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ORIGINAL ARTICLE

Evaluation of the Analytical Properties of the Diagon CoagXL Coagulation Analyzer

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SUMMARY

Background: **This study aims to evaluate the analytical properties of the DIAGON CoagXL (Budapest, Hungary) coagulation system.**
Methods: **The study includes a total of 212 normal, 49 pathologic plasma samples sent to our laboratory. The partial thromboplastin time (PTT) and activated partial thromboplastin time (aPTT) measurements were performed on the Diagon CoagXL and Stago StaR coagulometers. The precision, method comparison, carry-over, activity determination, and reference range verification studies were performed with Diagon CoagXL, the test analyzer.**
Results: **In the precision study performed with normal and pathologic plasma samples for the PT and aPTT tests, the within-day coefficient of variation (CV%) was 1.9 in the normal and 0.68 in the pathologic plasma for the PT, and for the aPTT it was 0.61 in the normal and 0.9 in the pathologic plasma. The between-day CV% was 1.6 in the normal plasma and 5.5 in the pathologic plasma for the PT and 3.7 in the normal plasma and 2.1 in the pathologic plasma for the aPTT. In the comparison study, the entire group mean ± standard deviation (mean ± SD) value for the INR was found to be 3.13 ± 1.26 in the CoagXL and 2.67 ± 0.82 in the StaR analyzer. The difference between these values was statistically significant ($p < 0.006$). For aPTT, mean ± SD value was found to be 39.44 ± 25.02 seconds (sec) in the CoagXL analyzer and 43.4 ± 27.63 sec in the StaR analyzer. The difference between these values was not statistically significant ($p > 0.5$). In the carryover study, the carryover value was -0.16 for the PT and 0 for the aPTT, which was under the allowable limit value (< 3 SD). In the percent activity determination study, regression equation of prothrombin activity (%) versus time (sec) was found as $y = 341.6567 \pm 37.1920x + 1.0913x^2$ ($R^2 = 0.97$). The reference range verification analyses reveal that the manufacturer ranges were acceptable.**
Conclusions: **Verification studies of CoagXL analyzer system was acceptable. But in comparison studies of PT we saw that there are still problems with recommended INR system.**
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INTRODUCTION

Clot-based tests, such as PT and aPTT, test the time interval from the onset of coagulation to clot formation. Prothrombin time is a single-stage test based on the time required for the formation of a fibrin clot following the addition of tissue thromboplastin, phospholipid, and calcium to a decalcified thrombocyte-poor plasma. aPTT is the measurement of the coagulation time fol-

lowing the addition of phospholipid, intrinsic pathway activator, and calcium. The time required for the formation of clot is determined [1].

There are two different methods used in most laboratories: photo-optical and mechanical. In the photo-optic method, a photo-sensitive sensor detects a decrease in the light transmission or an increase in the light scattering with the formation of a clot as the endpoint [1,2]. In the mechanical method, a magnetic sensor follows the metal ball within the test solution. When a clot occurs, the change in ball movement is detected by the sensor. An advantage of the mechanical method is that bilirubin, lipid, and hemoglobin do not interfere as is the case in the optical methods [2]. However, irregularities in fibrin and fibrin degradation products as in dysfibrinogenemia and sepsis may cause unreliable results in the mechanical method [3,4].

The PT test gives information on the extrinsic and common coagulation pathways. This test is used especially in monitoring the anticoagulant therapy with vitamin K antagonists.

The thromboplastin reagent used in the PT measurement includes the tissue factor and coagulant phospholipids. Many commercial thromboplastins are prepared from mammalian tissues containing tissue factor and phospholipids. A thromboplastin preparation, which consists of a single tissue extract, is called 'pure'. The preparation is called 'combined' if it additionally contains the Factor V and the absorbed bovine plasma as a fibrinogen source. Thromboplastins have sources such as human, bovine, and rabbit according to the species; brain, lung, and human placenta according to the tissue they are obtained from. The recombinant human thromboplastin is produced by the recombinant DNA methods in *E-Coli* or insect cells and the lipid is added *in vitro* [5].

Because of manufacturing differences among commercial PT kits, different PT values can be obtained with the same sample. The World Health Organization introduced the International Normalized Ratio (INR) system in 1983 for standardization of PT assays [6]. Reagent kits are calibrated according to an international reference thromboplastin, and a specific international sensitivity index (ISI) value is determined for each reagent. INR is calculated by the formula $INR = (PT/MNPT)^{ISI}$. Thus, INR is supposed to give the PT value to be obtained as if the international reference thromboplastin is used in the patient test. The MNPT (mean normal PT) is the geometric mean of the prothrombin time values of the healthy adult population. The geometric mean of the prothrombin time of the fresh plasma received from at least 20 healthy adults from both genders is recommended for determining the MNPT [5]. The ISI is the mathematical formula between the clotting time (sec) obtained with the use of the primary international reference thromboplastin and the thromboplastin reagent used [5]. International reference thromboplastin is prepared from at least 20 healthy people and 60 patients who use 60 vitamin K antagonist, as recommended by WHO. ISI values are specific for the combination of the reagent thromboplastin and the analyzer together [7]. However, it is not considered sufficient by some researchers. The specific ISI value is recommended for each new thromboplastin lot [8]. As the ISI value decreases, the thromboplastin sensitivity increases and expresses unit prothrombin activity in a longer time interval. Recombinant human thromboplastin is the most sensitive thromboplastin and its ISI value is closest to 1 [7].

In this study, we evaluated the performance characteristics of the DIAGON CoagXL coagulation system.

MATERIALS AND METHODS

In the present study, normal samples were selected from the samples sent to the central laboratory of Dr. Lütfi Kırdar Kartal Training and Research Hospital for coagulation analysis in November 2016. The blood samples were taken from the antecubital vein after 8 - 12 hour fasting into 1.8 mL Golden Vac (Huangyan, China, lot no. B:160710) tubes with 3.2% (109 mmol/L) sodium citrate with a ratio of 9/1:blood/citrate. The samples were centrifuged at 1,500 x g for 15 minutes. Hemolyzed, lipemic, and icteric samples were excluded. The samples were selected after the results were evaluated. The study includes a total of 212 normal samples and 49 pathologic samples. The MNPT, precision, method comparison, carry-over, activity determination, and reference range studies were completed in a maximum of 4 hours after samples were taken. Samples were divided into portions and stored at -20°C for between-day precision studies.

Diagon CoagXL (Budapest, Hungary), the test analyzer, is a fully automated coagulation analyzer which uses the optical method. The Dia-PT R (lot no. 960420) kit was used for the PT on this analyzer. The lot-specific ISI value of this kit was 1.08, using human thromboplastin produced from *E. coli* with recombinant DNA technology. Plasma and reagents were heated to 37°C, and the measurement was started when the 100 µL of prothrombin reagent was added to the 50 µL of sample. The clotting time was determined and expressed in seconds. The Dia-PTT Liquid (lot no. 960510) kit was used for the aPTT test. The reagent content is rabbit brain phospholipid, and the activator is ellagic acid. In the study, the plasma and reagents were heated to 37°C. Fifty microliters of sample and the 50 µL of aPTT reagent were added to the vessel and incubated for 3 minutes. Then, 50 µL of $CaCl_2$ (lot no. 960626) reagent was added and the timer started. The clotting time was determined and expressed in seconds.

The analyzer used for the comparison was the StaR (Paris, France) fully automated coagulation analyzer of Diagnostica Stago, which we use for routine coagulation tests in our laboratory. The analyzer measures using the mechanical method. The reagent Sta Neoplastin CI plus (lot no. 250832) was used in this analyzer for

the PT. This reagent consists of lyophilized thromboplastin prepared from rabbit brain tissue and calcium. The ISI value was 1.24. The STA - CK Prest® (lot no. 250995) reagent was used in this analyzer for the aPTT. This reagent contains lyophilized cephalin obtained from rabbit brain and buffered kaolin [9]. The $CaCl_2$ reagent is STA-$CaCl_2$ 0.025 M (lot no. 251330).

In the MNPT study for CoagXL analyzer, the geometric mean of the PT values obtained from 20 healthy plasma samples was used. The INR values were calculated from the formula (patient PT/MNPT)^{ISI} [5].

The precision study was conducted separately for within-day and between-day evaluations, according to CLSI Guidelines. One normal (9.24 ± 0.18 seconds for PT, 28.6 ± 0.17 seconds for aPTT) and one pathologic (20.29 ± 0.14 seconds for PT, 35.27 ± 0.31 seconds for aPTT) plasma pool was prepared. The PT and aPTT were measured 20 times from 2 samples to determine the within-day precision. For between-day precision, normal and pathologic samples were tested 3 times per day for 5 days. The mean, SD, and CV% values were calculated (CV = SD/mean x 100) [10].
The allowable total day-to-day coefficient of variation (CV) of the system was less than 5% with the normal and abnormal control plasmas [11].

In the comparison study, chosen according to the laboratory results, PT was measured in a total of 44 samples (10 with INR < 2, 30 with 2 > INR < 4, and 4 with INR > 4) and aPTT in a total of 40 samples (20 normal and 20 pathologic) on a CoagXL [12].

In the carry-over study, 10 pathologic (P) and 11 normal (N) plasmas were measured in the order of N1/N2/N3/P1/P2/N4/P3/P4/N5/N6/N7/N8/P5/P6/N9/P7/P8/N10/P9/P10/N11 [10]. N plasma values measured after N, and N plasma values measured after P were compared. The allowable error limit was < 3 SD for each parameter [13].

In the activity determination study the manufacturer's calibrator (Dia-cal lot no. 960119) and diluent (Dia-imidazole lot no. 950726) were used**.** From the calibrator (100% activity) dilutions of 3/4, 1/2, 2/5, 1/3, 1/4, and 1/8 were automatically performed by the analyzer. All samples were measured twice. The obtained data were assessed in regression analysis.

In reference range verification study, each of PT and aPTT studies included 20 samples from subjects with normal biochemistry, urine, and coagulation test results. All the reference subjects' results were within the reference interval given by the manufacturer for PT test. For aPTT only one subject was outside of the range. Therefore, the reference ranges were considered acceptable for our population (CLSIC28-A2) [14].

Statistical analysis
The statistical analyses were performed with the Medcalc software 17.5.5 (Belgium) version. $p < 0.5$ was accepted as statistically significant.
The Independent *t*-test, Bland-Altman analysis, Passing-Bablock Regression and Concordance Correlation Coefficient (CCC) were used for comparison. Bland-Altman analysis evaluates the difference between the measurements according to the arithmetic mean of the two methods. The difference % (difference/mean x 100) was shown as recommended. The mean difference ± 1.96 SD limits were shown as the limits of agreement. The Passing-Bablok Regression calculates a regression equation between the two methods and gives confidence intervals for a constant or a proportional bias. The concordance correlation coefficient (CCC) is an index used for the evaluation of the concordance. Those with CCC > 0.99 were interpreted as perfect, between 0.99 - 0.95 as good, between 0.94 - 0.90 as medium and < 0.90 as poor correlation.

RESULTS

MNPT study
The geometric mean of the PT values obtained from 20 healthy plasma samples was found to be 8.82 seconds and accepted as the MNPT value.

Precision study
The within- and between-day CV% values of normal and pathologic plasmas are shown in Table 1.

Comparison study
In the entire group, the mean ± SD values for the PT-INR were found to be 3.13 ± 1.26 in the CoagXL and 2.67 ± 0.82 in the StaR analyzer ($p < 0.006$). The Bland-Altman analysis results are shown in Figure 1. The mean bias (limits of agreement) values in the entire group was found as -13.3% (+7.5 and -34) in Bland-Altman analysis. When the graphic was examined, it was seen that the mean bias of the INR values > 2.5 shifted towards more negative values. The mean bias values of INR < 2, 2 < INR < 4, and INR > 4 were found to be -5.7% (+6.3 and -17), -13.7% (+3.7 and -31), and -30.1% (-6.2 and -54.1), respectively. The CCC value of the entire group was found as 0.80; INR < 2, 2 < INR < 4, and INR > 4 were 0.84, 0.65, and 0.07, respectively. The constant error was -0.64, (CI% -0.91 to -0.39) and the proportional error was 1.41, (CI% 1.29 - 1.51) for the INR in the Passing-Bablock analysis. The results of the Passing-Bablock analysis are shown in Table 2. The mean ± SD values were found to be 39.44 ± 25.02 sec in the CoagXL and 43.4 ± 27.63 sec in the StaR analyzer for the aPTT in the entire group ($p > 0.5$). The Bland-Altman analysis results are shown in Figure 2. The mean bias (limits of agreement) was found as 2.2% (+25.7 and -21.4) for the aPTT. The Bland-Altman graphic shows that the mean bias % values are different

Table 1. Within- and between-day precision study.

		Within day			Between day		
		Mean	SD	CV%	Mean	SD	CV%
PT	Normal	9.24	0.18	1.9	9.78	0.15	1.61
	Abnormal	20.29	0.14	0.68	22.31	1.24	5.51
APTT	Normal	28.6	0.17	0.61	29.94	1.16	3.73
	Abnormal	35.27	0.31	0.90	36.48	0.76	2.18

Table 2. Passing-Bablock regression analysis.

	Intercept (a)	95% CI	Slope (b)	95% CI
aPTT	-9.01	-13.29 to -4.86	1.24	1.10 to 1.37
INR	-0.64	-0.91 to -0.39	1.41	1.29 to 1.51

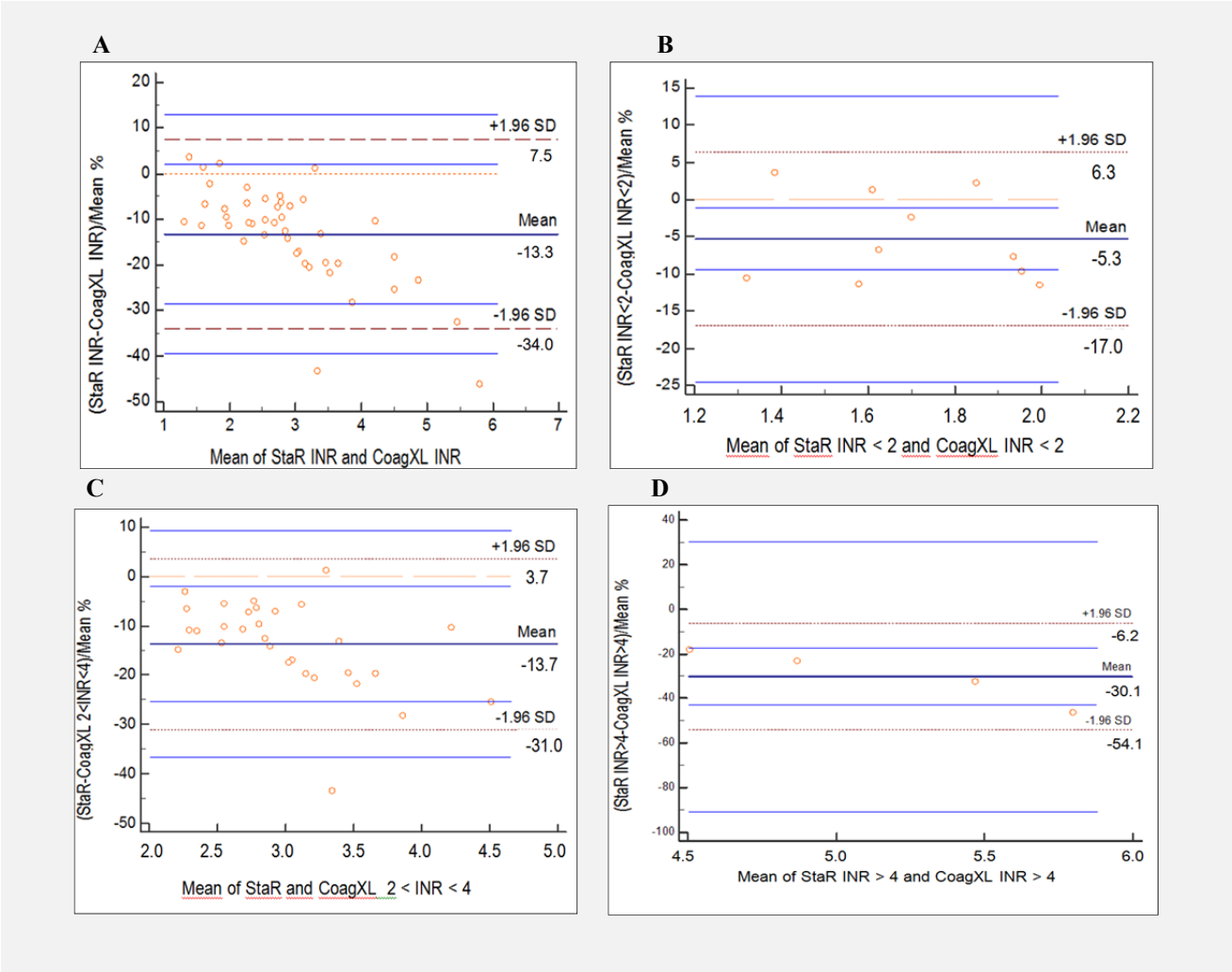

Figure 1. Bland-Altman Graphics (INR).

(A) Entire group INR, (B) INR < 2, (C) 2 < INR < 4, (D) INR > 4.

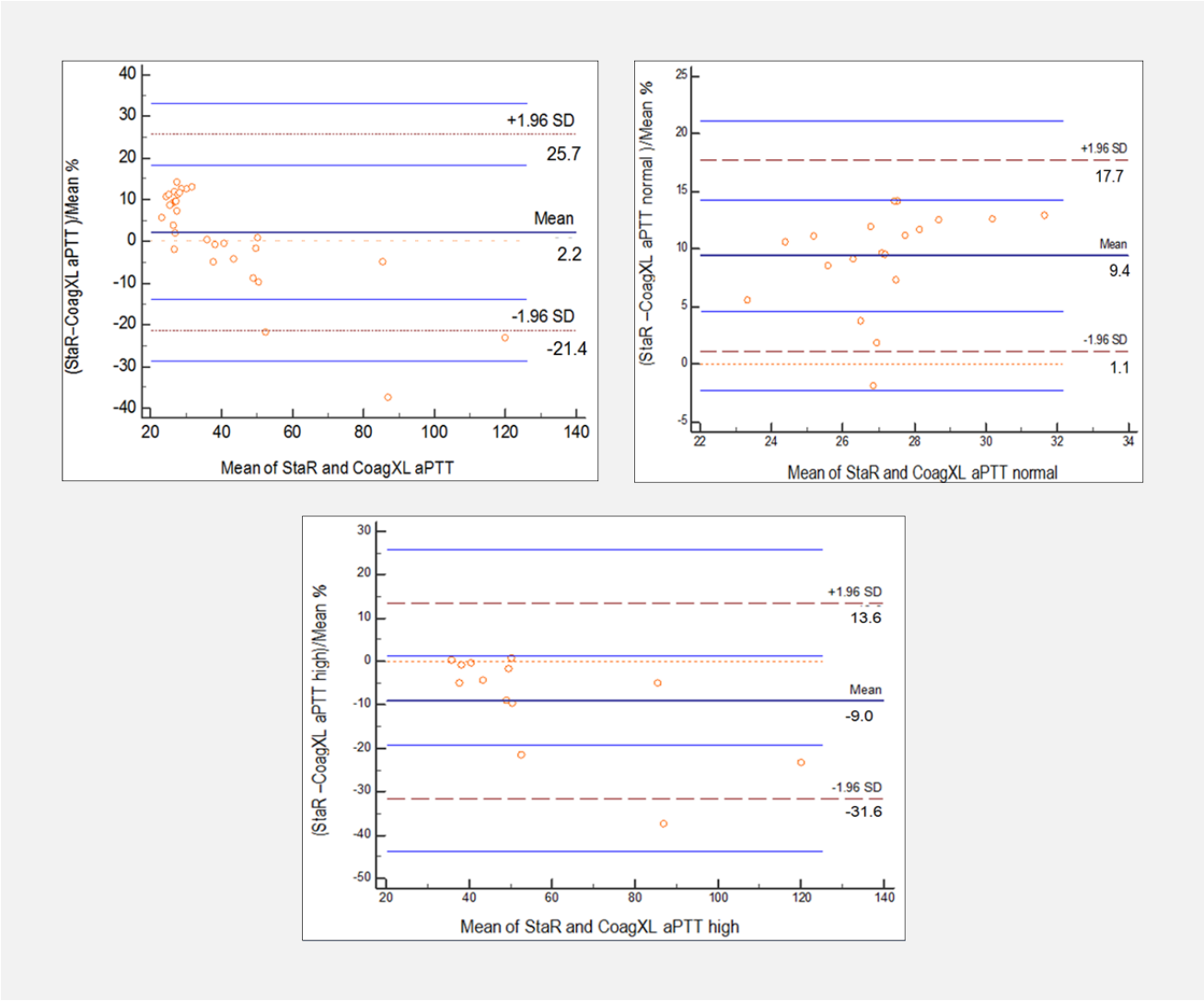

Figure2. Bland-Altman Graphics (aPTT).

(A) Entire group aPTT, (B) Normal values aPTT, (C) high values aPTT.

in normal and pathologic samples. The mean bias (limits of agreement) values for normal and high values were found as 9.4% (17.7 and + 1.1) and -9% (+13.6 and -31.6) in the result of the separate evaluation of the two groups. The CCC value of the entire group was 0.98. The CCC values were 0.41 and 0.87 for the normal and high aPTT values, respectively. In the Passing-Bablock analysis, the constant (intercept = -9.01, CI% -13.29 and -4.86) and proportional (slope = 1.24, CI% 1.10 - 1.37) errors were found for the aPTT. The results of the Passing-Bablock analysis are shown in Table 2.

Carryover

The mean ± SD were found as 9.76 ± 0.18 second and 9.6 ± 0.07 second (N-N and P-N, respectively) for PT and as 27.2 ± 0.14 second and 27.2 ± 0.18 second (N-N and P-N, respectively) for aPTT. The carryover value was -0.16 for the PT and 0 for the aPTT. The carryover value was under the limit value (< 3 SD) for both tests.

Reference range verification study

The values of 20 healthy people were used for the reference range verification. None of the values exceeded the reference intervals given by the manufacturer (0.8 - 1.2) for INR; only one sample exceeded the manufacturer's range (23.2 - 35.2 seconds) for aPTT. Therefore, the recommended reference ranges were considered acceptable.

DISCUSSION

Between-day CV% values obtained in the precision study for PT and aPTT were in accordance with the limit recommended by CLSIH4-A2 (5%), except for an abnormal level PT. Although the allowable total within-day coefficient of variation (CV) of the system was found higher than that given by the manufacturer, it compatible with the value accepted in the literature [15, 16].

In the comparison results, the PT-INR values of two analyzers showed a significant difference ($p < 0.006$). The CoagXL gave higher results in all INR values. The difference between aPTT values measured with the two analyzers was not statistically significant ($p > 0.5$). The normal values were measured higher in the Stago analyzer, while the pathologic values were measured higher in the CoagXL analyzer. However, the bias was under 15% for both normal and pathologic groups.

Because of the difference between the commercial PT kits, different PT values can be obtained with different reagents in the same sample. The World Health Organization set the International Normalized Ratio (INR) system in motion in 1983 to overcome this variability [6]. An ISI value given for a particular analyzer-thromboplastin combination is not considered sufficient by some researchers [8]. Recently, some manufacturers have offered "analyzer-specific" and "thromboplastin-specific" ISIs. However, it was shown that this did not provide sufficiently standardized INR values [14]. Same combination of thromboplastin and coagulometer might give different ISI values in different laboratories.

Recently, the determination of local ISI with certified commercial plasmas has provided advantages in the development and simplification of INR accuracy. The Clinical and Laboratory Standard Institute (CLSI) recommends local ISI determination with certified plasmas in each coagulometer-thromboplastin combination in clinical laboratories [18,19]. The correct ISI value should be specified for each new thromboplastin lot. In general, when the recombinant thromboplastin is used, the abnormal prothrombin activity is expressed in a longer time interval and this allows for a more precise analysis [7].

In a study of 67 samples, Barcellona et al. investigated which of the recombinant thromboplastin (ISI = 0.82) and rabbit thromboplastin (ISI = 1.46) showed better performance. Despite high ISI values of the rabbit thromboplastin, they did not find a significant difference between the two kits ($p = 0.19$) [19]. In an intra- and inter-assay precision study, Martinez Brotons et al. compared human recombinant thromboplastin and a high sensitivity rabbit brain reagent. Recombinant thromboplastin reagent showed better intra- and inter-assay CV's especially in abnormal PT values. They observed a higher sensitivity in dysfibrinogenemia and in patients receiving anticoagulant therapy, but a lower sensitivity in patients on heparin therapy [20].

CONCLUSION

Verification studies of CoagXL analyzer system was acceptable. But in comparison studies of PT we saw that there are still problems with the recommended INR system.

Declaration of Interest:
The authors declare no conflicts of interest.

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