Performance Evaluation of the Coapresta®2000 Automated Coagulation Analyzer for Screening Coagulogram

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ABSTRACT

Objective: The Coapresta®2000 (CP) is an automated analyzer which can perform various coagulation assays. The author aimed to validate the CP performance for screening coagulogram testing, including APTT, PT, and fibrinogen assays, under our local situation such that the data could be applied to the laboratory in Thailand.
Methods: The real patients' samples and control materials were used to evaluate the CP in terms of precision, reference range, comparability to the validated analyzer Sysmex® CS-2100i (CS), carryover, and turnaround time.
Results: The highest percentages of coefficient of variation of APTT, PT, and fibrinogen were 1.05, 3.92 and 2.53 for within run and 2.15, 2.71 and 6.53 for between run precision studies. The correlation coefficients of PT and fibrinogen between CP and CS were 0.99, while it was 0.77 for APTT. Considering warfarin dose adjustment, 6% of patients might require dose adjustment when using CP instead of CS. Carryover was not observed. The turnaround time of first specimen was less than 6 minutes for APTT and 3 minutes for PT and fibrinogen.
Conclusion: The performance of CP for screening coagulogram in terms of precision and carryover was acceptable. The turnaround time was short. The comparability between CP and CS was excellent for PT and fibrinogen, but was fair for APTT. The participation of external quality assurance scheme is needed to assess the accuracy.

Keywords: Coapresta®2000, automated analyzer, performance evaluation, screening coagulogram

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INTRODUCTION

Screening coagulogram, which comprises activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time or fibrinogen assay, is one of the commonly requested tests in clinical practice for assessing bleeding risk. Accurate laboratory results are very important tools to guide the diagnosis and management of bleeding disorders as well as for anticoagulant control. According to the development of analytical methodology, there are 2 methods for screening coagulogram, which are the traditional manual tilt-tube and automated analyzer. The former is very useful in many circumstances, e.g. when one encounters with interferences in plasma. However, the precision and accuracy of manual tilt-tube technique depend on expertise of each staff. Furthermore, the process usually takes time and effort, so it is impractical for a large hospital. However, these disadvantages have been improved by advanced technology of automated analyzers. To date, most laboratories use automated analyzers as a main method. Many instruments developed by various

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manufacturers are available on the market.[1] Each instrument has different advantages and drawbacks. Before implementation of any instrument for regular use, it is recommended that each laboratory should evaluate that analyzer to assure the appropriate function under local laboratory environment.[2-4]

The Coapresta®2000 (Sekisui Medical Co. Ltd., Tokyo, Japan), or CP, is a fully automated analyzer which can perform various coagulation assays. Only a study by Ohsaka et al., demonstrated the acceptable performance of CP.[5] However, this instrument has just started marketing and has not yet been evaluated by any local laboratory in Thailand. In addition, previous study compared CP with an instrument which is not the commonly used one in Thailand.[6] Therefore, the author aimed to validate the CP to assess the performance of screening coagulogram testing, including APTT, PT, and fibrinogen assays, under our local situation, so that the data could be applied to the laboratory in Thailand.

MATERIALS AND METHODS

Analyzers and reagents

The evaluated analyzer was CP, which is designed to perform various coagulation assays including screening coagulogram based on photo-optical endpoint detection. The reagents used for APTT, PT, and fibrinogen in the CP system were Coagpia®APTT-N lot 821RBK, Coagpia® PT-N lot 833RBK, and Coagpia® Fbg lot 837RLJ (Sekisui Medical Co. Ltd., Tokyo, Japan), respectively. The CP was compared with the previously validated analyzer Sysmex® CS-2100i (Sysmex® Company, Kobe, Japan), or CS, in a comparison study.[7] The reagents used for APTT, PT, and fibrinogen in the CS system were Thromborel®S, Dade® ActinFS, and Dade® Thrombin Reagent (Siemens Healthcare Diagnostics, Marburg, Germany), respectively. The CS has been regularly checked for quality by both internal quality control and external quality assurance scheme.

Performance evaluation

Performance evaluation included precision study, reference range determination, comparison study, carryover study, and turnaround time evaluation. The blood samples used in these studies, except for the precision study which used quality control materials, were the real patients' samples collected in the 3.2% sodium citrate tubes. The protocol of evaluation is a part of method validation which was applied according to previous recommendations.[2,3,8]

Precision study

Forty quality control materials, which were composed of 20 Control Plasma N and 20 Control Plasma P (Dade-Behring, Marburg, Germany), were used. These control materials were analyzed repeatedly for APTT, PT, 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 percentages of coefficient of variation (% CVs) were calculated.

Reference range determination

Samples from 40 apparently normal subjects were tested for APTT, PT, and fibrinogen by CP and CS. Ages of subjects varied from 18 to 60 years old. The exclusion criteria for these subjects were a history of bleeding or thrombotic disorders, acute illness, pregnancy, hormonal contraceptive use, and any medication taking. Mean and standard deviation were calculated and reference value for each test was demonstrated as mean ± 2SD.

Comparison study

Forty patients' samples were tested for APTT, PT, and fibrinogen levels by CP and CS simultaneously over several days. Then the correlation and agreement of both analyzers were demonstrated by correlation coefficient (r^2) and Bland and Altman plot for each test parameter. Fifty samples of patients who receiving long term warfarin therapy were tested for PT-international normalized ratio (PT-INR) by CP and CS. The clinical agreement between methods in terms of decision making for warfarin dose adjustment was analyzed. Given that the therapeutic range is PT-INR by the CS of 2 to 3 units and the trigger level of dose adjustment is the PT-INR outside

the therapeutic range of ± 0.2 PT-INR units, the numbers of samples that would affect dosage changes when using CP were counted.[8]

Carryover study

Ten samples with normal APTT were collected. Each sample was divided into 3 aliquots. Then 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.

Turnaround time evaluation

Ten pooled normal samples were collected into a rack and then assayed continuously for APTT, PT, and fibrinogen levels. The time starting from the beginning of the first sample operation to the report of each sample in the same rack was recorded and defined as analytical turnaround time.

Statistical analysis

The Microsoft Office Excel version 2007 was used to calculate means, standard deviations, % CVs, as well as to prepare the linear regression lines, r^2, and Bland and Altman plots for comparability determination. The SPSS software, version 16.0 for Windows, was used for normality testing of the distribution (Kolmogorov–Smirnov test) for reference range determination and paired t-test for equivalence testing in carryover study.

Ethical consideration

This study is considered as a "Research with Exemption" category by Siriraj Institutional Review Board (SIRB Protocol No. 398/2556).

RESULTS

Precision study

The within-run and between-run % CVs of APTT, PT, and fibrinogen assays for 2 levels of control materials are demonstrated in Table 1.

Reference range determination

There was no outlier, defined as the observation whose the value is different from its closest observation more than one-third of the range of all observations, for APTT, PT, and fibrinogen by both CP and CS. The APTT, PT, and fibrinogen values were normally distributed. The reference ranges, defined by mean ± 2SD, are shown in Table 2. All of these were within the ranges which were determined by the manufacturers.

Comparison study

The linear regression lines and r^2, which demonstrate the correlation of APTT, PT, and fibrinogen between CP and CS, are shown in Fig 1. The Bland-Altman plots are shown in Fig 2. To determine the clinically relevant agreement in terms of warfarin monitoring, the samples with PT-INR ranged from 2.04 to 9.68 by CS were included. Six percent (3/50) of PT-INR by CP were found to be discordant with the PT-INR by CS and could affect the dose adjustment.

Carryover study

The APTT was more than 180 seconds in the second aliquot (plasma with heparin spiking) of all samples. No significant prolongation of

TABLE 1. Percentages of coefficient of variation (% CVs) of activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen assays.

Test parameters	% CV			
	Within-run		Between-run	
	Normal level	Abnormal level	Normal level	Abnormal level
APTT	0.70	1.05	2.50	2.15
PT	1.91	3.92	1.06	2.71
Fibrinogen	1.98	2.53	6.53	4.11

TABLE 2. Reference ranges of activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen assays by Coapresta®2000 (CP) and Sysmex® CS-2100i (CS).

Test parameters	Reference ranges (Mean ± 2SD)	
	CP	CS
APTT	25.3 to 39.7 seconds (32.5 ± 7.2 seconds)	23.4 to 31.4 seconds (27.4 ± 4 seconds)
PT	10.7 to 12.9 seconds (11.8 ± 1.1 seconds)	11 to 13.4 seconds (12.2 ± 1.2 seconds)
Fibrinogen	166.8 to 414.2 mg/dl (290.5 ± 123.7 mg/dl)	136.9 to 430.9 mg/dl (283.9 ± 147mg/dl)

APTT in the third aliquot compared to the first aliquot in each sample was observed. The 95% CI of the difference was between -1.48 and 1.88 with the p value of 0.79.

Turnaround time evaluation

The analytical turnaround time of the first and the tenth samples in the same rack was 5.7 and 7.1 minutes for APTT, 2.5 and 4.3 minutes for PT, and 2.4 and 3.8 minutes for fibrinogen assay.

DISCUSSION

In this study, the performance of the CP analyzer in various aspects was assessed. For precision assessment, it is recommended that the within-run and between-run % CVs of the assays should not be more than 25% and 33% of allowable total error defined by the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88).[9] The % CVs of CP were less than those values, which are 3.75% for APTT and PT, and 5% for fibrinogen assay in the within-run precision and 5% for APTT and PT, and 6.67% for fibrinogen assay in the between-run precision. These findings were found in both normal and abnormal levels of control materials. Therefore, the precision of CP was acceptable for APTT, PT, and fibrinogen assays.

The reference ranges of APTT, PT, and fibrinogen assays of CP were determined in this study. These ranges are helpful for a small laboratory which uses the same analytical system to transfer them for the routine use. It should be emphasized that these ranges are specific only to the evaluated system and they cannot be used interchangeably with different analyzers, reagent types, or reagent lots.[2,3]

The APTT, PT, and fibrinogen of CP were compared to those of the validated system, the CS. The correlations of PT and fibrinogen were excellent (r^2 of 0.99 for both assays) while the correlation of APTT was fair (r^2 of 0.77). It was also noted that the upper limit of APTT reference range from CP was much higher than that from CS. For the agreement analysis, results of most specimens were within 95% agreement for APTT, PT, and PT in the Bland and Altman plots. However, the mean biases between the systems may be clinically significant. These discrepancies could be explained by the different reagent constituents, especially for the phospholipid component, between the two systems. The use of a correction factor to convert the clotting time from one system to another is discouraged. As a result, laboratories should not use multiple analyzers or reagents at the same time. The differences of clotting time did not indicate the inaccuracy of the assays and the interpretation of coagulogram for clinical decision making is usually based on the reference ranges. To verify the accuracy of assays, participation of an external quality assurance scheme (EQAS) to compare the results with the peer group is crucial.[10] Considering warfarin dose adjustment, 6% of patients might require dose adjustment when the CP was used instead of the CS. Although this is a small number of patients, over- or under-anticoagulation could occur. The laboratory should communicate clearly

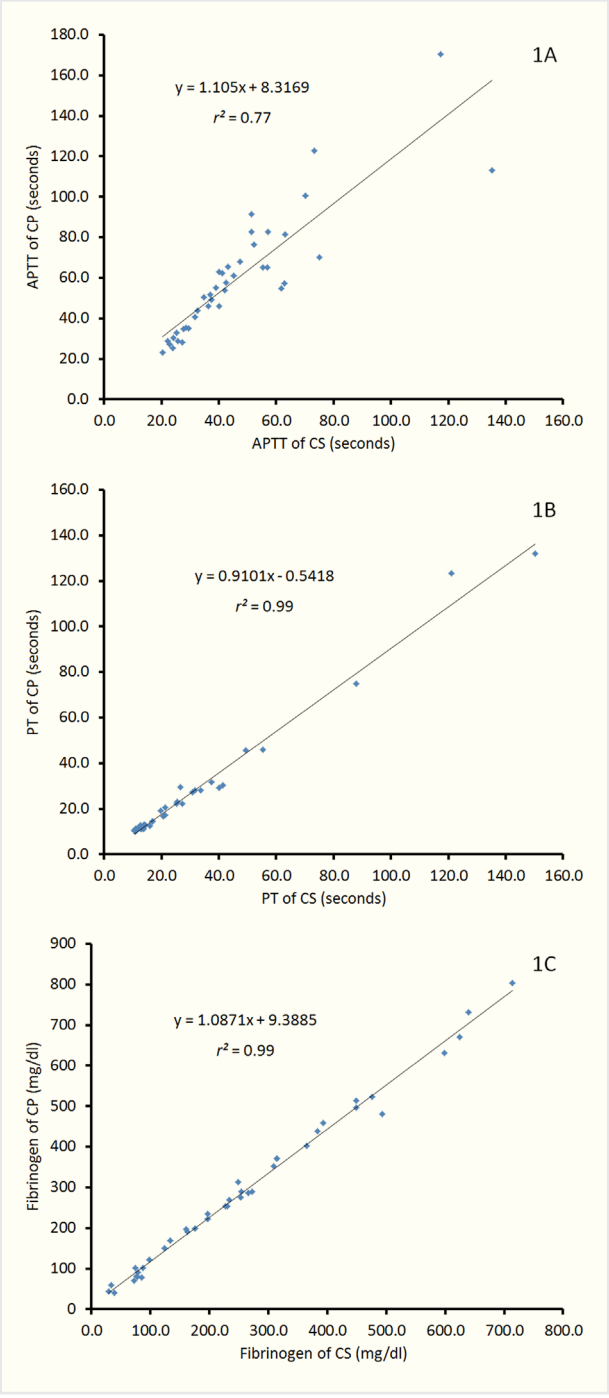

Fig 1. Linear regression lines and correlation coefficients (r^2) of screening coagulogram determined on Coapresta®2000 (CP) and Sysmex® CS-2100i (CS). Fig 1A, 1B, and 1C for activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen, respectively

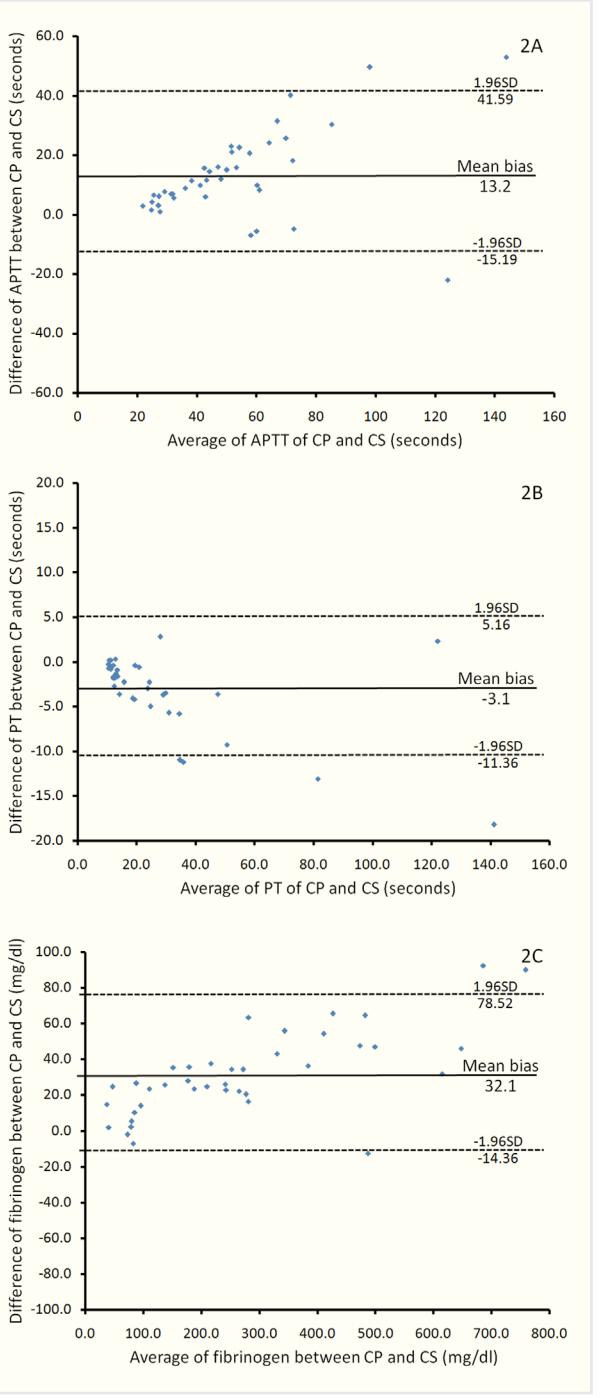

Fig 2. The Bland and Altman plots of the differences of screening coagulogram between those determined on Coapresta®2000 (CP) and Sysmex® CS-2100i (CS). Fig 2A, 2B, and 2C for activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen, respectively.

to the clinicians after implementing the new system and the anticoagulated patients should be observed closely.

The carryover of specimen by the CP was not observed in this study. The turnaround time of APTT, PT, and fibrinogen testing by CP was short. The first specimen could be finished at less than 6 minutes for APTT and less than 3 minutes for PT and fibrinogen. These features are very useful for the emergency cases such as the stoke

fast track patients. Furthermore, the batch testing was also quick and this would improve the overall turnaround time for screening coagulogram in a laboratory. However, only the normal specimens were used to evaluate the turnaround times. Therefore, the turnaround time in a real situation, which has both normal and abnormal specimens, may be longer than that observed in this study.

In conclusion, the CP is a fully automated coagulation analyzer which can perform various coagulation assays including screening coagulogram. The performance of CP in terms of precision and carryover was acceptable. The short turnaround time of screening coagulogram performed by this analyzer was very useful. The comparability of PT and fibrinogen between those performed by CP and CS was excellent, but for the APTT testing it was only a fair correlation. The participation of an EQAS is needed to assess the accuracy of CP.

Conflict of interest

There is no conflict of interest in any financial or commercial aspect regarding the results of this study.

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