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ORIGINAL ARTICLE

Performance Evaluation of Sysmex CN-3000 and Stago STA R Max Coagulation Analyzers and Interference of Hemolysis

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SUMMARY

Background: **In this study, our purpose was to evaluate the analytical performances of the STA R Max and CN-3000, and compare the results of both for PT, aPTT, fibrinogen, D-dimer, and factor VIII, and also to show the influence of hemolysis on PT, aPTT, and fibrinogen assays.**
Methods: **Three hundred ninety-five randomly-selected blood samples from residual material from Istanbul Faculty of Medicine, Central Laboratory workflow comprised the study group. PT, aPTT, fibrinogen, D-dimer, and factor VIII activity were done using both analyzers. Analytical performances were determined through precision, linearity, and comparability studies. Artificial hemolysis was performed through freezing-thawing and mechanical-sheer methods.**
Results: **Intra-assay and between-day CVs% of PT and aPTT were lower than 5% for STA R Max and CN-3000. Only the within-run and between-day CVs% of fibrinogen and the between-day CVs% of D-dimer were higher than 5%, but in acceptable targets. Intra-assay and between-day CVs% of FVIII on the CN-3000 were 3.5% and 12.3% at the low and 2.5% and 5.3% at high level, and 1.8% and 3.7% at the low and 6.3% and 5.9% at high level on the STA R Max. The comparison results of PT, aPTT, fibrinogen, and D-dimer were good (r > 0.91), also good correlations were obtained for FVIII activity > 40 IU/dL and FVIII between 5 - 40 IU/dL (r = 0.89). The results of the hemolysis study were within acceptable limits of the recommended criteria of Fraser and the manufacturer.**
Conclusions: **CN-3000 and STA R Max coagulation analyzers are accurate and highly precise systems for safe use in clinical diagnostic applications. The interferences obtained for both analyzers were found to be within accepted targets.**
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KEY WORDS

coagulation parameters, Sysmex CN-3000, Stago STA R Max, method comparison, interference of hemolysis

INTRODUCTION

Nowadays, automated coagulometers are an indispensable component of modern laboratories. In the last decade, the development in laboratory technology in terms of methodology and test panels, increase in coagulation test volume, and especially urgent requests, have led to the need for high-performance systems with shorter turnaround time. However, evaluating the precision of the systems used in clinical laboratories and ensuring

their sustainability is becoming increasingly time-consuming and complex. Furthermore, in automatic coagulometers, in addition to common screening tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and fibrinogen measurement, various specific tests such as D-dimer, protein C and S, antithrombin III, and factor analysis reveal the necessity of systems to combine different techniques such as chromogenic, coagulation, and immuno-turbidimetric/chemiluminescence in a single system.

In modern coagulation analyzers, mostly two different methods are used to measure coagulation tests based on optical and mechanical clot detection [1]. In the photo-optical method, the detection of clot formation is provided by measuring an alteration of optical density (OD) in a test sample. Therefore, a decrease or change in optical density shows the endpoint of coagulation in seconds. However, in the mechanical method, clot formation is obtained by monitoring the alteration of the movement of a steel ball using a magnetic sensor due to fibrin strands formed. Some studies have reported that optical and mechanical clot detection methods have similar performances in terms of correlation, accuracy, and precision [1-3], whereas others reported the superiority of mechanical detection over the photo-optical method in the measurement of turbid samples [4,5].

In this study, we evaluated the analytical performances of Stago STA R Max, which is currently used in our laboratory, and Sysmex CN-3000. The CN-3000 is a newly introduced analyzer by Sysmex Corp., which has multi-wavelength technology (340, 405, 575, 660, 800 nm) combined with advanced clot detection capability to reduce interfering effects of hemolysis, icterus, and lipemia (HIL) [6]. The STA R Max® is an automated coagulation analyzer that uses a mechanical clot detection method and also integrates chromogenic assays and turbidimetric immunoassays in one system. Additionally, both systems have pre-analytical processing units such as HIL and cap-piercing modules.

In recent years, advancement in laboratory technology has led to a significant reduction in analytical errors in hemostasis assays. However, this situation has revealed the importance of managing the preanalytical phase accurately, such as sample collection, processing, and transport, which is important in terms of the quality and reliability of the test. In preanalytical errors, hemolysis is the most common cause of interference with the most prominent effect on coagulation tests.

Hemolysis resulting from the breakdown of red blood cells (RBCs) and the release of hemoglobin and intracellular contents of RBCs into the plasma often causes inconsistent results by causing biologic and/or spectrophotometric interference [5]. Spontaneous hemolysis can emerge during sample drawing, transportation to the laboratory or processing [7]. In hemolysis, free hemoglobin released from lyzed red blood cells may cause higher absorbance due to adding absorption at the wavelengths used for the optical detection of the measured test in the analyzer. The phospholipid membrane and intracellular contents of lysed erythrocytes may also cause increasing interference by activating thrombocytes and the coagulation pathway [7]. Until recently, hemolysis detection was only made through visual inspection based on the individual experience of laboratory technicians. However, today, the removal of individual subjectivity due to visual inspection and the reliable determination of cut-off values for interfering substances that may affect the test results are performed with HIL modules, which are widely used in the preanalytical modules of clinical chemistry systems, and recently applied to coagulation analyzers.

In this study, our purpose was to evaluate the analytical performances of the STA R Max and CN-3000 analyzers, compare the results of both for and D-dimer, factor VIII, and also to show the influence of hemolysis on PT, aPTT, and fibrinogen assays.

MATERIALS AND METHODS

Three hundred ninety-five randomly-selected blood samples from residual material of Istanbul Faculty of Medicine, Central Laboratory workflow comprised the study group. Blood samples were collected into tubes containing 0.109 mol/L sodium citrate (Becton Dickinson, Plymouth, UK), immediately centrifuged at 2,500 g for 15 minutes, and supernatants were used for the measurement of routine clotting tests (PT, aPTT, fibrinogen, D-dimer), and the assesment of factor VIII activities using a STA R Max (Stago, Asnieres sur Seine, France) and CN-3000 (Sysmex Corp., Norderstedt, Germany). For FVIII activity, supernatants were stored at -80°C and thawed at 37°C in a waterbath before testing.

Analytical performances of the analyzers were determined through precision, linearity, and comparability studies.

Precision studies were performed by using manufacturers' low and high controls (n = 20) on consecutive days following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines EP5-A-2 [8]. Data were used for the calculation of variation coefficient (CV%) and total standard deviations (SDs) [8].

Linearity studies were performed with the serial dilutions of patient samples with a high level according to the the Clinical and Laboratory Standards Institute (CLSI) document by EP06-A [9]. The analyzer's primary diluting fluids were used as the diluent. Seven-point graphical dilution curves were plotted, and regression analyses were performed [9].

For the method comparison study, samples were assessed on the STA R Max and CN-3000 consecutively within two hours of arrival at the laboratory. For method comparison of the two analyzers, D-dimer levels were evaluated as lower than 1,000 µg/L and higher than 1,000 µg/L due to recommendations to exclude thromboembolic diseases [10,11]. Factor VIII activities were evaluated in three groups; 40 IU/dL above, between 5 - 40 IU/dL, and below 5 IU/dL due to the im-

portance of sensitivity of low values of FVIII activity, because our laboratory works as a hemophilia center. The comparison results were evaluated by Deming regression and Bland-Altman analyses. The bias and 95% confidence interval (CI) were calculated by Bland-Altman analysis according to the CLSI document EP9-A3 [12].

Assays

Coagulation tests on the STA R Max (PT, aPTT, fibrinogen assays) were performed using electromagnetic mechanical detection of clotting time, and D-dimer levels were assessed using turbidimetric immunoassays with all reagents from Stago (Stago, Asnieres sur Seine, France) (Table 1).

FVIII activities were measured in a one-stage assay using both analyzers PT, aPTT, fibrinogen, D-dimer levels, and FVIII activities were performed on the CN-3000 using the photo-optical method with reagents from Siemens (Siemens Healthcare, Marburg, Germany). All assays were performed according to the manufacturer's instructions. The factor VIII activities were measured using a one-stage clotting assay on both analyzers. For the measurement of FVIII activity on the CN-3000, actin FSL, ellagic acid, and immundepleted FVIII plasma from Siemens (Siemens, Marburg Germany), Dade® CA System (Owren's veronal buffer, Siemens), and calcium chloride (CaCl2) 0.025 mol/L were used. For the STA R Max analyzer, STA®-C.K. Prest® and immunodeficient FVIII from Stago were used.

Hemolysis study

In vitro hemolysis was done using two methods reported by Lippi et al. [13-15].

Nonhemolyzed samples from 15 healthy subjects were selected to prepare plasma pools, then each pool was separated into six 2-mL aliquots. The first tube was used as a non-hemolyzed sample for measuring PT and aPTT levels.

In vitro hemolysis was performed through freezing-thawing and mechanical-sheer methods. The freezing-thawing method is carried out by freezing whole blood samples in citrated tubes at -70°C for 24 hours [13].

A mechanical-sheer effect was created by mechanical rupture of the cells by passing the sample through a needle (3 times and 7 - 8 times), the type of syringe used (insulin type, 0.5 mL), as well as the thickness of the needle (30 gauge) [14,15]. Then, the in vitro hemolyzed and normal samples were centrifuged at 2,500 g for 10 minutes to remove the cell debris, and the hemoglobin levels of the supernatants were measured using a LH 780 from Beckman Coulter.

In vitro hemolyzed samples were prepared by adding hemolysate to a pool of plasma samples; free hemoglobin levels ranged from 0.16 g/L to 5.0 g/L in each tube. This study was approved by the Ethics Committee of Istanbul Faculty of Medicine (No.: 52059)

Statistical interpretation

Statistical analyses were performed using the MedCalc 15.2.2 software package (MedCalc Software, Ostend, Belgium). Deming regression analysis and Bland-Altman analysis were used to evaluate the comparability of the methods. $p < 0.05$ was considered statistically significant.

For hemolysis, the Westgard criteria and the criterion of + 10% change from the non-hemolyzed samples were used [16,17].

RESULTS

Precision results are shown in Table 2 A - B. Intra-assay and between-day CVs% of PT, aPTT, and D-dimer were lower than 5% for STA R Max and CN-3000 using manufacturers' low and high controls. Only the intra-assay and between-day CVs% of fibrinogen using STA R Max were 11.1% and 8.9% at low level, respectively, and the between-day CV% of D-dimer was 6.0% for STA R Max. Intra-assay and between-day CVs% of FVIII on the CN-3000 were 3.5% and 12.3% at low level and 2.5% and 5.3% at high level, and for the STA R Max 1.8% and 3.7% at low level and 6.3% and 5.9% at high level. All parameters showed good linearity between measuring ranges for both analyzers ($R^2 > 0.97$). The results of method comparison studies were as follows: for PT (n = 390): y = 0.9297x-0.1934 (CI: -2.0199 to 1. 6332), r = 0.98, bias 5.3%; for aPTT (n = 384): y = 0.9198x-2.0252 (95% CI: -9.118 to 5.0757), r = 0.91, bias 14.3 %; for fibrinogen (n = 304): y = 1.030x-45.369 (95% CI: -95.7074 to 4.9695), r = 0.89, bias: 8.1%; and for D-dimer (n = 118) y = 1.3361x-165.570 (95% CI: -330.6310 to -0.3810), r = 0.93, 8.9%, D-dimer < 1000 µg/L (n = 101); y = 1.566 x-240.484 (95% CI: -358,9268 to -122,0406), r = 0.82, bias:1.9%. For factor VIII activity, comparison studies were performed for FVIII activities higher than 40 IU/dL (n = 27) and between 5 - 40 IU/dL (n = 12) and lower than 5 IU/dL (n = 16). For FVIII activity higher than 40 IU (n = 27): y = 0.2563 + 0.9419x, (95% CI: -27.5489 to 28.01616), r = 0.89; for F VIII between 5 - 40 IU (n = 12): y = -10.7005 + 1.5787x, (95% CI: -22.2462 to 0.8453), r = 0.89; and for activities lower than 5 IU (n = 16): y = -0.1021 + 1.0537x, (95% CI: -1.6177 to 1.4135), r = 0.79.

Evaluation of hemolysis study

The results of the hemolysis study is presented in Figure 3 A - C using multiple determinations of a single plasma pool. The supernatant hemoglobin levels ranged from 0.16 g/L to 5.0 g/L. Based on our results, the PT levels of hemolyzed pairs were significantly prolonged compared with non-hemolyzed pairs using the CN-3000, but no significant changes were observed in aPTT and fibrinogen levels. However, the aPTT levels shortened, whereas fibrinogen levels increased in hemolyzed samples compared with non-hemolyzed pairs using the

Table 1. Description of analyzers, reagents, and calibrators used in this study.

Tests	Stago Reagents STA R Max	Stago Calibrator	CN-3000 Reagents	CN-3000 Calibrator
Prothrombin time	neoptimal	unicalibrator	thromborel S	PT multicalibrator
aPTT	cephascreen	unicalibrator	actin FS	-
Fibrinogen	STA-Liquid fibrinogen	unicalibrator	dade thrombin	multicalibrator
D-Dimer	liatest D-dimer plus	-	INNOVANCE® D-Dimer	INNOVANCE® D-Dimer calibrator
Factor VIII	STA-C.K. Prest immunodeficient FVIII	unicalibrator	actin FSL immundepleted FVIII	standard human plasma

Table 2. Intra-assay [A] and between-day [B] coefficient of variation [CV%] for PT, aPTT, fibrinogen, D-dimer and factor VIII using STA R Max, and CN-3000 coagulation analyzers.

	STA R Max				CN-3000			
	Low		High		Low		High	
[A] Intra-assay	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%
Fibrinogen (mg/dL)	102.7 ± 11.4	11.1	330.9 ± 6.8	2.1	86.8 ± 2.0	2.3	263.3 ± 6.9	2.6
D-dimer (µg/L)	270 ± 0	0	2,200 ± 60	2.7	327 ± 9	2.9	2,700 ± 67	2.5
F VIII (IU/dL)	46.6 ± 0.84	1.8	106.9 ± 3.98	3.7	29.6 ± 1.0	3.5	93.6 ± 2.4	2.5
aPTT (s)	32.8 ± 0.5	1.5	50.9 ± 0.5	1.1	20.3 ± 0.08	0.4	37.0 ± 0.3	0.7
PT (s)	13.65 ± 0.1	1.1	24.85 ± 0.6	2.5	10.6 ± 0.07	0.7	19.1 ± 0.08	0.4
[B] Between-day								
Fibrinogen (mg/dL)	107.2 ± 9.6	8.9	298.8 ± 18.7	6.2	87.5 ± 4.6	5.2	263.6 ± 11.2	4.2
D-dimer (µg/L)	250 ± 15	6	2,180 ± 104	4.8	320 ± 19	5.8	2,650 ± 160	6
F VIII (IU/dL)	40.7 ± 2.6	6.3	93 ± 5.5	5,9	27.6 ± 3.4	12.3	91.4 ± 2.9	5.3
aPTT (s)	34.2 ± 1.6	4.8	51.3 ± 2.3	4.5	25.1 ± 0.27	1.1	44.4 ± 1.4	3.1
PT (s)	38.6 ± 1.5	3.9	82.7 ± 7.5	9.1	12.8 ± 0.5	3.7	20.3 ± 0.5	2.4

STA R Max. However, all changes seen in PT, aPTT, and fibrinogen levels were within 10%.

DISCUSSION

In this study, we compared the results of routine coagulation tests and factor VIII activities using automated coagulation analyzers, which used photo-optical (CN-3000) and mechanical methods (STA R Max) in the detection of fibrin formation. Imprecision results of PT, aPTT, D-dimer, and fibrinogen were lower than the accepted goals reported (< 10%) except between-day CVs% of fibrinogen [16,17]. The results were similar to the findings of previous studies [1,19-23], but the between day CVs% of fibrinogen and D-dimer were higher than the results of previous studies using STA R Max [22]. However, in a study evaluating the performance of the STA R Max analyzer, it was reported that the intraassay and interassay CVs% of D-dimer were 7.9% and 8.6%, higher than our results [23]. Gardiner et al. also reported similar imprecision results for PT, aPTT, and fibrinogen with the Clauss method, and D-dimer using a CS-5100 analyzer and CN-6000 [6]. In a study by the World Federation of Haemophilia (WFH) EQA programs, the acceptable CVs for PT and aPTT were reported as 10.1 - 20.4%, with larger variations for D-dimer (up to 47%) assays [24,25]. PT, aPTT, and fibrinogen showed good correlation and agreement between the two analyzers, correlation coefficients were between 0.89 - 0.98. However, the biases were 5.3% for PT, 14.3% for aPTT, and 8.1% for fibrinogen which were higher than recommended [16,17]. The reason of less satisfactory correlation and bias in aPTT results can be explained by different measurement methods, phospholipid sources, lupus anticoagulant (LA) sensitivity,

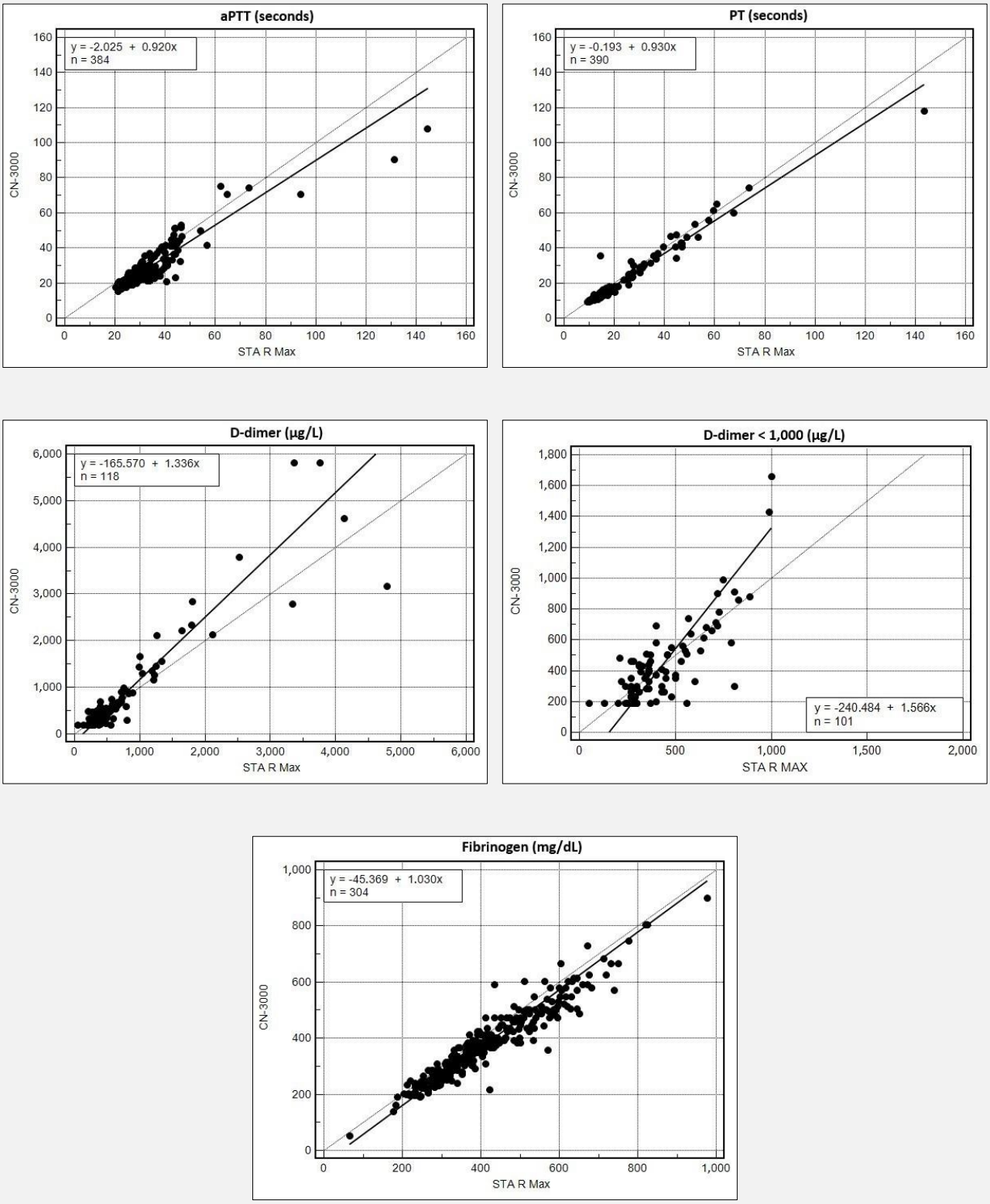

Figure 1. Comparison of the CN-3000 with the STA R Max: results of PT, aPTT, fibrinogen, and D-Dimer.

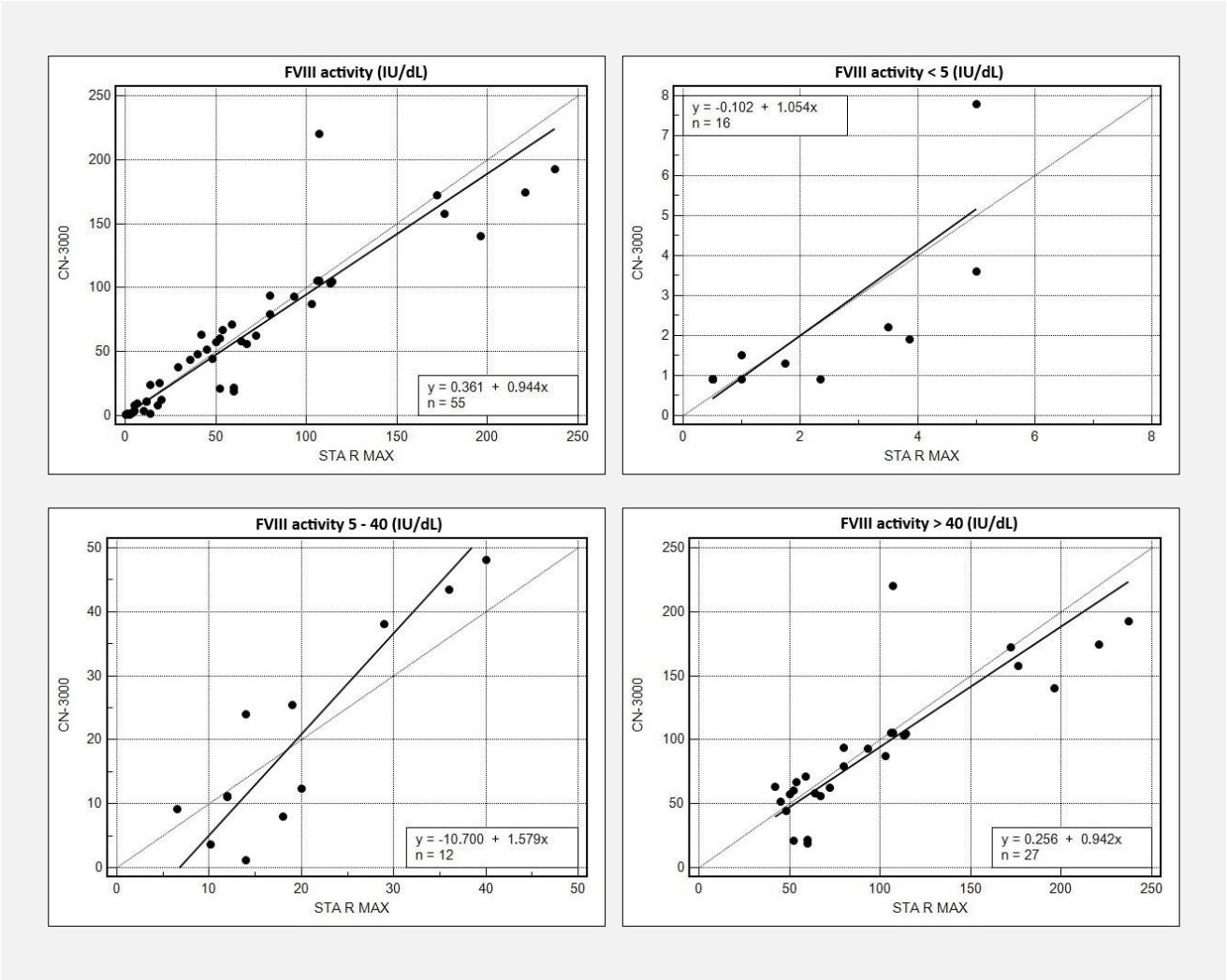

Figure 2. Comparison of the CN 3000 with the STA R Max: results of Factor VIII activity.

and different contact factors used for method comparison [26,27]. In our study, a polyphenolic compound as an activator with a rabbit brain-derived phospholipid was used in the cephascreen reagent from Stago, and ellagic acid with a plant-derived phospholipid was used in Actin FS from Siemens [28,29]. The cephascreen reagent, containing silica as an activator, is superior to actin FS in LA sensitivity, and the plant origin and high phospholipid concentration of the actin-FS reagent explain its limited sensitivity to human protein-phospholipid compounds [27]. However, there are studies reporting similar or better correlation coefficients for PT, aPTT, and fibrinogen between mechanical and photo-optical assays compared with our results [1,26]. Lippi et al. also reported better correlation coefficients for PT than aPTT (r = 0.74) and fibrinogen values between STA R Max and the ACL Top, similar to our results [26]. Depending our findings, precision results were lower than 10% for D-dimer. Even though the cut-off value of D-dimer is 500 µg/L for our laboratory which is the most commonly used value to exclude VTE and PE, the studies have revealed that using an age-adjusted, higher D-dimer cut-off in patients ≥ 50 years seems to be confident. There are studies that recommend higher cut-offs for the patients older than 60 years [10,11,30]. Because of the differences in the decision limit, the method comparison and bias were evaluated in two groups; the bias % of the entire group was 8.9%, reduced to 1.9% for the samples with D-dimer levels lower than 1,000 µg/L, both groups showed good correlation between both analyzers (r = 0.93 and 0.82). These results once again reveal the importance of inter-laboratory variation and note the importance of harmonization in coagulation results to ensure accurate diagnosis and follow-up of clinical results.

The intra-assay and between-day CVs of FVIII were lower than 3.7% and 6.5% for both analyzers, in accordance with findings of previous studies which were lower than accepted goal, except between-day CV of CN-3000 [6,17,31,32]. The comparison studies for

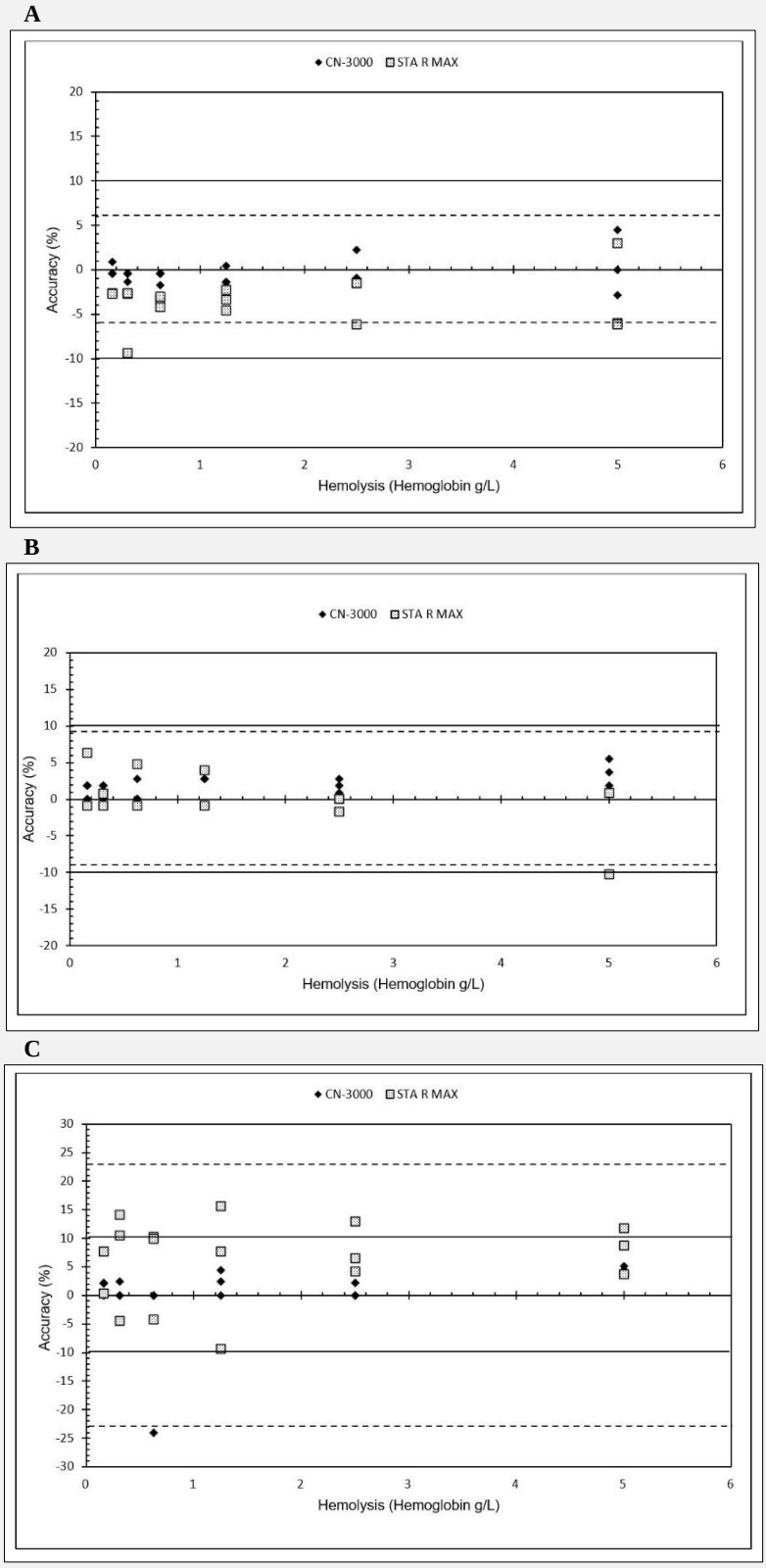

Figure 3. The interfering effect of hemolysis on: (A) activated partial thromboplastin time, (B) prothrombin time, (C) fibrinogen with the results of multiple measurements of a single plasma pool using CN-3000 and STA RMax analysers.

Acceptable limits recommended by manufacturer's (continuous line) and Fraser's (dashed line) are plotted.

FVIII activities showed good correlation and agreement (r = 0.89) between both analyzers for the FVIII activities higher than 40 IU/dL and between 5 - 40 IU/dL. The correlation for FVIII activities lower than 5 IU/dL was less satisfactory but at an acceptable level (r = 0.79). The previous studies performed using optical systems have shown lower correlation coefficients for FVIII levels lower than 10 IU/dL in accordance to our findings [33,34]. However, when we evaluated all groups, correlation coefficients increased to 0.93, in accordance with the findings of Gardiner et al. (r = 0.96) [6].

When we evaluated the impact of in vitro hemolysis on PT and aPTT results, which was prepared using two techniques including mechanical-sheer and freeze-thaw cycles, we observed prolonged PT duration and no significant changes in aPTT and fibrinogen levels in hemolyzed samples using the CN-3000, whereas shortened aPTT and increased fibrinogen levels were demonstrated in hemolyzed samples using the STA R Max. The important issue in interference studies is the selection of the ideal technique, which includes biologic interferences that mimic the natural biologic environment that potentiates the coagulation pathway with the effect of membrane phospholipids. Lippi et al. reported prolongation of PT, shortening of aPTT, and lower fibrinogen at 0.9 g/L hemoglobin using an optical analyzer [35]. However, some studies performed with mechanical clot detection have shown a nonsignificant small decrease in aPTT levels [36,37], other studies showed significantly lower aPTT levels, in line with our results [38]. Additionally, there are studies demonstrating no changes or longer duration in PT and aPTT levels [36,39]. Hedeland et al. reported no significant changes in PT levels with INR < 2.0, but prolonged PT levels with INR > 2.0 [40]. The results of these studies revealed the effect of interfering substances on the test results varied according to the nature of the interfering substance, its level, and the reagent used. Accordingly, the European Federation of Clinical Chemistry and Laboratory Medicine working group recommended the estimation of cut-off and reporting of hemolytic test samples together with warnings and comments [41].

In conclusion, CN-3000 and STA R Max coagulation analyzers are accurate and highly precise systems for safe use in clinical diagnostic applications. The comparison results of CN-3000 were good for routine screening parameters including PT, aPTT, fibrinogen, D-dimer, and also factor VIII with a mechanical-based analyzer (STA R Max), but the biases were higher than expected.

When the interfering effect of hemolysis was evaluated, the impact of obtained interferences was within accepted targets and not at a level affecting clinical results with either analyzer. However, the most important issue is to establish the accurate cut-off values and to apprise the laboratory technicians about the clinical relevance of hemolysis tests.

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