and manufacturers were advised to inform their clients of the type and magnitude of units that the assay generated and to inform the laboratory to use this information in reporting D-dimer data. Despite this effort, reevaluation of the issue in 2007 and 2011 demonstrated that there was still evidence of confusion on the part of some laboratories regarding the type of units, potentially resulting in the bimodal distributions of proficiency testing data for some methods. The bimodal peaks were separated by a 2-fold difference in mass per volume values, suggesting that some variability arises from confusion of DDU and FEU, which have molecular weights that differ by approximately 1-fold. Whether this confusion regarding the type of units contributes to the bimodal distribution of the data reported here merits further study and provides impetus for international standardization of D-dimer units.

Occasionally, when laboratories change methods for detecting an analyte, the units for reporting the results are also changed, requiring that the clinician think differently about the result. If a clinician is accustomed to seeing D-dimer results with an upper limit of 0.5 mg/L FEU for the reference interval (RI), then when the laboratory implements changes, the upper limit of the RI may become 500 ng/mL FEU. These limits represent the same value, of course, but because the numbers vary by 3 orders of magnitude, the clinician may be confused. In this setting, the laboratory will be tempted (and often does) to mathematically convert the data in order for clinicians to interpret values within a range with which they are accustomed. Such a practice does have some value but also presents problems. For every method used for performing D-dimer testing, such mathematical change of both the type and magnitude of units occurs in some laboratories. Such changes, made by as many as one-third of laboratories in 2004, can lead to errors as evidenced by the increased failure rates among laboratories making such changes. It is encouraging to see that the number of self-reporting laboratories that were changing results had fallen to 12.6% by 2007, but it has remained at that level in 2011. This suggests that interventions (Participant Summary reports, letter to manufacturers, literature) may have had a positive impact; however, nearly 28% of laboratories are still converting the data. Recently, the Clinical and Laboratory Standards Institute (CLSI) published guidelines for the use of D-dimer in the exclusion of VTE. The CLSI recommends that laboratories use the type and magnitude of units that are generated by the assay and that laboratories not convert either the type or magnitude of the units.[20] Data presented here support that recommendation. Although there is no direct evidence, the error rates seen in proficiency testing will, very likely, lead to similar errors in patient reporting. There may be compelling reasons for laboratories to convert the data, but they must be aware of the risks associated with this conversion on proficiency testing success and, potentially, patient reporting.

The CLSI guidelines emphasize the critical importance of determining both the RI and the threshold for the exclusion of VTE.[20] It is convenient if the upper limit of the RI is the same as the threshold for exclusion of VTE; however, this is

Table 7. Information From Package Inserts: 2012					
D-dimer Assay	Unit Type	Units	Threshold Provided	No.	FDA Approval/Clearance for VTE Evaluation
bioMerieux VIDAS/miniVIDAS[a]	FEU	ng/mL	Yes	331	Exclusion
Alere Triage D-dimer Test[b]	DDU	ng/mL	No	466	Aid in diagnosis[c]
Diagnostica Stago LIA[d]	FEU	ug/mL	Yes	1127	Aid in diagnosis
HemosIL D-dimer[e]	DDU	ng/mL	Yes	517	Exclusion
HemosIL D-dimer HS[e]	DDU	ng/mL	Yes	161	Exclusion
Roche Cardiac Reader[f]	NP	ug/mL	No	20	None
Roche Tinaquant[f]	FEU	ug/mL	Yes	156	Exclusion
Siemens Diagnostic Stratus CS[g]	FEU	ng/mL	Yes	145	Aid in diagnosis
Siemens Diagnostics[g]	FEU	mg/L	Yes	600	Exclusion
Siemens Diagnostics Advanced[g]	FEU	mg/L	Yes	330	Aid in diagnosis
Total				**3853**	

Abbreviations: DDU, D-dimer unit; FDA, US Food and Drug Administration; FEU, fibrinogen equivalent unit; NP, information not provided; VTE, venous thromboembolism.

[a] bioMerieux, Marcy l'Etoile, France.
[b] Alere San Diego, San Diego, California.
[c] The FDA indicated that this method was cleared for substantial equivalence to an approved method; however, the package does not provide the threshold aiding the diagnosis of VTE. The package insert also does not include the clinical performance characteristics of the test such as negative predictive value, sensitivity, and specificity.
[d] Diagnostica Stago, Asnières sur Seine, France.
[e] Instrumentation Laboratory Company, Bedford, Massachusetts.
[f] Instrumentation Laboratory Company, Bedford, Massachusetts.
[g] Siemens Healthcare Diagnostic Products, Marburg, Germany.

often not the case. Clinicians and, sometimes, laboratories falsely conclude that if a value is within the RI ("normal"), it is below the threshold for exclusion of VTE. In this study, nearly 30% of laboratories were using a threshold that was higher than that recommended by the manufacturer. This may lead to false-negative results and place patients at risk for undiagnosed and untreated VTEs.

When queried regarding the method used to set the threshold for exclusion of VTE, most were using the recommendation of the manufacturer or data from the literature. However, many laboratories set the threshold on the basis of their own local data and most did so from a sample size that would be unlikely to reach the regulatory standard. Whether random sampling of true-positive and true-negative subjects or the random sampling of test-negative subjects is used to set the threshold for exclusion,[21] estimating the required sample size for establishing negative predictive value (NPV) depends on several variables.[22] If one demands, in addition to high sensitivity (0.97) and high NPV (0.98), a specificity of 0.40 or higher and power that exceeds 0.80, then the sample size will be very high, as great as 2500 to 3000. However, even though the requirement for NPV may be 0.98, tests may set the threshold for exclusion very low to ensure the minimum false-negative test (accepting a lower specificity), such that the actual NPV will be greater than 0.99. The CLSI guidelines recommend that laboratories use the threshold set by the manufacturer. The reason is that to be approved by the FDA for evaluation of patients with VTE, the manufacturer must meet stringent criteria. Management studies performed by manufacturers for FDA approval in labeling the method for use in the exclusion of VTE require separate studies for deep venous thrombosis (DVT) and pulmonary embolus (PE), require testing at a minimum of 3 sites, require that testing be performed only on patients with a low or intermediate clinical pretest probability of thrombus/embolus (not high probability), and require that those patients without VTE be followed up for a minimum of 3 months to confirm that no thrombosis has occurred. The results of such a study must reach an NPV of 97% (lower end of 95% confidence interval [CI] of the NPV $\geq$95%) and sensitivity of 95% (lower end of 95% CI of sensitivity $>$90%). To achieve this goal, studies require approximately 300 cases each of PE and DVT. For an assay to be cleared by the FDA to make the claim "aid in diagnosis," a study demonstrating that an assay is substantially equivalent to an approved assay is required. Those requirements for the D-dimer assay are available in the CLSI document.[20]A slightly more rigid criterion is recommended by the CLSI guidelines, that of a NPV of 98%, the level adopted in the United Kingdom.[20] Achieving such a study locally is impractical in nearly all clinical laboratories, thus the manufacturers' threshold for the exclusion of VTE is strongly recommended.

Conclusions from the 2007 study summarized in Table 5 include the following: (1) many D-dimer methods (at least 15) are used by clinical laboratories in the United States, (2) method-specific reproducibility is low for a sizeable number of methods and laboratories, and (3) methods show marked variation in reproducibility at increasing concentrations of D-dimer.

These findings are similar to those reported by external quality assessment programs in the Netherlands (ECAT Foundation), Germany (INSTAND e. V.), and Italy (CISMEL).[17,18] Poor standardization of the D-dimer assay appears to be an international issue and is not limited to certain geographic regions.

Systematic bias indicates that certain assay variables are poorly controlled among methods. Candidate variables include calibration, antibody specificity, assay format, and interfering substances. Calibration appears to be important, since the use of a disseminated intravascular coagulation plasma pool as a common calibrator substantially decreases the bias between multiple methods.[16] Despite ongoing efforts within the coagulation community, there is still no international standard/calibrator for D-dimer.[16,23–26] Consequently, manufacturers are developing their own kit calibrators on the basis of different, proprietary, in vitro fibrinolysis methods without linkage to a common standard.

Antibody specificity also appears to play a role in systematic bias, since different methods show varying reactivity toward D-dimer antigen present in high-molecular-weight versus low-molecular-weight fibrin fibers.[16] Data reported here support variation in antibody specificity as well because methods show differing response to increasing D-dimer concentration.

One practical implication of high systematic bias is that D-dimer threshold values for the exclusion of VTE will be different for each assay and cannot be used interchangeably. Clinical laboratories need to take this into consideration when evaluating threshold values derived from the peer-reviewed literature, package inserts from kit manufacturers, and in-house studies.

Methods with low reproducibility are of particular concern. The lower the reproducibility, the higher the frequency of erroneous interpretations when using stringent D-dimer threshold values in excluding VTE. In this data set, more than 700 clinical laboratories are using methods with low reproducibility on samples with moderately elevated D-dimer levels. Laboratories need to carefully consider assay reproducibility, particularly at the threshold for VTE exclusion, when selecting a particular test for clinical use. The recommended CV for the assay performance at the threshold for VTE exclusion is 7.5%.[20] External quality assurance data such as those reported in this study, as well as in-house validation data, can help guide laboratories in this decision.

Finally, to date, none of the semiquantitative methods are sufficiently sensitive to use for the evaluation of VTE and should not be used for that purpose. Some point-of-care, rapid methods have been reported to be used successfully in patients with low clinical probability of disease, but few of these assays have been cleared by the FDA for aid in diagnosis of VTE. It is a significant concern that there continue to be laboratories that use semiquantitative assays for the exclusion of VTE. These assays have not demonstrated sufficient sensitivity for this application and using them for this purpose may place patients at risk.

When the FDA approves or clears D-dimer assays, the labeling for "indications for use" may be either for "exclusion of DVT or PE" or for "aid in diagnosis of DVT or PE." The criteria for the former indication (described above) are more rigorous than the criteria for the latter.[20] These are not the same levels of clearance; the differences in the clearance for these indications for use are substantial. Evaluation for DVT and PE each require separate studies. However, the major difference is that to be approved for exclusion, testing is performed only on patients with low or intermediate pretest probability and outcomes are compared to imaging studies, with a required 3-month follow-up of the imaging studies yielding negative results to confirm the negative results. In

Table 8. Summary of Observations and Associated Recommendations	
Observations	**Recommendations**
Some laboratories are using inappropriate D-dimer methods for exclusion of VTE.	Use only a quantitative assay with FDA approval or clearance for evaluation of VTE.
Some laboratories use a threshold for VTE exclusion that is not appropriate for the method.	Use the threshold for VTE evaluation that has been determined by the manufacturer.
Some laboratories change the type and/or magnitude of units of D-dimer that are (is) generated by the method.	Use the type and magnitude of unit that are recommended by the manufacturer in the package insert.
Some laboratories are not clear regarding the difference between the reference interval and the threshold for exclusion of VTE.	Be sure that the reference interval for the test and the threshold for VTE exclusion are reported and that the values are clearly distinguished.
Some manufacturer's package inserts still lack clear instructions and essential information, and some laboratories still misuse methods with clear instructions.	Manufacturers need to be aware that use of the D-dimer assay methods is variable. It is important that package insert instructions and information be clear, and laboratories are encouraged not to deviate from those instructions. Laboratories must follow the instructions provided by the manufacturers via the package inserts.
There is wide variation in reproducibility among the various quantitative D-dimer assays in current use.	The international hemostasis community should continue its efforts to develop an international standard for D-dimer. Information in this article provides further evidence of the need for such a standard.

Abbreviations: FDA, US Food and Drug Administration; VTE, venous thromboembolism.

contrast, pretest probability is not a required entry criterion for aid in diagnosis clearance and no follow-up of patients without VTE is required. Validation of aid in diagnosis involves comparison to a predicate device (correlation with a test previously approved for exclusion), demonstrating "substantial equivalence."[20] The current study did not address practices of laboratories using methods with different levels of approval or clearance by the FDA; however, it is clear that methods with both levels of clearance, as well as methods that are not approved or cleared by the FDA for use in exclusion of VTE, are being used for that purpose. Regardless of the method used, the laboratory must use care in setting the threshold, following guidelines for VTE exclusion in order to provide optimal patient care.[20]

From the data presented, one can draw upon a number of observations with associated recommendations (summarized in Table 8).

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