From Hemophilia to Deep Venous Thrombosis Patient Samples: How to Perform an Easy Coagulometer Validation Process According to Available Guidelines

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Abstract
Validation protocols for the evaluation of coagulometers are needed to help professionals select the most suitable system for their regular laboratory routines. The objective of this study was to show how high standard protocols for the coagulometer validation process can fit into the daily laboratory routine. For this study, 45 healthy individuals and 112 patient samples were analyzed. From the patient samples, 51 were investigated for deep venous thrombosis, 27 for coagulopathy, 19 for antivitamin K therapy, and 15 for hemophilia. For the assessment, the performance of the 3 coagulometers and 1 point-of-care device was considered. One of the coagulometers was a new acquisition evaluated for precision, linearity, throughput, and carryover in the first moment, and the new coagulometer was then compared with the other well-established equipment in the laboratory. In normal plasma, coefficient of variation was ≤ 1.8% for total precision in screening tests and ≤ 3.5% for within-run precision in specific assays. For prothrombin time/international normalized ratio, no significant difference was found when comparing methods. Our study showed how to compare the capacity of a reagent in order to discriminate patients with severe hemophilia from patients with moderated hemophilia, and the κ coefficient agreement was 0.669 (95% confidence interval: 0.3-1.0; P < .001). D-dimer evaluated in patients with deep venous thrombosis and controls showed a 20% discrepancy between the methods. In our experience across Latin America, the number of laboratories that has performed this process is limited. In this study, we demonstrated how to adapt the validation process for the hemostasis laboratory routine to help the professional chose the best and more suitable option.

Keywords
labatory, quality control, validation, coagulometer

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Introduction
There is an increasing number of new automated coagulometer available. For this reason, a validation protocol for the combination of the equipment and reagents is critical to ensure the quality of laboratory processes. The manufacturer’s information regarding coagulometer characteristics is a guidance for the laboratory professional.1 However, local validation protocols for the coagulometer are needed to help professionals select the most suitable system for their regular laboratory routine. This validation should be conducted with samples from both healthy individuals and patients in different clinical situations, using parallel methodologies and reference reagents.1-3 Some important points to consider are knowing

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how to select the coagulometer, how to plan and understand the steps of the validation, and how to adapt the requirements of the validation protocol for the routine assays in the laboratory. Validation processes are the only tool able to demonstrate whether the chosen coagulometer and reagents are adequate and reliable.5,6 There is little practical information on how a hemostasis laboratory can evaluate a coagulometer during an initial purchase or in the need of a replacement. In this study, 3 coagulometers and 1 point of care were evaluated in parallel to demonstrate how the coagulometer evaluation should be performed and how the available guidelines and scientific studies contributed to validation process.

Methods
Study Design

Three coagulometers and 1 point-of-care equipment were use in this study to demonstrate how to perform a coagulometer validation. One of them, Q Smart system compact equipment (low median port), was selected as a new coagulometer to replace the 2 other systems (median high port) and compared with point-of-care equipment. The performance evaluation of the Q Smart system included an analytical assessment (reference range determination, precision, linearity, throughput, and carryover) as well as a comparison with the 3 other equipment (Bland-Altman analysis, sensitivity, and specificity). The assays were selected to represent an evaluation of all types of hematology–hemostasis laboratory methods, such as clotting, chromogenic, and immunoturbidimetric.

Guidelines Needed for Coagulometer Validation

The use of guidelines to proceed the best evaluation in this setting is mandatory. The mean needed protocols are (1) Protocol for the Evaluation, Validation and Implementation of Coagulometers; Approved Guideline (H57-A) 2008; (2) Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline (EP5-A2). 2005; and (3) Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach; Approved Guideline (EP5-A2). 2003. All of these guidelines are from the Clinical and Laboratory Standards Institute (CLSI).5,6,7

Human Plasma Samples

Blood samples from healthy individuals, consecutive outpatients, antivitamin K (AVK)-anticoagulated patients in therapeutic range, patients with hemophilia A, patients with deep venous thrombosis (DVT), and patients under coagulopathies and thrombosis investigation were collected in 0.109 M sodium citrate tubes as recommended by the CLSI H21A5.9 Samples were fractionated by centrifugation at 2500g for 10 minutes at room temperature. Blood processing was completed within 2 hours from extraction. Plasma fractions were stored at −80°C until the assay was performed.

Reagents and Equipment

The Q Smart system (Diagnostic Grifols, Barcelona, Spain) comprises the Q Smart analyzer and the following DG reagents (Diagnostic Grifols) for each test: DG-PT RecombiLIQ for prothrombin time (PT), DG-APTT Synth for activated partial thromboplastin time (APTT), DG-TT L Human for thrombin time (TT), DG-APTT Synth/DG-FVIII for FVIII, DG-Latex DDimer for d-dimer, and DG-Chrom AT L for Antithrombin(II). ACL TOP 500 analyzer and Hemosil reagents from Instrumentation Laboratory (Bedford, Massachusetts) were used for comparison in the following tests: Hemosil Recombiplastin 2G for PT, Hemosil APTT-SP (liquid) for APTT, Hemosil Thrombin Time for TT, and Hemosil APTT-SP (liquid)/Hemosil FVIII Deficient Plasma for FVIII. For comparison in the d-dimer and AT tests, the BCS XP coagulometer and reagents were used (Siemens AG, Munich, Germany). Additionally, the PT assay was also performed with the CoaguChek XS point-of-care_POC (Roche, Indianapolis, Indiana).

Analytical Assessments

Reference range. In order to verify the normal ranges informed by the company, 20 samples from healthy individuals for PT, APTT, and TT screening test were evaluated, and the normal distribution between results was calculated using Shapiro-Wilk test, mean ± 2 standard deviations (SDs).

Precision analysis. The total precision (reproducibility) analysis was also based on the CLSI EP-5A protocol and was assessed using newly reconstituted lyophilized normal (DG-C1) and abnormal (diluted; DG-C2) commercial plasmas for screening tests, PT, APTT, and TT. The analyses were carried out in 4 runs (intra-routine) on 10 different days (inter-routine), with each run consisting of 2 replicates of the 2 different levels (normal and abnormal).

Linearity analysis. Three different methods of equipment measurement (FVIII, d-dimer, and AT) were evaluated for linearity using lyophilized reference plasma as a calibration material (DG-Ref [Diagnostic Grifols] for FVIII and AT; DG-d-Dimer Ref [Diagnostic Grifols] for d-dimer). Five calibration curves were performed for each screening assay, with at least 5 dilution points in duplicate.

Throughput. The throughput rate was calculated based on the number of samples processed during half an hour, and the results were reported as the total number of test performed per hour, evaluated using different test profiles: PT and PT + APTT. Additionally, the time to report the first result of each test profile was measured.

Carryover. The carryover was assessed by measuring the sequence A1-A2-B1-B2-B3 in duplicate, where A were heparinized samples (A code) with final concentration of heparin 1 IU/mL, whereas B was a plasma sample with normal results for
dilutions with buffer were carried out for each sample: 1/2, 3 different dilutions from the same sample. Three different regression, slope ratio, and coefficient of variation (%). In general, the parameters used for evaluation when the samples and standard curve present a parallelism curve are sample and standard plasma curve. The analyses are carried out automatically by the software from each coagulometer. The d-dimer assay results obtained with the Q Smart and the comparison system (BCS XP coagulometer) were evaluated using samples from healthy individuals (n = 25) and patients with DVT (n = 51). The ability of both systems to discriminate between patients with DVT and healthy individuals was assessed. The AT test was evaluated in a group of patients (n = 37) who were under DVT investigation. In this setting of patients, the Q Smart and BCS XP systems were compared and evaluated using a Bland-Altman analysis.

**Method Comparison Study**

The screening assays PT, APTT, and TT were performed in a routine assay with a number of 69 consecutive outpatients and evaluated with the comparison system according to Bland-Altman analysis. The international normalized ratio (INR) of PT test was performed in samples from AVK patients (n = 19) within the normal dose range, and the results obtained in the Q Smart were compared according to the instrument used (ACL TOP 500 and point-of-care device: CoaguChek XS) and the therapeutic range (INR < 2.0, INR: 2.0-3.0, and INR: 3.0-4.0). The FVIII clotting factor assay comparison was performed on samples from patients presenting different coagulopathies profiles (hemophilia A, thrombotic antiphospholipid syndrome [APS], and patients under investigation). In hemophilia A samples (n = 15), the ability of the Q Smart and the comparison analyzer to distinguish and classify patients according to severity (severe, moderate, and mild) was evaluated, as well as the coefficient of agreement between both systems. Samples from patients under bleeding investigation (n = 27) and thrombotic APS (n = 9) were used to assess the quality of the FVIII assay through the analysis of parallelism curves on specific software of Q Smart in comparison with a reference system (ACL TOP 500, coagulometer). The purpose of this analysis was to evaluate whether the fit curve from patients presented the same fit curve from standard plasma (in other words, whether both curves were parallel; Figure 1). In general, the parameters used for evaluation when the samples and standard curve present a parallelism curve are sample regression, slope ratio, and coefficient of variation (%) between 3 different dilutions from the same sample. Three different dilutions with buffer were carried out for each sample: 1/2, 1/4, and 1/8. The factor assays were then tested automatically using both equipment parts. The analyses are based on checking whether times obtained from each patient plasma dilution are parallel with the standard plasma curve. The analyses are carried out automatically by the software from each coagulometer. The d-dimer assay results obtained with the Q Smart and the comparison system (BCS XP coagulometer) were evaluated using samples from healthy individuals (n = 25) and patients with DVT (n = 51). The ability of both systems to discriminate between patients with DVT and healthy individuals was assessed. The AT test was evaluated in a group of patients (n = 37) who were under DVT investigation. In this setting of patients, the Q Smart and BCS XP systems were compared and evaluated using a Bland-Altman analysis.

**Statistical Analysis**

The data sets are expressed as mean with SD when data were normally distributed and median value with range when the normal distribution was not available. The method comparison study, the Spearman coefficient of rank correlation, concordance kappa coefficient, and the bias analyses evaluated according to Bland and Altman were used to test the clinical agreement between methods. Statistical analysis was performed using the GraphPad Prism, version 5, for Windows (GraphPad Software Inc, La Jolla, California). P < .05 was considered statistically significant.

**Results**

**Analytical Assessment**

The reference range determined for PT, TT, and APTT with 20 healthy donor samples and the mean normal PT and SD for APTT and TT ratio are shown in Table 1. The coefficient of variation (CV%) of total precision for each screening test ranged from 0.8% for APTT in intra-routine normal plasma to 3.2% for TT in inter-routine abnormal plasma. Details are shown in Table 2. The within-run precision for specific assays presented values with a CV% range from 0.1% to 3.5% for FVIII, 0.1% to 0.9% for d-dimer, and 0.1 to 0.9% for AT, respectively. The linearity of assays was acceptable with no significant nonlinearity. Regarding throughput, the number of tests completed in 1 hour of continuous processing was 80 for PT and 84 for PT/APTT. The time to report the first result was 3 minutes 25 seconds and 8 minutes 10 seconds for PT and PT/APTT, respectively. No carryover was observed (1.69%).

**Method Comparison Study**

**Prothrombin time, APTT, and TT tests.** Bland-Altman results of the PT, APTT, and TT tests obtained on consecutive outpatients using the Q Smart and the comparative system (ACL TOP 500) are shown in Figure 2. In the PT plot, 3 of 67 data points exceeded the lower limit of agreement; in the APTT plot, 2 of 69 data points exceeded the limits of agreement: 1 exceeded the upper limit and the other the lower limit; and in the TT plot, 4 of 63 data points exceeded the lower limit. The PT/INR
results obtained on samples from the 19 patients with AVK oral anticoagulation with 2 different systems, laboratory coagulometers (Q Smart and ACL TOP 500) and the POC point-of-care device (CoaguChek XS), are shown in Figure 3, according to the therapeutic range used. The main indications for anticoagulation of patients were prophylaxis of venous thromboembolic events, atrial fibrillation, and prosthetic heart valves. There were 4 patients with INR from POC with <2.0, 9 patients with INR from POC with 2.0 to 3.0, and 7 patients with INR from POC 3.0 to 4.0. No significant differences were observed between the test results within a determined therapeutic range when performed by different analyzers.

FVIII assay. Parallelism curve analysis for the evaluation and classification of samples from 15 patients with hemophilia A using the FVIII test with both systems is shown in Table 3. The kappa coefficient agreement was 0.669 (95% confidence interval [CI]: 0.3-1.0; \( P < .001 \)); 3 patients with moderate hemophilia presented results close to borderline (5 UI/dL). All patients classified as severe and mild hemophilia were aligned between systems. The 9 patients under thrombosis investigation were evaluated to test the reproducibility with high levels of protein. All results presented values higher than the normal level in both systems. In order to improve the quality of FVIII assay analyses, 27 patients under coagulopathy investigation were evaluated with parallelism curve analysis. Q Smart software and IL software showed concordance with 22 of 27 samples. Of 5, 4 nonconcordant results were abnormal (nonparallel) for Grifols software and normal (parallel) for IL software, whereas 1 sample was normal for Q Smart software and abnormal for IL software. All these 5 nonconcordant results showed variation in the dilutions, which were not linked with unspecific antibodies.

d-dimer assay. Significant differences between systems were found in d-dimer assay (\( P < .0001 \)). The median values for patients with DVT (n = 51) and healthy individuals (n = 25) were 524 ng/mL fibrinogen equivalent units (FEU) and 335 ng/mL FEU, respectively, when using the Q Smart and 860 ng/mL FEU and 250 ng/mL FEU when using the BCS XP coagulometers (Figure 4). The percentage of alignment between
systems in patients with DVT was 80%, considering the cutoff value of each system (500 ng/mL: FEU Siemens system and 400 ng/mL: FEU Grifols system). The receiver operating characteristic (ROC) curve generated for each system is shown in Figure 5. Sensitivity was 39.0% and specificity 76.0% for Grifols system and 33.0% and 88.0%, respectively, for the Siemens system. The area under the ROC curve in the Grifols system was of 0.7859 with 95% CI = 0.6839 to 0.8879 and \( P < .0001 \), whereas the Siemens system showed an area of 0.5573 with 95% CI = 0.4271 to 0.6874 and \( P = 0.1258 \).

**Table 3. Factor VIII Determination Comparison Using One-Stage Method.**

| Hemophilia A Severity | n   | Q Smart    | ACL TOP 500 |
|-----------------------|-----|------------|-------------|
| Severe                | 3   | 0.4 (0-0.6) | 0.1 (0-0.1) |
| Moderate              | 5   | 6.2 (10-2.9) | 2.7 (1.5-4.2) |
| Mild                  | 7   | 14.5 (10.4-22.5) | 9.5 (6.2-14.3) |

*Kappa coefficient agreement: 0.669 (95% confidence interval: 0.3-1.0; \( P < .001 \)).

The AT results obtained using the Q Smart and BCS XP systems were compared, and a significant correlation was observed in samples from healthy individuals (\( r = 0.7165 \); \( P < .0001 \)).

**Discussion**

According to the local validation protocol used in this study, all characteristics related to the equipment and reagent met the requirements. In general, results of 3% for screening tests and 5% for specific assays in a total precision evaluation are considered acceptable according to the recommendations for hemostasis laboratories.\(^1\) In our study, the precision values met these targets. In addition, good linearity and low CV% between the calibration curves were also observed, and no carryover was found.\(^1,^{10}\) The analytical performance of Q Smart, the new one, was comparable with that of other analyzers.\(^6,^{11}\) In this study, we were able to evaluate patients undergoing AVK therapy with 3 different systems: 2 automated coagulometers and 1 point-of-care device. Regarding the PT/INR and the recombinant thromboplastin reagent, no significant differences were found when comparing Q Smart with either ACL TOP using ISI reagent at approximately 1.0 or with point-of-care CoaguChek XS. All therapeutic ranges were evaluated and were found to be satisfactory; however, studies have shown a general agreement along therapeutic ranges between 2 and 3 INR and discrepancies for results higher than 3.0.\(^{12-14}\) In this study, patients with INR value that were in therapeutic range obtained with the point-of-care device showed the same therapeutic range values for both Grifols and Instrumentation Laboratory systems. Regarding the tests used for thrombosis investigation, concordance between methods in d-dimer of patients with DVT was 80%. Currently, high variability is found for the evaluation of different d-dimer reagent, and 2 main issues remain problematic: the current lack of uniformity in the type and magnitude
of units used for reporting results and the lack of a calibrator that can be used to standardize the assays. Patient plasma contains a mixture of fibrin fragment complexes, generated by plasmin on intravascular and extravascular clot-derived fibrin, as well as circulating soluble fibrin leading to a different composition of antibodies used by manufacturers of D-dimer kits. Same initiatives for harmonization of D-dimer reagents have been carried out by the National Institute for Biological Standards and Control and by the International Society on Thrombosis and Haemostasis. Considering the high variation observed in the context of D-dimer reagents, it is important to note that Grifols and Siemens reagents evidenced a difference between patients with DVT and healthy individuals with acceptable value for ROC curve area, however with different sensitivity and specificity. Regarding the method comparison for FVIII, this study was able to evaluate parallelism of curves by means of Q Smart software to ensure quality in factor determination. As expected, when determining FVIII, the activity of protein should present a linear relation between both clotting times and standard dilutions of plasma. An important prerequisite for this assay is the parallelism of curves between standard plasma and patient plasma in different dilutions. When non-parallelism of curves is observed, the reason may be an inadequate preanalytical condition of samples, inadequate reagent quality, or the presence of unspecific antibodies. In such a case, a new sample should be taken to check whether the abnormal results were an artifact or unspecific antibody interference. When a similar result is obtained with different dilutions of the sample, an investigation of unspecific antibodies should be carried out. There are significant quality analytical benefits with parallelism analyses. Factor assays should always be performed in at least 3 different dilutions to check whether there is parallelism between curves. In addition, this procedure of parallelism analysis that has been traditionally performed manually is time-consuming. Therefore, the automation becomes a convenient alternative for routine laboratory analyses. In this aspect, coagulometers with this type of software available adds a higher level of quality in factor determinations. In conclusion, the validation process to evaluate a new coagulometer or a replacement is a very important issue. In our experience across Latin America, the number of laboratories that has performed this process in their daily practice is limited. The first reason is the difficulty to put in practice the recommendations from guidelines due to the time-consuming, cost, and no quality control experience. It is important to note that the protocols should be adapted according the laboratory routine; however, a standard process should be used in order to have consistent information to perform a statistical evaluation. Empirical and subjective data do not help the laboratory to evaluate process quantitative measurement procedures. Many studies have demonstrated that some reagents may present different compartments according to the equipment and process adopted by the laboratory, confirming the need for independent validation carried out in the daily practice routine. This study demonstrated how guidelines could be adapted according to lab necessity and presented the minimal prerequisites to this end. By selecting patients with hemophilia A and patients with DVT, the laboratories were capable of evaluating 2 important areas of diagnosis and of checking all measurement parameters of the equipment. Furthermore, the study demonstrated that a low median port equipment could be adapted in a laboratory in order to replace more robust equipment and that the validation process could help the laboratory evaluate the cost–benefit relationship.

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Declaration of Conflicting Interests

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References

1. Gardiner C, Kitchen S, Dauer R, Kottke-Marchant K, Adcock D. Recommendations for evaluation of coagulation analyzers. Lab Hematol. 2006;12(1):32-38.
2. Gardiner C. Guidelines for evaluation of coagulation analyzers and coagulation testing. In: Kottke-Marchant K, Davis B, eds. Laboratory Hematology Practice. West Sussex, United Kingdom: Wiley-Blackwell; 2012; 543-551.
3. Clinical and Laboratory Standards Institute (CLSI). Protocol for the Evaluation, Validation and Implementation of Coagulometers; Approved Guideline (H57-A). Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
4. Kitchen S, Woolley A. Evaluation of the Q analyzer, a new cap-piercing fully automated coagulometer with clotting, chromogenic, and immunoturbidometric capability. Blood Coag Fibrinol. 2013;24(1):28-34.
5. Marlar RA, Gausman JN, Engel JW. Validation of hemostasis and coagulation assays: recommendations and guidelines. Semin Thromb Hemost. 2014;40(2):186-194.
6. Badrick T. The quality control system. T Clin Biochem Rev 2008;29(suppl 1):S67-S70.
7. Clinical and Laboratory Standards Institute. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline (EP5-A2). Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
8. Clinical and Laboratory Standards Institute. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP5-A2). Wayne, PA: Clinical and Laboratory Standards Institute; 2003.
9. Clinical and Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline (H21-A5). Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
10. Broughton PMG. Carry-over in automatic analysers. J Autom Chem. 1984;6(2):94-95.
11. Toulon P, Fischer F, Appert-Flory A, Jambou D. Evaluation and performance characteristics of the Q hemostasis analyzer, an automated coagulation analyzer. Thromb Res. 2014;133(5):927-935.
12. Meneghelo ZM, Barroso C, Liporace IL, Cora A. Comparison of the international normalized ratio levels obtained by portable coagulometer and laboratory in a clinic specializing in oral anticoagulation. Int J Lab Hematol. 2015;37(4):536-543.
13. Kalcçi M, Yesin M, Gürsoy MO, et al. Comparison of the INR values measured by CoaguChek XS coagulometer and conventional laboratory methods in patients on VKA therapy. Clin Appl Thromb Hemost. 2017;23(2):187-194.
14. Iijima S, Baba T, Ueno D, Ohishi A. International normalized ratio testing with point-of-care coagulometer in healthy term neonates. BMC Pediatr. 2014;14(1):179.
15. Kahler ZP, Kline JA. Standardizing the D-dimer assay: proposing the d-dimer international managed ratio. Clin Chem. 2015;61(5):776-778.
16. Lippi G, Cervellin G, Casagrandi I, Morelli B, Testa S, Tripodi A. D-dimer testing for suspected venous thromboembolism in the emergency department. Consensus document of AcEMC, CIS-MEL, SIBioC, and SImEL. Clin Chem Lab Med. 2014;52(5):621-628.
17. Gaffney PJ, Edgell T, Creighton-Kempsford LJ, Wheeler S, Tarrelli E. Fibrin degradation product (FnDP) assays: analysis of standardization issues and target antigens in plasma. Br J Haematol. 1995;90(1):187-194.
18. Nieuwenhuizen W. A reference material for harmonisation of D-dimer assays. Fibrinogen Subcommittee of the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis. Thromb Haemost. 1997;77(5):1031-1033.
19. Verbruggen B, Meijer P, NováKova I, Van Heerde W. Diagnosis of factor VIII deficiency. Haemophilia. 2008;14(suppl 3):76-82.
20. Ruinemans-Koerts J, Peterse-Stienissen I, Verbruggen B. Non-parallelism in the one-stage coagulation factor assay is a phenomenon of lupus anticoagulants and not of individual factor inhibitors. Thromb Haemost. 2010;104(5):1080-1082.