PT and aPTT Evaluation of Automated Coagulation Analyzer, Sysmex CA-7000

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CA-7000 (Sysmex Corporation, Kobe, Japan) is a completely automated coagulation analyzer that performs clotting, chromogenic and immunological tests. We evaluated the analytical performances of prothrombin time (PT) and activated partial thromboplastin time (aPTT), and the usefulness as the emergency coagulation analyzer.

Neoplastin Cl Plus (Diagnostica STAGO, France) and Thromborel S (ISI 1.01, Dade Behring, USA) were used for measurement of PT, and PTT A 5 (Diagnostica STAGO), Actin FS (Dade Behring) and CaCl2 (Dade Behring) for aPTT. We evaluated the precision and accuracy of PT and aPTT. Comparison study was performed by comparing the results of STAGO (Diagnostica STAGO) with those of CA-7000. Interferences of hemoglobin, lipid and bilirubin in the PT and aPTT measurements were examined. The results measured by commercial control plasma (Coag-N and Coag-P, Diagnostica STAGO) with designated values showed satisfactory accuracy. The coefficients of variation (CV) of both within-run and between-day precision for CA-7000 were below 2%. The comparison study indicated the good correlation (R^2>0.97) between results of STAGO and those of CA-7000. Various concentrations of hemoglobin, lipid and bilirubin affected no influence on PT and aPTT results.

We concluded that CA-7000 offered excellent performances and provided high throughput for stat coagulation tests. CA-7000 is expected to contribute to the highest efficiency in clinical laboratories.

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Key Words CA-7000, Automated Coagulation Analyzer, PT, aPTT

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INTRODUCTION

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are used for screening of coagulopathy and monitoring of anticoagulant therapy. Establishment of suitable reference ranges for each laboratory, standardization of assay methods and quality controls are required because those assays are affected by pre-analytical variables like sampling procedure, storage condition of specimens and analytical variables like instrumentation and reagents etc.[1)]

Our emergency laboratory used MLA 1000C (MLA, USA) with Neoplastin Cl Plus (Diagnostica STAGO, France) and PTT A5 (Diagnostica STAGO) for emergency testing of PT and aPTT. Comparative evaluation of its results with those of routine coagulation analyzer, STAGO (Diagnostica STAGO) has been performed consistently. Recently, CA-7000 (Sysmex Corporation, Kobe, Japan) has been introduced to our laboratory for emergency testing of PT and aPTT. We evaluated performances of CA-7000 for reproducibility, accuracy, precision and correlation with STAGO. In addition, International Sensitivity Index (ISI) of Neoplastin Cl Plus for CA-7000 was established to compare PT INR. Interferences by hemoglobin, lipid and bilirubin were compared on three analyzers, CA-7000, STAGO and MLA 1000C. Also we validated reference ranges with specimens from normal healthy individuals.

MATERIALS AND METHODS

Specimens

Inpatients' specimens and specimens of normal healthy individuals were collected in sample tubes containing CTAD (Citrate Theophylline Adenosine Diphyridamole, 0.109M) anticoagulant as ratios of 9:1. Those specimens were centrifuged for 15 minutes at 2500g and then supernatant fractions were analyzed. Commercial control plasmas Coag-N (Diagnostica STAGO) and Coag-P (Diagnostica STAGO) and normal pooled plasma (NPP) were used for accuracy and precision testing.

Instruments and Reagents

Thrombrel S (ISI 1.01, Dade Behring, USA) and Neoplastin Cl Plus (ISI 1.34) were used for PT measurements; Actin FS (Dade Behring), PTT A 5 and $CaCl_2$ (Dade Behring) were used for aPTT measurements on both CA-7000 and STAGO.

Reproducibility of detection channels

In-house normal pooled plasma was used to evaluate reproducibility of CA-7000 24 detection channels for PT with Thrombrel S and Neoplastin Cl Plus, for aPTT with Actin FS and PTT A 5.

Accuracy

Coag-N (PT 74-100%, aPTT 24-37sec) and Coag-P (PT 33-47%, aPTT 47-61sec) were analyzed 20 times consecutively on CA-7000. Mean value, standard deviation (SD) and coefficient of variation (CV) were calculated.

Precision

Coag-N, Coag-P and normal pooled plasma were analyzed 24 times, 4 times consecutively for 6 hours with 1-hour intervals in a day on CA-7000 (Within-day precision). Also Coag-N, Coag-P and normal pooled plasma were analyzed 24 times, once a day for 24 days on CA-7000 (Between-day precision). Mean values, SD and CV were calculated respectively.

Establishment of local SI value

We established local SI of Neoplastin Cl Plus (ISI for STAGO: 1.34) for CA-7000 by comparing with Thromborel S (ISI for CA-7000: 1.01). 85 specimens with different PT measurement values were analyzed by both reagents on CA-7000. Local SI of Neoplastin Cl Plus for CA-7000 was established from calculated slope and intercept by following formula: Local SI = Known ISI × slope. Local SI of Neoplastin Cl Plus for CA-7000 was validated by comparing INR results between CA-7000 and STAGO after adapting local SI.

Correlation

1) PT: 203 specimens (***Table 1***) were analyzed with Neoplastin Cl Plus on STAGO and CA-7000. PT % and PT INR results were compared.

2) aPTT: 188 specimens (***Table 2***) were analyzed with PTT A 5 on STAGO and CA-7000. Results from two analyzers were compared.

Effects of interfering substances

1. Hemoglobin

Normal healthy individual's plasma (PT 11.1sec, aPTT 33.6sec) and normal healthy individual' plasma with 1 g/dL of hemoglobin (PT 11.2sec, aPTT 33.8sec) were mixed to be final hemoglobin concentrations at 0, 100, 200, … , 1000 mg/dL. Each mixture was analyzed 3 times consecutively for PT with Neoplastin Cl Plus and aPTT with PTT A 5 on CA-7000.

2. Lipid

Normal healthy individual's plasma with 177 mg/dL of triglyceride and patient's plasma with 1317 mg/dL of triglyceride(***Table 3***) were mixed to be final triglyceride concentrations at 177, 291, 405, 519, 633, 747, 861, 975, 1089, 1203 and 1317 mg/dL. Each mixture was analyzed for PT and aPTT on CA-7000, STAGO and MLA 1000C.

3. Bilirubin

Normal healthy individual's plasma diluted with saline to obtain PT result similar to the patient's PT and patient's plasma (Bilirubin 38.3 mg/dL) (***Table 4***) were mixed to be final bilirubin concentration at 0.4, 3.83, 7.66, 11.49, 15.32, 19.15, 22.98, 26.81, 30.64, 34.47, and 38.3 mg/dL. Each mixture was analyzed for PT and aPTT on CA-7000 and STAGO.

Validation of reference ranges

Plasmas collected from 30 healthy adult men and 30 healthy adult women with no coagulopathy were analyzed on CA-7000 for PT and aPTT to validate current our reference ranges (PT 70-140%, aPTT 30.5-45.0sec) by applying Mean ±2SD.

Statistical analyses

All data were processed statistically by Microsoft, Excel.

RESULTS

Reproducibility of detection channels

Normal pooled plasma was analyzed 24 times on CA-7000 to assess reproducibility of 24 detection channels. CV were 0.58% and 1.30% for Thromborel, S and Neoplastin Cl Plus, 0.69% and 0.42% for Actin FS and PTT A 5, respectively (***Table 5***).

Accuracy

Commercial control materials, Coag-N and Coag-P were analyzed 20 times consecutively on CA-7000. All results were within assigned values. CV for Cang-N and Coag-P were 0.69%, 0.98% for PT and 0.32%, 0.53% for aPTT, respectively (***Table 6***).

Precision

1. Within-day precision

Coag-N, Coag-P and normal pooled plasma were analyzed 24 times, 4 times consecutively for 6 hours with 1-hour intervals in a day on CA-7000. CV were 0.69%, 1.53%, 1.89% for PT and 0.55%, 1.37%, 1.05% for aPTT, respectively (***Table 7***).

2. Between-day precision

Coag-N, Coag-P and normal pooled plasma were analyzed 24 times, once a day for 24 days on CA-7000. CV were 1.70%, 1.50%, 1.54% for PT and 0.90%, 1.27%, 1.43% for aPTT, respectively (***Table 8***).

Establishment of local SI value

85 specimens with different PT measurement values were analyzed with Neoplastin Cl Plus and Thromborel S (ISI for CA-7000: 1.01) on CA-7000. Local SI value of

Table 1 *Distribution of specimens analyzed in PT measurements*

PT (Activity)	Below 44%	45 - 70%	71 - 100%	Over 100%
Case No.	40	61	88	14

Table 2 *Distribution of specimens analyzed in aPTT measurements*

aPTT (sec)	Below 30	30 - 45	46 - 70	Over 70
Case No.	2	105	66	15

Table 3 *PT and aPTT results of normal plasma and patient plasma used in mixtures for lipid interference*

Normal plasma (Triglyceride: 177 mg/dL)						Patient's plasma (Triglyceride: 1317 mg/dL)					
PT(sec)			aPTT(sec)			PT(sec)			aPTT(sec)		
CA-7000	STAGO	MLA 1000C	CA-7000	STAGO	MLA 1000C	CA-7000	STAGO	MLA 1000C	CA-7000	STAGO	MLA 1000C
11.0	11.0	11.6	33.8	33.2	35.2	11.8	11.9	12.7	44.0	39.3	40.8

Table 4 *PT and aPTT results of normal plasma and patient plasma used in mixtures for bilirubin interference*

Diluted normal plasma (Bilirubin 0.4mg/dL)				Patient's plasma (Bilirubin 38.3 mg/dL)			
PT(sec)		aPTT(sec)		PT(sec)		aPTT(sec)	
CA-7000	STAGO	CA-7000	STAGO	CA-7000	STAGO	CA-7000	STAGO
17.7	17.1	57.1	61.2	18.9	18.4	54.5	55.8

Table 5 *Reproducibility of CA-7000 detection channels for PT and aPTT*

(N=24)

	PT						aPTT	
	Thromborel S			Neoplastin Cl Plus			Actin FS	PTT A 5
	Sec	%	INR	Sec	%	INR	Sec	Sec
Mean	12.7	80.3	1.13	12.4	93.2	1.06	44.3	44.5
SD	0.07	0.81	0.01	0.16	1.87	0.02	0.30	0.19
CV (%)	0.58	1.02	0.47	1.30	2.00	1.53	0.69	0.42

Table 6 *Accuracy of PT and aPTT on CA-7000*

(N=20)

	Coag-N		Coag-P	
	PT (%)	aPTT (sec)	PT (%)	aPTT (sec)
Mean	95.6	32.7	43.2	48.7
SD	0.66	0.11	0.42	0.26
CV (%)	0.69	0.32	0.98	0.53

Table 7 *Within-day precision of PT and aPTT on CA-7000*

(N=24)

	Coag-N		Coag-P		Normal pooled plasma	
	PT (%)	aPTT (sec)	PT (%)	aPTT (sec)	PT (%)	aPTT (sec)
Mean	95.3	32.8	43.3	49.0	93.1	38.9
SD	0.66	0.18	0.66	0.67	1.75	0.41
CV (%)	0.69	0.55	1.53	1.37	1.89	1.05

Table 8 *Between-day precision of PT and aPTT on CA-7000*

(N=24)

	Coag-N		Coag-P		Normal pooled plasma	
	PT (%)	aPTT (sec)	PT (%)	aPTT (sec)	PT (%)	aPTT (sec)
Mean	93.7	32.9	42.8	48.9	93.1	39.3
SD	1.59	0.30	0.64	0.62	1.44	0.56
CV (%)	1.70	0.90	1.50	1.27	1.54	1.43

Neoplastin Cl Plus for CA-7000 was calculated by following formula.

Natural logarithm of PT (sec) of Neoplastin Cl Plus
=1.173467 × Natural logarithm of PT (sec) of Thromborel S – 2.24112
Slope = 1.173467
Intercept = -2.24112

Local SI of Neoplastin Cl Plus
= Thromborel S ISI × Slope = 1.185202 ≒ 1.19

After applying calculated local SI of Neoplastin Cl Plus, specimens were analyzed both on CA-7000 and STAGO, and then results were compared (***Fig. 1***). Mean and SD of INR differences were 0.05 and 0.16 each.

Correlation

For PT, total 203 specimens were analyzed with Neoplastin Cl Plus on CA-7000 and STAGO. R^2 of PT % was 0.9783 and R^2 of PT INR was 0.9751 (***Fig. 2a, b***). For aPTT, total 188 specimens were analyzed with PTT A 5 on CA-7000 and on STAGO. R^2 of aPTT was 0.9907 (***Fig. 2c***).

Effects of interfering substances

1. Hemoglobin

Normal healthy individual's plasma (PT 11.1sec, aPTT 33.6sec) and normal healthy individual' plasma with 1 g/dL of hemoglobin (PT 11.2sec, aPTT 33.8sec) were mixed to be final hemoglobin concentrations at 0, 100, 200, … , 1000 mg/dL. Each mixture was analyzed 3 times consecutively for PT with Neoplastin Cl Plus and aPTT with PTT A 5 on CA-7000. Hemoglobin had no influence on the PT and aPTT results up to 1g/dL (***Figs. 3 and 4***).

2. Lipid

Normal healthy individual's plasma with 177 mg/dL of triglyceride and patient's plasma with 1317 mg/dL of triglyceride were mixed to be final triglyceride concentrations at 177, 291, 405, 519, 633, 747, 861, 975, 1089, 1203 and 1317 mg/dL. Each mixture was analyzed 3 times consecutively for PT and aPTT on CA-7000, STAGO and MLA 1000C. The interference of lipid for PT and aPTT on CA-7000 was below equality to STAGO. Especially on PT, CA-7000 showed the minimal influence in three analyzers. (***Figs. 5 and 6***).

3. Bilirubin

Normal healthy individual's plasma diluted with saline and patient's plasma (bilirubin 38.3 mg/dL) were mixed to be final bilirubin concentration at 0.4, 3.83, 7.66, 11.49, 15.32, 19.15, 22.98, 26.81, 30.64, 34.47, and 38.3 mg/dL. Each mixture was analyzed 3 times consecutively for PT and aPTT on CA-7000 and STAGO. The interference of the bilirubin for PT and aPTT on CA-7000 was below equality to STAGO. (***Figs. 7 and 8***).

Validation of reference ranges

Plasmas collected from 30 healthy adult men and 30 healthy adult women with no coagulopathy were analyzed for PT and aPTT on CA-7000. Mean±2SD were 86-110% for PT, 32-42sec for aPTT. Therefore current our reference ranges (PT 70-140%, aPTT 30.5-45.0sec) were validated.

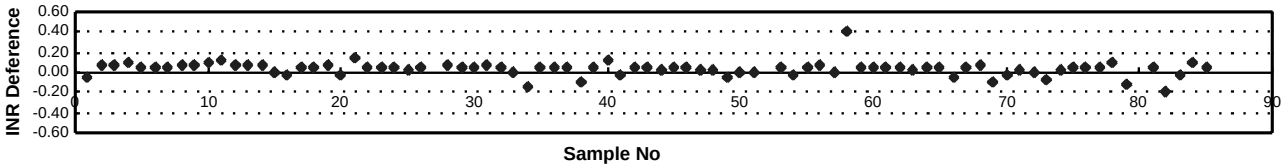

Fig. 1 *INR difference between STAGO and CA-7000 after applying local SI*

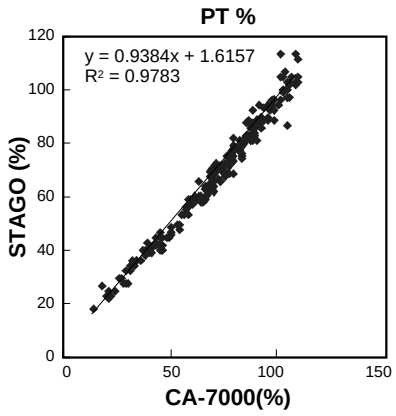

Fig. 2a *Correlation of PT %*

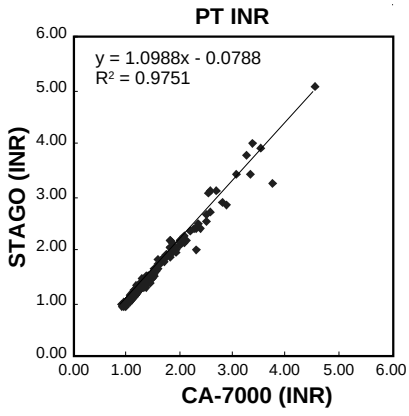

Fig. 2b *Correlation of PT INR*

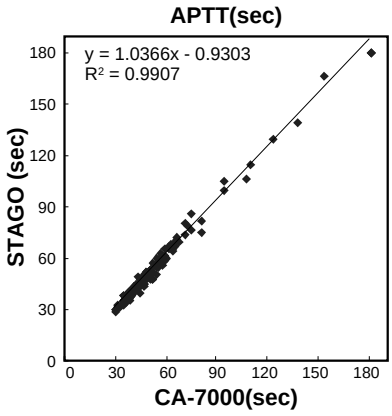

Fig. 2c *Correlation of aPTT*

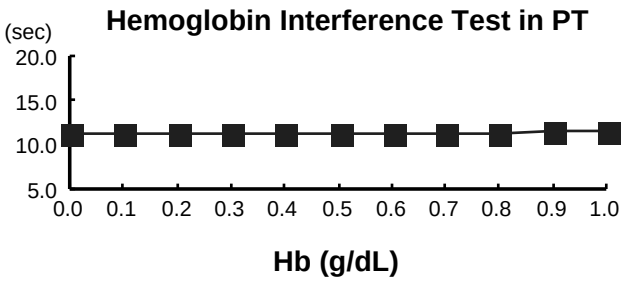

Fig. 3 *Hemoglobin interference in PT*

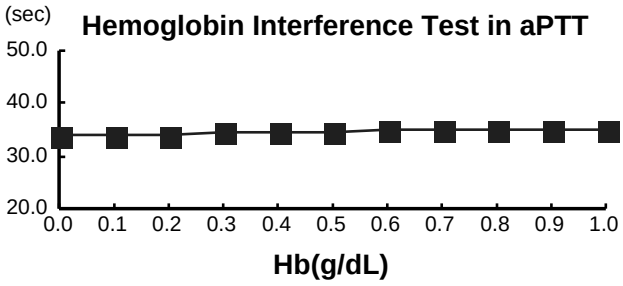

Fig 4 *Hemoglobin interference in aPTT*

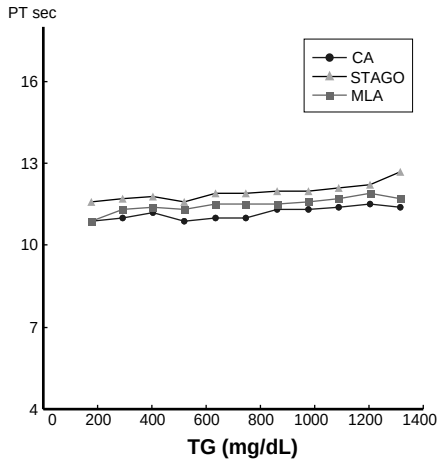

Fig. 5 *Lipid interference in PT*

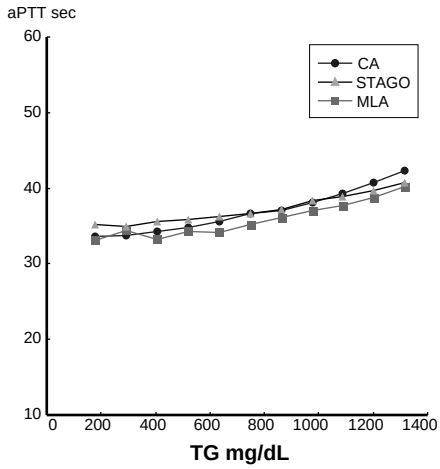

Fig. 6 *Lipid interference in aPTT*

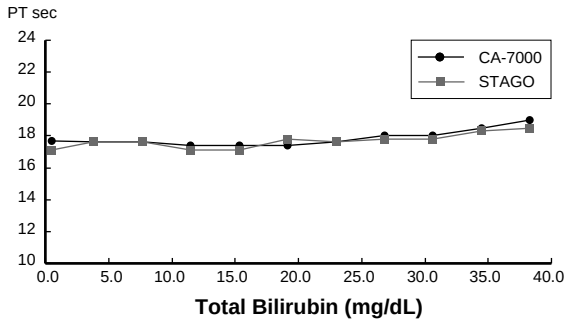

Fig. 7 *Bilirubin interference in PT*

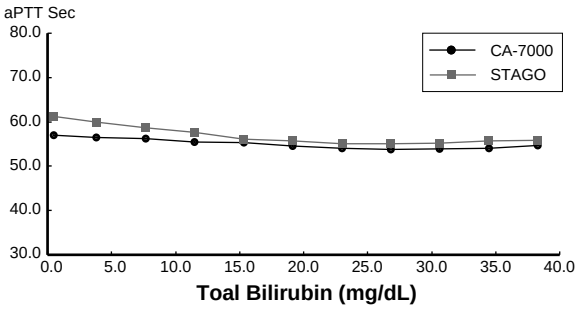

Fig. 8 *Bilirubin interference in aPTT*

DISCUSSION

For emergency coagulation tests, the coagulation analyzer has emerged that measures PT and aPTT rapidly and accurately with efficiency, shortened turn around time (TAT) and cost saving[5].

Our laboratory used MLA 1000C as the emergency coagulation analyzer and results of MLA 1000C had been compared consistently with results of routine coagulation analyzer, STAGO. However, MLA 1000C was worn out, CA-7000 has been introduced to improve the performance of emergency coagulation tests and to shorten TAT. STAGO adapts viscosity method utilizing mechanical principle that clotting time is measured when rotation of stainless ball in a cuvette is stopped. This mechanical principle has the advantage that is almost not affected by interfering substances such as hemoglobin, lipid and bilirubin, which affect photometric detection adapted by MLA 1000C. CA-7000 has adapted both of percentage end point method and light scattering method. When the reagent is added to sample, turbidity change is occurred and the change is detected by incident light from light source, then converted to electric signal. CA-7000 enables fast measurements and shortened TAT by three sample pipettes, five reagent pipettes and cap piercing system[1]. With 24 detection channels, CA-7000 has throughput of 560 tests per hour when PT and aPTT are measured simultaneously. Reproducibility of 24 detection channels with two kinds of PT and aPTT reagents each was good as CVs of 0.47-2.00% for PT and CVs of 0.42-0.69% for aPTT.

Two kinds of commercial control plasmas were analyzed 20 times consecutively. All results were within assigned values. CVs were below 2.0%. Referring Lee, et al.[1], CVs of three kinds of plasmas were 0.80-3.78% and especially, CV of abnormal control plasma was over 5.00%. Beckala, et al.[2] and Lewis, et al.[3] suggested that the precision is good when CV of within-day precision is below 3% and CV of between-day precision is below 5%. Therefore, it is considered that we had excellent precision performance of CA-7000 in this study. In correlation study between CA-7000 and STAGO, R^2 of PT%, PT INR and aPTT were 0.978, 0.9751 and 0.991. We assumed that those good correlation coefficients were induced because same STAGO reagents were used on both instruments although both instruments have different detection principles.

PT results depend on the sensitivity of thromboplastin reagent. Because each reagent has different sensitivity, there are differences on PT second and PT %(activity) by different reagents, therefore it is difficult to compare those results directly. INR report is important to stan-

dardize inter-laboratory measurement values, especially for monitoring of oral anticoagulant therapy like wafarin[9]. We established ISI of Neoplastin Cl Plus (ISI for STAGO: 1.34) for CA-7000 by comparing with Thromborel S (ISI for CA-7000: 1.01) that has known ISI for CA-7000. 85 specimens were analyzed by both reagents on CA-7000. Calculated local SI of Neoplastin Cl Plus for CA-7000 was 1.19. After applying local SI of Neoplastin Cl Plus for CA-7000, specimens were analyzed both on CA-7000 and STAGO. Mean PT INR difference between two instruments was 0.05, which was not considerable.

In interference study, CA-7000 showed no considerable interference effects by hemoglobin, bilirubin and lipid on PT. Especially, CA-7000 showed minimal lipid interference on PT among three analyzers, CA-7000, STAGO and MLA 1000C. Also CA-7000 showed no considerable interference effects by hemoglobin, bilirubin and lipid on aPTT like STAGO, which adapts mechanical method that is relatively not affected by interfering substances.

PT and aPTT results of plasmas collected from 30 healthy adult men and 30 healthy adult women were 86-110% for PT and 32-42sec for aPTT. Both results are within our current reference ranges (PT 70-140%, aPTT 30-45sec).

CA-7000 is a fully automated coagulation analyzer that performs fast and multi-parameter analyses. The analyzer showed excellent accuracy and precision. The effects of interfering substances were minor unlike in other instruments adapting optical measurement principle. Also CA-7000, a random access analyzer equipped with cap piercing system enables shortening of TAT and protects operators from potentially biohazard specimens. We concluded that CA-7000 is suitable as the emergency coagulation analyzer.

ACKNOWLEDGEMENTS

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