Influence of Plasma Interferences on Screening Coagulogram and Performance Evaluation of the Automated Coagulation Analyzer Sysmex® CS-2100i

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ABSTRACT

Background: Plasma interference is a problem for coagulation analyzers using a photo-optical detection method. Sysmex® CS-2100i is a fully-automated coagulation analyzer which has been developed to reduce this problem.
Objective: To evaluate the influence of interferences on prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen analyzed by Sysmex® CS-2100i. The performance of this analyzer was also assessed in this study.
Methods: Pooled plasma samples spiked with interfering substances including hemoglobin, bilirubin and lipid were used in the interference study. Real patients' samples with these interferences were also tested. Control materials and pooled samples were used for precision, comparison, and carryover studies.
Results: The PT, APTT, and fibrinogen could be analyzed in both plasma samples with interference added and real abnormal patients' plasma samples. The deviation of PT, APTT, and fibrinogen levels was not obvious, except for an upward trend of fibrinogen level with increased hemoglobin. The highest % CV of PT, APTT, and fibrinogen were 1.24, 3.18, and 4.31, respectively for within-run and 2.32, 2.11, and 6.06, respectively for between-run precision analysis. The correlation coefficients of PT, APTT, and fibrinogen between Sysmex® CS-2100i and Sysmex® CA-1500 were 0.99, 0.99, and 0.98 with intra-class correlation coefficients of 0.99, 0.99, and 0.98 respectively. No significant carryover of specimen was observed.
Conclusion: Sysmex® CS-2100i could analyze both samples with artificial interfering substance added and real abnormal patients' samples. This machine also had an acceptable performance by our evaluation.

Keywords: Coagulation analyzer, Sysmex® CS-2100i, prothrombin time, activated partial thromboplastin time, fibrinogen, and plasma interferences

To date, most laboratories use automated analyzers or coagulometers to analyze screening coagulogram, including prothrombin time (PT) and activated partial thromboplastin time (APTT). Thrombin time (TT) or fibrinogen assay are also included in this kind of test in some institutes. In general, the principle of detection system can be divided into two categories: electromechanical and photo-optical methods.[1] Both methods have different advantages and limitations. It is known that the photo-optical method is limited by a high degree of colored or particulate interferences in plasma samples such as lipemic, icteric, or hemolytic substance. Coagulation test results from these samples could lead to erroneous or undetectable results. The degree of interferences which limit the analytical capability of each analyzer varies depending on the analytical system including analyzer and reagent. In a previous study, the prevalence of plasma samples with interferences sent to coagulation laboratories was as high as 19.5%, 0.3%, and 0.3% for hemolytic, lipemic, and icteric specimens respectively.[2] We have also encountered this problem in our laboratory. Some screening coagulogram could not be analyzed especially in specimens with a high level of interference. Sysmex® CS-2100i is a new generation, fully-automated coagulation analyzer for which it is claimed that its multi-wavelength detection feature can reduce this pre-analytical problem.[3] To verify this advantage, we evaluated the effects of hemoglobin, lipid, and bilirubin on the limitations of analysis and the deviation of screening coagulogram results which were analyzed by Sysmex® CS-2100i from both interfer-

ing substance-added and patients' samples with abnormal characteristics. Furthermore, we also partially verified the performance status of this analyzer. This process was conducted to assure the appropriate function of new analyzer under a local laboratory environment.[4]

MATERIALS AND METHODS

Interference study

The influence of interferences, both interfering substance-added and patients' samples with abnormal characteristics were evaluated. Residual 3.2% (0.109 mol/L) sodium citrated patients' samples with normal PT, APTT, fibrinogen (normal pooled plasma, NPP) were collected and added with Interference Check. A Plus (Sysmex® Company, Kobe, Japan). This set of control material comprises of bilirubin (F: free type, C: conjugated type) and hemolytic hemoglobin. Twenty percent intralipid (Sino-Swed Pharmaceutical, Wuxi Jiangsu, China) was used to evaluate the effect of lipemic interference. The serial dilutions of these interfering substances added to NPP were performed according to the manufacturer's direction. After that, PT, APTT and Clauss fibrinogen assays were performed in all specimens by the clot-based principle with photo-optical detection by Sysmex® CS-2100i. The reagents used in this study were Thromborel® S (Siemens Healthcare Diagnostics, Marburg, Germany) lot number 545348 for PT, Dade® Actin® FS (Siemens Healthcare Diagnostics, Marburg, Germany) lot number 538407 for APTT, and Dade® Thrombin Reagent (Siemens Healthcare Diagnostics, Marburg, Germany) lot number 537978 for fibrinogen assays. Percentages of deviation of PT, APTT, and fibrinogen from the baseline sample (plasma without interfering substance) were calculated at various concentrations of interferences. A Roche Modular E170 clinical chemistry analyzer was used to determine the amount of interfering substances in every specimen including triglyceride, total bilirubin and hemoglobin level. The latter was analyzed by a Serum Index assay for hemoglobin level. This assay was validated and there was a good correlation between the hemoglobin level ranging from 0 to 1,450 mg/dl and H index shown in an information sheet provided by the company. One unit of H index is approximately equivalent to 1 mg/dl of hemoglobin.[5]

Apart from the testing of NPP with interference added, 3.2% sodium citrated patients' samples with abnormal characteristics observed by gross inspection were also evaluated. These samples, which included 8 hemolytic, 12 icteric, and 2 lipemic samples, were the residual plasma samples from routine coagulation testing. These samples were analyzed immediately or frozen at -70°C in a refrigerator until analysis. These samples were tested for PT, APTT and fibrinogen as well as for the interference levels (total bilirubin, hemoglobin, and triglyceride).

Performance evaluation

Commercial quality control materials and NPP were used to assess the performance status of the Sysmex® CS-2100i. The protocol of evaluation was a part of method validation which was applied according to previous recommendations.[6,7]

Precision study

Forty quality control materials, 20 normal levels from Control Plasma N (Dade-Behring, Marburg, Germany) and 20 pathological levels from Control Plasma P (Dade-Behring, Marburg, Germany), were evaluated. These control materials were analyzed repeatedly for PT, APTT,

and fibrinogen levels in a single run of 20 measurements (within-run precision) and in the morning and evening over a period of 10 consecutive days (between-run precision). Then the percentages of coefficient of variation (%CV) were calculated.

Comparison study

Forty one residual patients' samples from routine coagulation analysis were tested for PT, APTT, and fibrinogen level by Sysmex® CS-2100i and Sysmex® CA-1500 (our current analyzer) over several days. Then the correlation and agreement of both analyzers were demonstrated by correlation coefficient (r^2) and intra-class correlation coefficient (ICC) for each test parameter.

Reference range

Citrated plasma samples from 35 apparently healthy subjects with ages range from 18 to 60 years old were tested for PT and APTT. The mean and standard deviation were calculated and for each test reference values were demonstrated as mean ± 2SD.

Carryover study

Twenty citrated plasma samples with normal APTT were collected. Each sample was divided into 3 aliquots. Then unfractionated heparin (Heparin Leo®, LEO Pharma, Ballerup, Denmark) at the concentration of 0.7 unit/ml was spiked into the second aliquot of each sample. APTT on the first, second and third aliquots were analyzed in order. Equivalence testing between APTT of the first and third aliquots were performed.

Statistical analysis

The SPSS software, version 16.0 for Windows, was used for statistical analysis of r^2 and ICC for comparative study, mean and standard deviation for the reference range determination, and paired T-test for equivalence testing for the carryover study. For agreement analysis, an ICC criterion of Landis and Koch was applied.[8] Briefly, ICC 0.00 to 0.20 means slight agreement, ICC 0.21 to 0.40 means fair agreement, ICC 0.41 to 0.60 means moderate agreement, ICC 0.61 to 0.80 means substantial agreement, and ICC 0.81 or above means almost perfect agreement.

Ethical consideration

This study was considered as a "Research with Exemption" category by Siriraj Institutional Review Board (SIRB Protocol No. 375/2553).

RESULTS

Interference study
- **NPP with interfering substance added**
  Interfering substances, including hemoglobin, bilirubin (both free and conjugate forms), and lipid (20% intralipid), were added to NPP in a serial dilution fashion. Characteristics of these samples have been shown in Fig 1. Trends of PT, APTT, and fibrinogen levels at various concentrations of interfering substances have been demonstrated in figure 2. The highest concentration of interfering substances were 503 mg/dl for hemoglobin in hemolytic samples, 14.07 mg/dl for total bilirubin in F-type icteric samples, 14.55 mg/dl for total bilirubin in C-type icteric samples and 978.8 mg/dl for triglyceride in lipemic samples. At these concentrations, the PT, APTT, and fibrinogen could be analyzed and reported. The percentages of deviation from baseline samples have also been indicated in Fig 2.
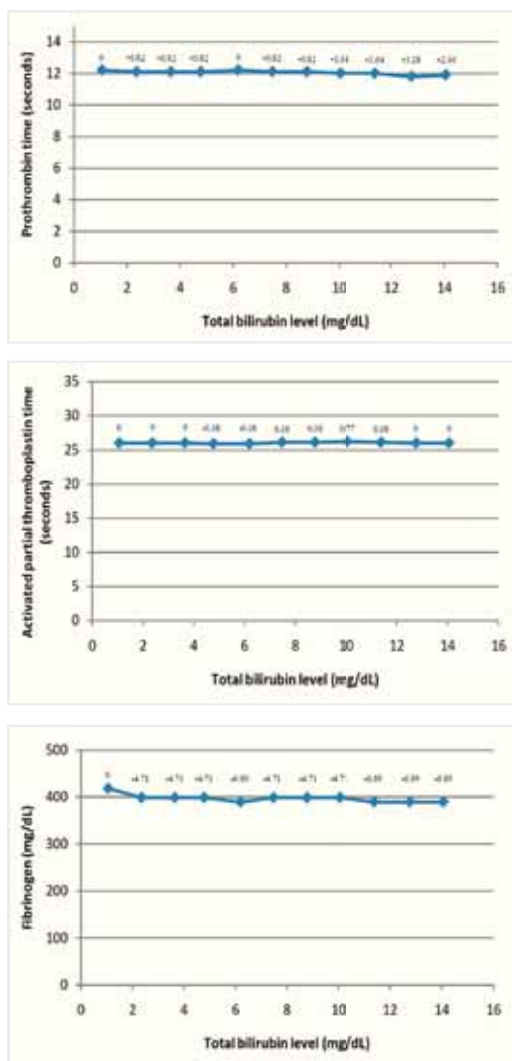
- **Abnormal patients' plasma**
 Characteristics of plasma samples, amount of
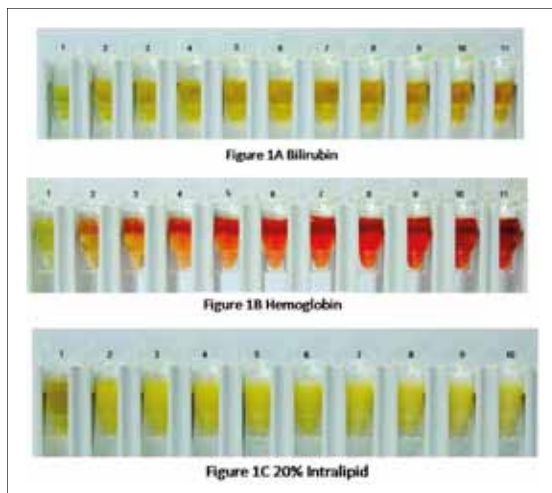
Fig 1. Characteristics of testing plasma samples added with increased concentrations of interfering substances: bilirubin (1A), hemoglobin (1B), and 20% intralipid (1C).

A. Free bilirubin (bilirubin-F)

Fig 2. The prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen levels and the percentages of deviation from baseline results at various concentrations of interfering substances: free bilirubin (A), conjugated bilirubin (B), hemoglobin (C), 20% intralipid (D).

B. Conjugated bilirubin (bilirubin-C)
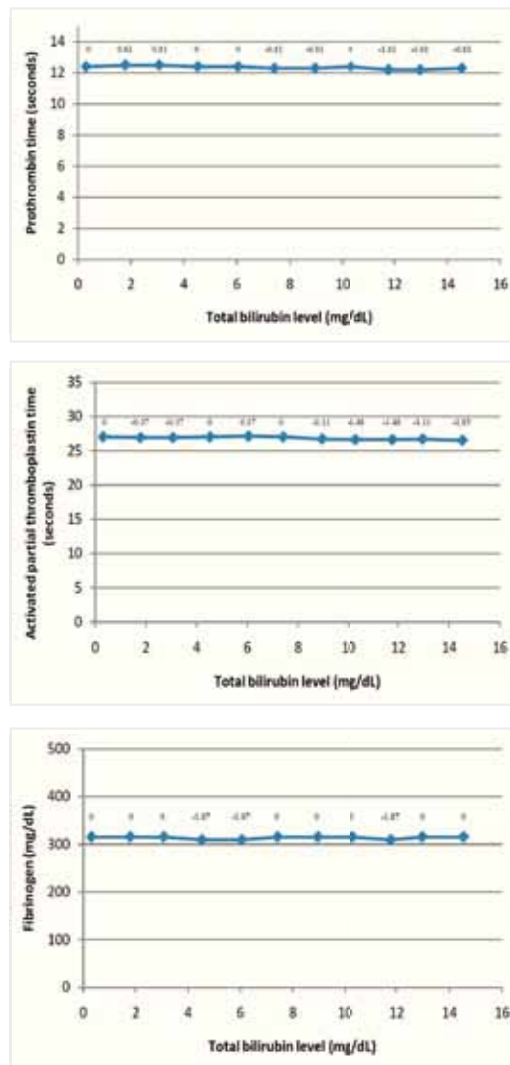
Fig 2. The prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen levels and the percentages of deviation from baseline results at various concentrations of interfering substances: free bilirubin (A), conjugated bilirubin (B), hemoglobin (C), 20% intralipid (D). (Continued)

interferences, and results of screening coagulograms (PT, APTT, and fibrinogen) have been demonstrated in Table 1. Eight hemolytic, 12 icteric, and 2 lipemic samples were chosen to be evaluated. The maximum concentrations of hemoglobin, total bilirubin and triglyceride in abnormal samples were 225, 36.32, and 439.1 mg/dl, respectively. All of these specimens could be analyzed and reported for PT, APTT, and fibrinogen with flagging of "abnormal plasma" which depends upon the type of interference.

Performance evaluation
### Precision study

The % CVs of control materials from both normal and pathological levels which were analyzed in a single run (within-run) and alternate run (between-run) have been shown in Table 2. The %CVs of PT, APTT, and fibrinogen ranged from 0.61% to 1.24%, 0.62% to 3.18%, and 2.42% to 4.31%, respectively for within-run and 1.60% to 2.32%, 1.07% to 2.11%, and 4.90% to 6.06%, respectively for between-run precision analysis.

C. Hemoglobin
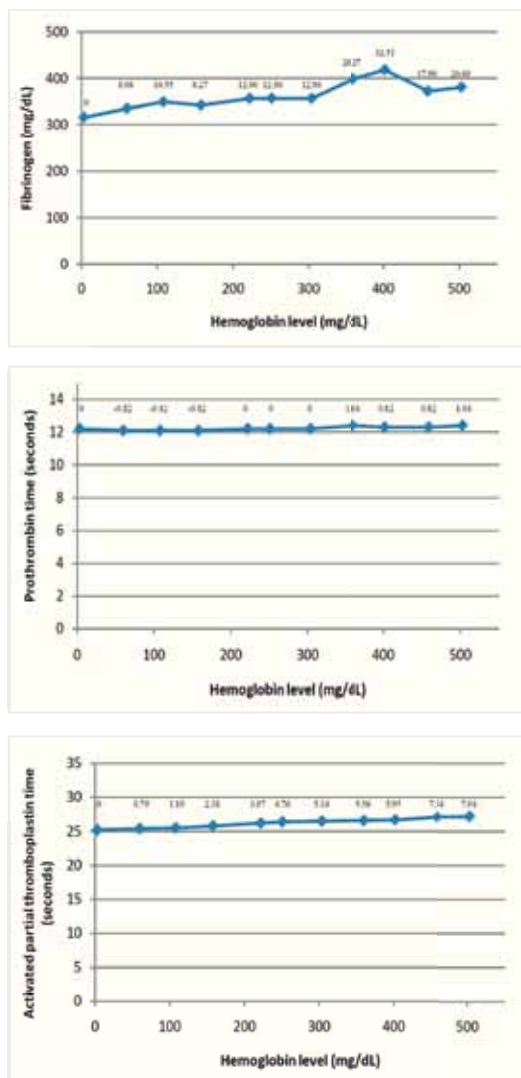
D. Lipemia (20% intralipid)
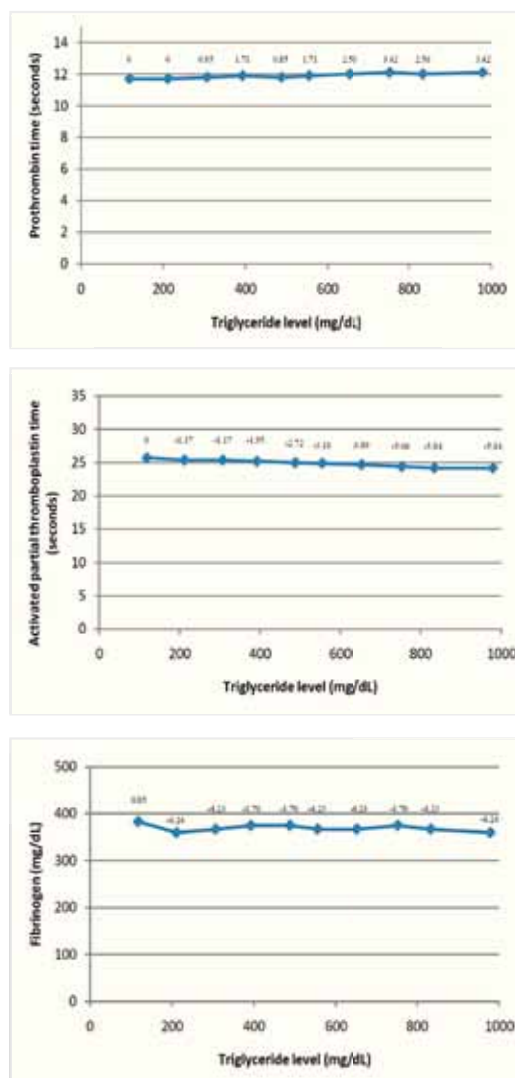
Fig 2. The prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen levels and the percentages of deviation from baseline results at various concentrations of interfering substances: free bilirubin (A), conjugated bilirubin (B), hemoglobin (C), 20% intralipid (D). (Continued)

Comparison study

The PT, APTT, and fibrinogen levels analyzed by Sysmex® CS-2100i and Sysmex® CA-1500 have been demonstrated in figure 3. The r^2 of PT, APTT, and fibrinogen assays between the two analyzers were 0.99, 0.99, and 0.98 respectively. The ICC of PT, APTT, and fibrinogen assays between the two analyzers were 0.99, 0.99, and 0.98 respectively ($p < 0.01$ for all).

Reference range

The reference ranges of PT were 10.75 to 12.89 seconds, and those of APTT were 21.77 to 29.81 seconds, for specific reagent lots.

Carryover study

The APTT was more than 180 seconds in the second aliquot (plasma with heparin spiking) of all samples. No significant prolongation of APTT in the third aliquot compared to the first aliquot in each sample was observed. The 90% CI of the difference was between -0.14 and 0.02, p value = 0.23.

DISCUSSION

In our study, we determined the influence of bilirubin, hemoglobin, and lipid on screening coagulogram including PT, APTT, and fibrinogen. We performed the analysis with both artificial interfering substance added and real patients' plasma with interferences. Theoretically, these kinds of substances influence the light transmission and affect the clotting time. Another hypothesis is that property of the interfering substance itself, such as phospholipid membrane from hemolytic red blood cell, may interfere with the coagulation reaction.[9,10] In our hospital, we encountered this problem especially when we analyzed patients' samples with high degrees of interferences. Sometimes, a false "no coagulation" report could occur. In general, there are many ways to correct this problem; for example, using an alternating principle like electromechanical clot detection method, ultracentrifugation to remove lipid particles, using blank measurement, changing

TABLE 1. Characteristics of samples, amount of interferences, and results of prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen levels of abnormal patients' samples.

| Plasma number	Characteristics of plasma	H index	Total bilirubin (mg/dl)	Triglyceride (mg/dl)	PT (second)	APTT (second)	Fibrinogen (mg/dl)
1	Hemolysis	56	0.16	197.7	11.4	26.3	452.4
2	Hemolysis	53	1.34	47.8	15.4	29.2	359
3	Hemolysis	51	5.91	97.6	22.8	40.4	504
4	Hemolysis	73	0.49	30.1	12.3	25.8	441.1
5	Hemolysis	70	0.32	65.7	26.5	40.6	650
6	Hemolysis	73	4.72	69	42.9	67.3	106.2
7	Hemolysis	85	0.39	98.9	29.9	35.3	536.8
8	Hemolysis	225	0.46	127.7	42.7	60.2	662.6
9	Icterus	0	18.82	129.5	18.6	34.1	176.6
10	Icterus	0	17.92	159.9	13.9	35.7	655.4
11	Icterus	0	11.60	246.8	11.5	29.6	464.3
12	Icterus	0	15.27	135.5	23.5	40.7	191.2
13	Icterus	0	36.32	160.7	13.2	25.3	259.9
14	Icterus	3	10.73	86.8	26.3	37.0	99.4
15	Icterus	0	32.60	98.6	28.6	41.8	135.3
16	Icterus	0	25.64	68.8	33.5	97.7	75.8
17	Icterus	0	30.76	104.5	13.5	31.7	178.9
18	Icterus	0	35.44	164.5	16.6	35.7	351.7
19	Icterus	0	27.72	182.2	15.0	24.5	163.8
20	Icterus	0	26.41	313.6	13.8	29.1	441.1
21	Lipemia	0	6.03	394.9	18.0	48.7	410.0
22	Lipemia	13	0.59	439.1	23.2	37.5	235.7

of the wavelength of detection, or using a multiple wavelength detection system. Sysmex® CS-2100i is a new generation, fully-automated blood coagulation analyzer developed by Sysmex Corporation. This coagulometer uses multi-wavelength detection which is capable of simultaneous analysis at five wavelengths, 340, 405, 575, 660, and 800 nm. When this property is combined with the "HIL detector", the appropriate absorbance is utilized for specific types of interference. It is claimed that using this feature can reduce the effect of interfering substances in sample. Previous studies from the company about the effect of hemolysis and lipids on clotting tests using Sysmex® CS-2000i/CS-2100i has shown that these machines were tolerant for the presence of high hemoglobin and lipids. In our study, the deviation of PT, APTT, and fibrinogen levels was not obvious except for an upward trend of fibrinogen level with an increased concentration of hemoglobin and slightly downward trend of APTT with an increased triglyceride. The maximum absolute deviation from baseline result was as high as 32.51% for fibrinogen level in the hemolytic sample. There was also a downward trend of APTT with a slightly increased triglyceride level. The absolute percentage of deviation was 5.84%. For the analysis of known abnormal patients' samples, the

screening coagulogram could be analyzed and reported in all samples. However, the confirmatory assay to determine the accuracy of results was not performed because of the inadequacy of the collected sample. Even though the analyzer could analyze and report the test results in plasma samples with interferences, the clot-based assay coagulogram should be interpreted cautiously in these samples especially fibrinogen assay in plasma with high hemoglobin. The sample quality checking system or HIL detection system, which is the feature installed in Sysmex® CS-2100i but not in Sysmex® CA-1500, is valuable in this setting. The clinician should be alerted if a flagging remark of any abnormal sample is shown. Cautious collection of new specimens to avoid this pre-analytical problem is recommended. If new specimens cannot be collected, a clinician has to determine whether the deviated lab result is clinically significant or not when the analyzer is used with the clot-based principle.

About performance evaluation, we performed a partial method verification of Sysmex® CS-2100i. The % CVs of PT, APTT, and fibrinogen for within-run precision study were all less than 25% of allowable total error defined by the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) which are 3.75% for PT and APTT, as well as 5% for fibrinogen assay.[11] Our %CVs were also lower than those reported by the manufacturer. For between-run precision, %CVs of PT, APTT, and fibrinogen were also less than 33% of allowable total error defined by CLIA'88 which are 5% for PT and APTT, as well as 6.67% for fibrinogen assay.[11] There was an excellent correlation with almost perfect agreement between Sysmex® CS-2100i and our previously verified analyzer, Sysmex® CA-1500 demonstrated by r^2 and ICC. Therefore, using Sysmex® CS-2100i instead of Sysmex® CA-1500 might not generate a significant change of test result. Furthermore, we were concerned about carryover of specimens because

TABLE 2. Percentages of coefficient of variation (% CV) of prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen assays.

	% CV			
	Within-run		Between-run	
	Control N	Control P	Control N	Control P
PT	0.61	1.24	1.60	2.32
APTT	0.62	3.18	1.07	2.11
Fibrinogen	2.41	4.31	4.90	6.06
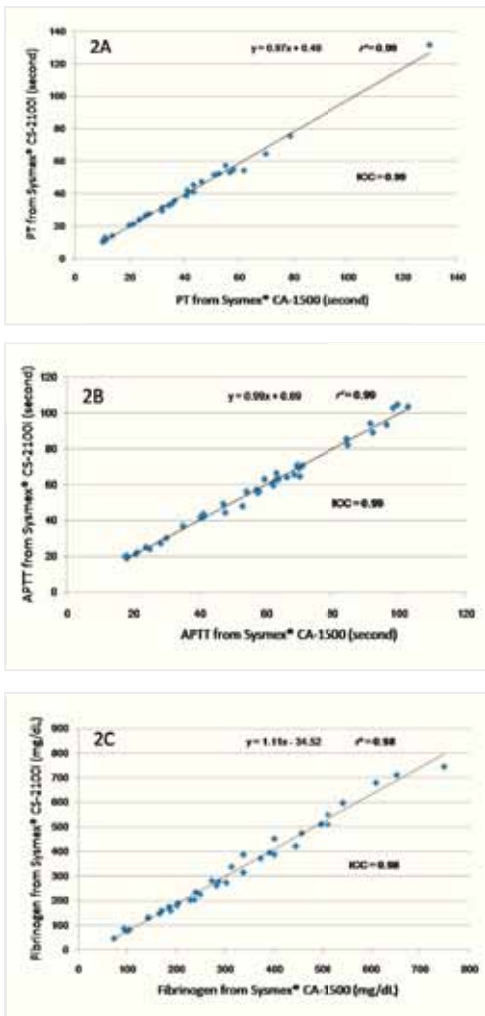
r^2 and ICC indicate correlation coefficient and intra-class correlation coefficient respectively.

Fig 3. Correlation and agreement between Sysmex® CS-2100i and Sysmex® CA-1500 (2A for prothrombin time (PT), 2B for activated partial thromboplastin time (APTT), and 2C for fibrinogen assay).

the Sysmex® CS-2100i analyzer uses a single probe for transfering both reagent and specimen which can generate carryover and give an inaccurate test result. However, significant carryover of samples was not observed in this study.

There are some limitations in our study. First, the concentrations of artificial interfering substances added to NPP were limited because we performed this study only according to the directions from the package insert of Interference Check. A Plus. Second, a limited number of real abnormal patients' samples was evaluated with no confirmatory assay in these samples due to the inadequacy of these specimens. Third, limited parameters of performance assessment were evaluated because some information such as specimen and reagent stability is already provided by manufacturer. Because of these, more abnormal patients'

sample evaluation with confirmatory assay is required to confirm the clinical advantage of Sysmex® CS-2100i in the reduction of false reports of plasma with interference. Further performance assessment of specific test parameters is required for comprehensive evaluation.

CONCLUSION

In our study, PT, APTT, and fibrinogen could be analyzed and reported by Sysmex® CS-2100i coagulation analyzer in plasma with certain amounts of interfering substances. The most deviation of the test result was observed in hemolytic plasma testing for fibrinogen level. In real patients' samples with interferences, the screening coagulogram could be analyzed also. However, more amounts of interfering substance and more numbers of abnormal patient's plasma are needed for evaluation of the clinical advantage in this viewpoint. About performance assessment, Sysmex® CS-2100i showed acceptable precision, excellent agreement with Sysmex® CA-1500 analyzer, and no significant carryover of samples. Further performance assessment of test parameters is required for comprehensive evaluation.

ACKNOWLEDGMENT

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