Basic evaluation of the CS-5100, a Fully Automated Blood Coagulation Analyzer

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A blood coagulation/fibrinolytic test is regarded as a clinically useful hemostatic test. This test is conducted to detect hemorrhagic or thrombotic predisposition, clarify pathological conditions in emergency medical care or before/after surgery, or monitor the course of treatment. Medical technologists are expected to quickly provide consistently accurate comprehensive measurement results. Generally, presently available fully automated blood coagulation analyzers adopt a complex system that enables high-speed processing and simultaneous measurement of multiple items required by the coagulation time method, synthetic substrate method and turbidimetric immunoassay. However, only a few models are compatible with the delivery line system that has been introduced to improve work efficiency. Analyzers that allow loading of general-purpose reagents are also limited. Recently, we introduced the CS-5100, a new fully automated blood coagulation analyzer, (hereinafter referred to as CS-5100; Sysmex Corporation, Kobe, Japan) that is compatible with the delivery line and accepts multiple samples for the evaluation of multiple measurements. The purpose of this integration of measurement systems was to utilize the work space more effectively and to improve work efficiency. We conducted a basic evaluation of the CS-5100 and here report the results.

We evaluated within-run reproducibility by calculating the coefficient of variability (CV) of each method, and obtained the following satisfactory results: ≤ 2% (coagulation method), ≤ 5% (synthetic substrate method) and ≤ 4% (turbidimetric immunoassay). In the dilution linearity test, satisfactory linearity was observed up to the following levels: 750 mg/dL (fibrinogen), 80 μg/mL [total fibrin/fibrinogen degradation products (T-FDP)] and 25 μg/mL (D-dimer). The minimum detection sensitivity was high in the case of measurements conducted on the same items. Clinical samples were used to compare the CS-5100 with conventional analyzers. Results of the evaluation of various items showed correlation coefficients that consistently exceeded 0.99.

Introduction of the CS-5100, an analyzer possessing the ability of high-speed processing of multiple samples/items, has enabled the integration of measurement systems, which has led to a reduction in hemostatic delivery line space by about 30%. A comparison was made between the CS-5100 and conventional analyzers with respect to TAT. Results showed no reduction in TAT with the CS-5100. Since the TAT required by the CS-5100 was equivalent to that required by conventional analyzers, the comparison results were considered to be satisfactory.

Key Words Fully Automated Blood Coagulation Analyzer (CS-5100), Multifunction/High-Speed Processing, Compatibility with Delivery Line, Multi-Wave Detection System, Sample Quality Check, Wavelength Switching

INTRODUCTION

The leading causes of death in Japan are cardiovascular and cerebrovascular disorders that are generally regarded as thrombotic diseases. Hemostatic tests that focus on coagulation and fibrinolysis are therefore important for predicting the development of thrombosis or monitoring antithrombotic treatment [1]. For emergency medical care or during the perioperative period, hemostatic treatment is indispensable and patient condition should be followed up carefully. This has resulted in an increased need for coagulation/fibrinolysis tests [2] and timely reporting of comprehensive laboratory results.

Currently, automated analyzers favored for hemostatic testing are multipurpose large-sized analyzers that enable the simultaneous measurement of multiple items required by the coagulation time method, synthetic substrate method and turbidimetric immunoassay, with additional efforts directed toward the acceleration of laboratory testing [3].

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At laboratories where many samples are delivered and measured, the delivery line system has been widely used to improve work efficiency, reduce testing time and minimize labor. Although many large-sized analyzers with multifunction/high-speed processing ability have been developed/marketed, only a few hemostatic analyzers are compatible with the delivery line. Moreover, only a limited number of analyzers allow loading of general-purpose reagents.

CS-5100, a fully automated blood coagulation analyzer, (hereinafter referred to as CS-5100; Sysmex Corporation, Kobe, Japan) adopts a multi-wave detection method [4] for its detector that is compatible with different measurement principles (coagulation method, synthetic substrate method, turbidimetric immunoassay and agglutination method). The CS-5100 is positioned as the highest model among the CS series due to its excellent ability to process complex orders. This multi-wave detection analyzer activates the sample quality check (HIL) function based on estimated levels of substances that can cause interference in the sample. The CS-5100 is also characterized by its wavelength switching detection function that serves in the measurement of lipemic or low fibrinogen-containing samples. Moreover, the CS-5100, which is compatible with main delivery lines, possesses a refrigerated cabinet that can accept various reagent vials, and incorporates a bar-code reader to enable efficient reagent control.

We conducted a basic evaluation of this new fully automated blood coagulation analyzer prior to its introduction, and here report the results.

MEASUREMENT PRINCIPLES AND CHARACTERISTICS

The CS-5100 adopts a transmitted light detection system as its principle for detection. The source light, which comes from a halogen lamp, is sorted with an interference filter into five light groups of different wavelengths (340, 405, 575, 660 and 800 nm). Optic fibers are used to deliver the light to the detector. Measurement samples were exposed to the transmitted light and the transmitted light at each wavelength was detected every 0.1 seconds (***Fig. 1***). After conversion of the transmitted light into an electric signal, coagulation time and concentration were calculated by means of signal analysis. This system is called the 'multi-wave detection system'. The CS-5100 has 20 photometric parts, all of which are compatible with the coagulation time method, synthetic substrate method and turbidimetric immunoassay.

If the coagulation time method is selected and the transmitted light intensity is inappropriate at the dominant wavelength, data at the secondary wavelength is automatically selected for the calculation of coagulation time. For measurement of fibrinogen, the dominant wavelength is adjusted to 405 nm and the secondary wavelength to 660 nm. For the measurement of other items required by the coagulation time method, the dominant wavelength is adjusted to 660 nm and the secondary wavelength to 800 nm.

The CS-5100 is characterized by its sample quality check function based on the multi-wave detection system. The distribution of absorbance level at multiple wavelengths was analyzed to predict the presence of interfering substances in the sample (e.g., hemolytics, icterus, lipemic substances).

ANALYZER AND MATERIALS

1. Analyzers and reagents

Analyzer: CS-5100

Reagents for analysis:

[Coagulation time method]

Prothrombin time (PT) - Thromborel S, Activated partial thromboplastin time (APTT) – Thrombocheck APTT-SLA, Fibrinogen (Fbg) – Thrombocheck Fib (L), Hepaplastin test (HPT) – Compound factor H 'Kokusai' (Sysmex Corporation)

[Synthetic substrate method]

Antithrombin III (AT-III) – Test Team S AT-III, Antiplasmin (APL) – Test Team S APL, Plasminogen

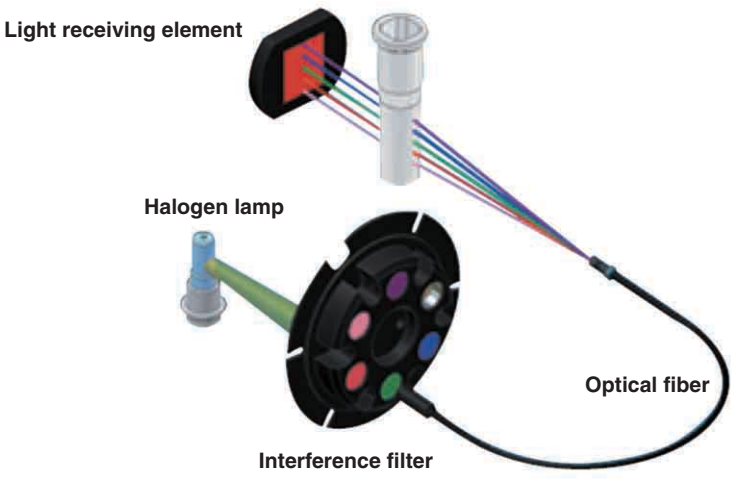


Fig. 1 *Multi-wave detection system*

(PLG) – Test Team SPLG, Protein C (PC) – Test Team S PC (SEKISUI MEDICAL Co., Ltd., Tokyo, Japan)
[Turbidimetric immunoassay]
Plasma total FDP (T-FDP) – Nanopia P-FDP (SEKISUI MEDICAL Co., Ltd.), D-dimer (DD) - LPIA-ACE D-D dimer II (Mitsubishi Chemical Medience Corporation, Tokyo, Japan)
Control analyzers (conventional analyzers): Coagrex-800 (blood coagulation analyzer; hereinafter referred to as CR 800, SEKISUI MEDICAL Co., Ltd.) – PT, APTT, Fbg, HPT measurement
BM-6010 (JEOL Ltd., Tokyo, Japan) – AT-III, APL, PLG, PC, T-FDP, DD measurement

2. Measurement samples

Plasma samples collected from inpatients and outpatients treated at Osaka University Hospital and sent to this laboratory (3.13% sodium citrate added) along with commercially available control plasma for the blood coagulation test were used as the measurement samples.

METHODS AND RESULTS

1. Within-run reproducibility

For items required by coagulation time and synthetic substrate methods, control plasma samples (Coagtrol IX-IIX; Sysmex) at two levels (normal range, abnormal range) were measured ten times each to evaluate within-run reproducibility. For the turbidimetric immunoassay, control plasma samples (FDP Control Neo; Sysmex Corporation) at two concentrations (L, H) were used in ten repeated measurements of T-FDP, and two different plasma samples (low value, high value) were used in ten repeated measurements of DD to evaluate within-run reproducibility.
The CV for the coagulation time method, the synthetic substrate method, and the turbidimetric immunoassay, ranged from 0.37% to 1.66% (***Table 1-A***), 1.31% to 4.92%, and 1.21% to 3.55% (***Table 1-B***), respectively.

Table 1-A *Within-run reproducibility (coagulation time method)*

Item	PT				APTT		Fbg		HPT	
	COAG・1X		COAG・2X		COAG・1X	COAG・2X	COAG・1X	COAG・2X	COAG・1X	COAG・2X
Unit	%	INR	%	INR	sec	sec	mg/dL	mg/dL	%	%
1	91.9	1.04	42.0	1.58	28.9	87.6	281.9	127.4	97.3	39.9
2	91.9	1.04	42.6	1.56	28.8	87.1	275.9	128.0	97.3	39.9
3	93.4	1.03	41.6	1.59	28.7	88.2	275.9	131.2	97.3	39.9
4	93.4	1.03	42.0	1.58	28.8	87.2	275.9	130.5	98.2	39.7
5	93.4	1.03	42.3	1.57	28.8	86.6	278.9	128.0	98.2	40.0
6	93.4	1.03	42.0	1.58	28.6	88.2	285.0	127.4	98.2	39.7
7	91.9	1.04	41.6	1.59	28.8	87.5	281.9	128.0	98.2	39.9
8	93.4	1.03	42.0	1.58	28.9	87.9	281.9	130.5	97.3	39.7
9	93.4	1.03	42.3	1.57	28.6	87.6	278.9	131.2	98.2	39.5
10	95.0	1.03	41.6	1.59	28.8	88.5	285.0	133.8	98.2	39.5
Mean	93.11	1.033	42.00	1.579	28.77	87.64	280.12	129.60	97.84	39.77
SD	0.97	0.0048	0.337	0.0099	0.106	0.580	3.552	2.154	0.465	0.177
CV%	1.04	0.47	0.80	0.63	0.37	0.66	1.27	1.66	0.48	0.44
MAX	95.0	1.04	42.6	1.59	28.9	88.5	285.0	133.8	98.2	40.0
MIN	91.9	1.03	41.6	1.56	28.6	86.6	275.9	127.4	97.3	39.5
RANGE	3.1	0.01	1.0	0.03	0.3	1.9	9.1	6.4	0.9	0.5

Table 1-B *Within-run reproducibility (synthetic substrate method, turbidimetric immunoassay)*

	Synthetic substrate method								Turbidimetric immunoassay			
Item	AT-III		APL		PLG		PC		T-FDP		DD	
	COAG・1X	COAG・2X	COAG・1X	COAG・2X	COAG・1X	COAG・2X	COAG・1X	COAG・2X	FDP(L)	FDP(H)	Low level sample	High level sample
Unit	%	%	%	%	%	%	%	%	μg/mL	μg/mL	μg/mL	μg/mL
1	96.8	29.8	102.5	38.7	99.1	33.3	92.8	32.1	9.2	42.2	1.00	13.40
2	95.5	28.4	108.6	37.9	99.9	33.7	96.2	31.9	9.1	41.9	1.10	12.80
3	97.3	29.7	102.2	36.0	99.7	33.7	95.5	32.8	9.0	41.7	1.10	13.25
4	99.7	31.8	111.2	37.6	101.4	34.1	94.9	31.6	8.5	42.0	1.10	13.55
5	96.6	28.2	105.1	38.9	100.3	33.2	90.9	32.4	9.0	41.4	1.07	13.62
6	96.7	28.8	106.9	37.2	100.4	32.6	94.0	33.0	9.1	41.5	1.03	13.40
7	99.5	30.3	106.4	35.9	100.3	33.4	94.5	33.7	9.1	42.7	1.03	13.18
8	98.6	29.5	104.2	32.6	103.6	34.0	94.8	32.1	9.4	41.5	1.07	13.36
9	98.8	27.6	104.4	36.4	100.5	33.6	93.5	32.4	9.2	40.9	1.03	12.69
10	97.6	28.3	104.0	37.4	102.2	34.9	95.1	31.3	8.7	41.4	1.07	12.99
Mean	97.71	29.24	105.55	36.86	100.74	33.65	94.22	32.33	9.03	41.72	1.059	13.250
SD	1.386	1.239	2.790	1.814	1.324	0.613	1.521	0.702	0.258	0.503	0.038	0.317
CV%	1.42	4.24	2.64	4.92	1.31	1.82	1.61	2.17	2.86	1.21	3.55	2.39
MAX	99.7	31.8	111.2	38.9	103.6	34.9	96.2	33.7	9.4	42.7	1.10	13.62
MIN	95.5	27.6	102.2	32.6	99.1	32.6	90.9	31.3	8.5	40.9	1.00	12.69
RANGE	4.2	4.2	9.0	6.3	4.5	2.3	5.3	2.4	0.9	1.8	0.10	0.93

2. Dilution linearity

To evaluate linearity, a sample containing a high concentration of Fbg was serially diluted 1/10 with Owren's veronal buffer (OVB; Sysmex Corporation). Linearity was confirmed up to a concentration of about 750 mg/dL (***Fig. 2***).

For T-FDP, a highly concentrated sample was diluted 1/5 with OVB. Satisfactory linearity was able to be confirmed up to a concentration of about 80 μg/mL (***Fig. 2***). Finally, a sample containing a high concentration of DD was diluted 1/10 with OVB. Satisfactory linearity was confirmed up to a concentration of about 25 μg/mL (***Fig. 2***).

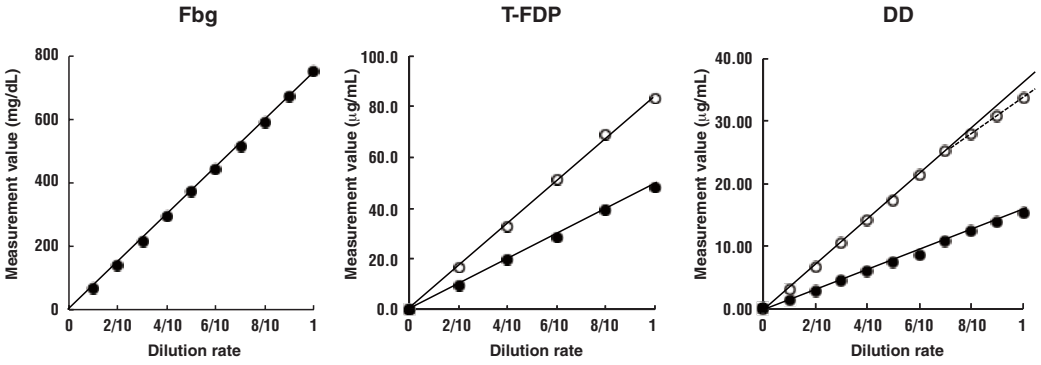
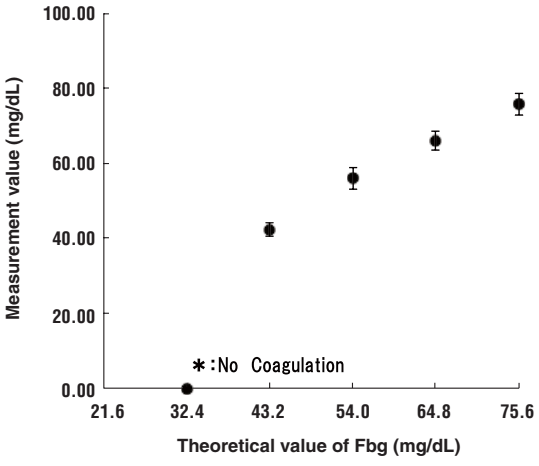


Fig. 2 *Dilution linearity*



Theoretical value	32.4	43.2	54.0	64.8	75.6
	conc.	conc.	conc.	conc.	conc.
1	∗	41.4	55	68.2	77.7
2	∗	43.2	58.1	66.9	75.5
3	∗	42.1	54.4	65.7	74.2
4	∗	41.1	55.1	63.6	74.5
5	∗	43.5	57.3	65.1	76.9
6	∗	41.9	58.3	65.5	75.3
7	∗	41.5	55.1	65.5	77.7
8	∗	42	56.7	65.5	73.7
9	∗	43.1	55.4	66.7	76.9
10	∗	43.2	54.6	66.5	74.5
MEAN	∗	42.3	56	65.92	75.69
SD	∗	0.87	1.47	1.24	1.50
CV	∗	2.07	2.62	1.87	1.98

∗ : No Coagulation : Slight Coagulation

Fig. 3-A *Minimum detection sensitivity (Fbg)*

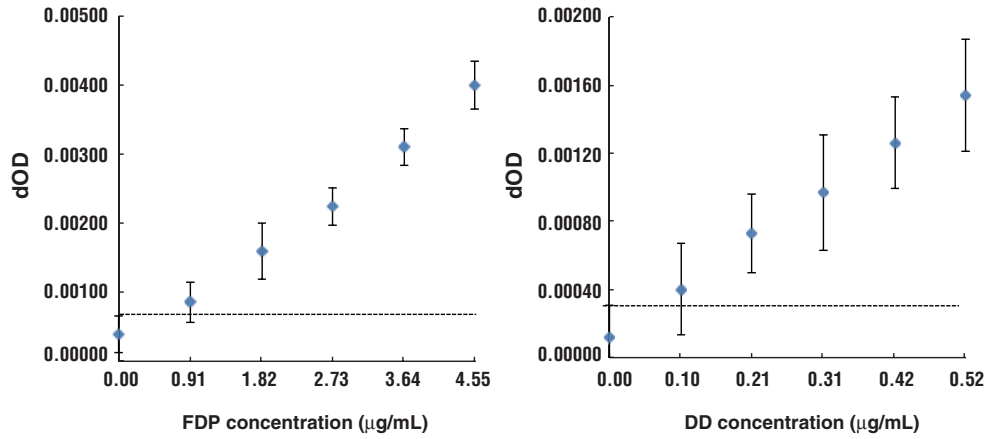


Fig. 3-B *Minimum detection sensitivity (T-FDP, DD)*

3. Minimum detection sensitivity

A sample containing a low concentration of Fbg was diluted with OVB to the specified concentrations. Each diluted sample was measured 10 times. The minimum detection sensitivity obtained with the ± 2SD method was 43.2 mg/dL. All measurements displayed flags indicating the presence of slight coagulation. Thus, all the measurement results of theoretical value (32.4 mg/dL) were judged to have no coagulation present (***Fig. 3-A***).
Similarly, a sample containing a low concentration of T-FDP was diluted with OVB to the specified concentrations and each diluted sample was measured 10 times. The minimum detection sensitivity obtained with the ± 2SD method was 1.82 μg/mL (***Fig. 3-B***). Finally, a sample containing a low concentration of DD was diluted with OVB to the specified concentrations and each diluted sample was measured 10 times. The minimum detection sensitivity obtained with the ± 2SD method was 0.2 μg/mL (***Fig. 3-B***).

4. Correlation with conventional analyzers

The correlation between the CS-5100 and the CR800 (SEKISUI MEDICAL Co., Ltd.), a conventional analyzer utilized for the items required by the coagulation time method; and between the CS-5100 and the BM-6010, a conventional analyzer utilized for the items required by the synthetic substrate method and turbidimetric immunoassay, were evaluated. A satisfactory correlation with the CR800 was observed from the following results: PT%: r = 0.993, y = 1.010x + 3.511, PT-INR: r = 0.997, y = 1.008x - 0.033, APTT: r = 0.996, y = 0.984x + 2.865, Fbg: r = 0.994, y = 1.156x - 29.410, HPT: r = 0.990, y = 0.889x + 8.720 (***Fig. 4-A***). Additionally, a satisfactory correlation with the BM-6010 was seen from the following results: AT-III : r = 0.996, y = 1.065x – 8.125, APL : r = 0.990, y = 0.913x + 8.018, T-FDP : r = 0.994, y = 1.122x + 0.174, PLG : r = 0.998, y = 1.029x – 0.716, PC : r = 0.998, y = 1.045x – 3.553, DD : r = 0.998, y = 0.906x + 0.353 (***Fig. 4-B***).

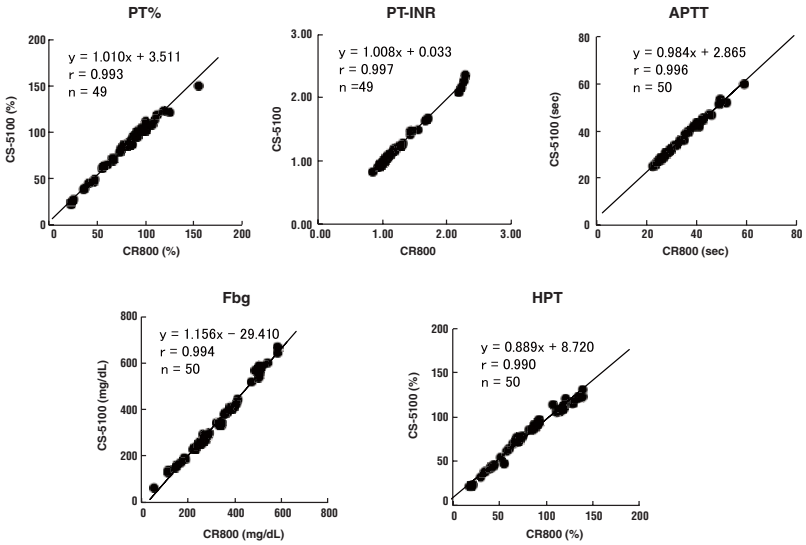


Fig. 4-A *Correlation with conventional analyzer (coagulation time method)*

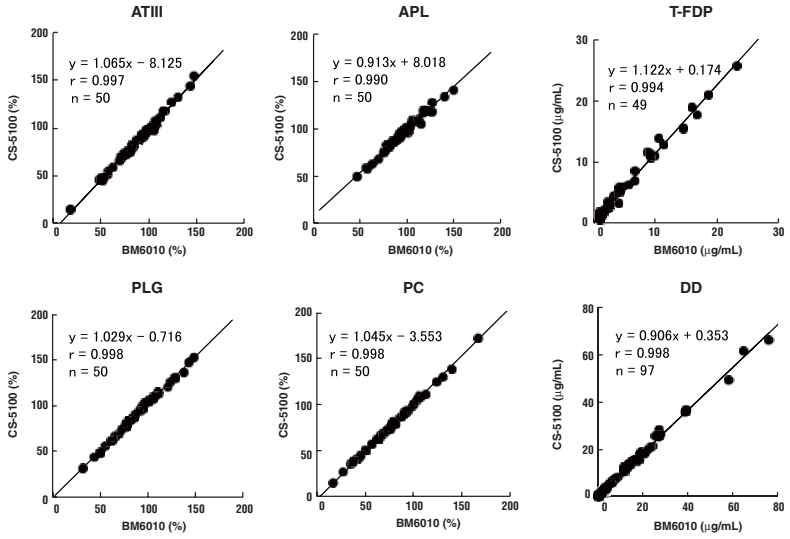


Fig. 4-B *Correlation with conventional analyzer (synthetic substrate method and turbidimetric immunoassay)*

DISCUSSION

In the present study, a basic evaluation of the CS-5100 was conducted prior to its practical introduction. Within-run reproducibility was evaluated by calculating the coefficient of variability (CV) of each method, which yielded the following satisfactory results: ≤ 2% (coagulation time method), ≤ 5% (synthetic substrate method) and ≤ 4% (turbidimetric immunoassay). Clinical samples were used to evaluate the correlation between the CS-5100 and conventional analyzers. Results from the evaluation of various items showed correlation coefficients that consistently exceeded 0.99, indicating good correlation. When evaluating APTT, a measurement value expressed by coagulation time, the correlation coefficient (0.996) was satisfactory; however, the normal time range was prolonged by about two seconds with the CS-5100. Due to this prolongation, the standard value should therefore be changed. The factors involved in this event likely reflect the differences between the CS-5100 and the CR800 in warming time and clotting point definition.

The dilution linearity test which employed serial 10-fold dilutions of a sample containing a high concentration of fibrinogen showed that accurate measurements could be obtained up to a concentration of about 750 mg/dL under the default measurement setting. Conducting the dilution linearity test on a sample containing a low concentration of fibrinogen showed that the acceptable range was from the measured value of minimum detection sensitivity to measurements obtained from 10 fold dilutions at concentrations exceeding 43.2 mg/dL. Additionally, the flag indicating the presence of slight coagulation was displayed. The wide range of measurement concentrations from 44 mg/dL to 750 mg/dL appeared to contribute to a reduction in retest rate. In the dilution retest, automated measurements were able to be conducted at concentrations of 25 mg/dL to 1500 mg/dL. The wide concentration range was also observed in the measurement of T-FDP and a reduction in retest rate is likely. In the measurement of DD, there was a tendency toward low values at concentrations exceeding around 25 μg/mL. Thus, compared with conventional analyzers, the CS-5100 did not contribute to further improvement in measurement range.

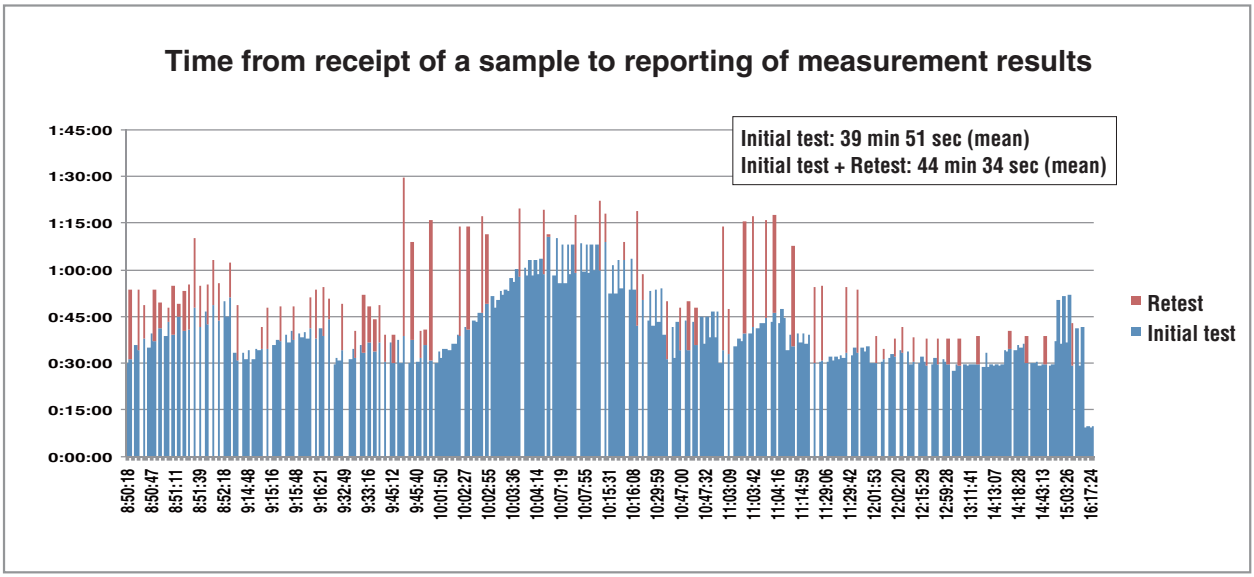
Table 2 shows conditions for hemostatic test operations on a day when a relatively large number of samples were

Table 2 *Condition of sample operation (May 14, 2012)*

Number of samples	278
Number of orders	897
Mean of order items	3.2
Number of retests	118
Retest rate	13.2%

Item	Number of initial tests	Number of retests
PT	262	39
APTT	227	41
Fbg	115	21
HPT	30	1
AT-III	40	3
APL	0	0
PLG	2	0
P-C	5	3
T-FDP	72	3
DD	144	7

	Number of samples	Number of tests
~ 9:00	24	109
9:00 ~ 9:30	27	79
9:30 ~ 10:00	23	62
10:00 ~ 10:30	72	243
10:30 ~ 11:00	18	60
11:00 ~ 11:30	43	151
11:30 ~ 12:00	1	5
12:00 ~ 12:30	16	50
12:30 ~ 13:00	10	24
13:00 ~ 14:00	8	20
14:00 ~ 15:00	22	32
15:00 ~ 16:00	12	30
16:00 ~	2	4



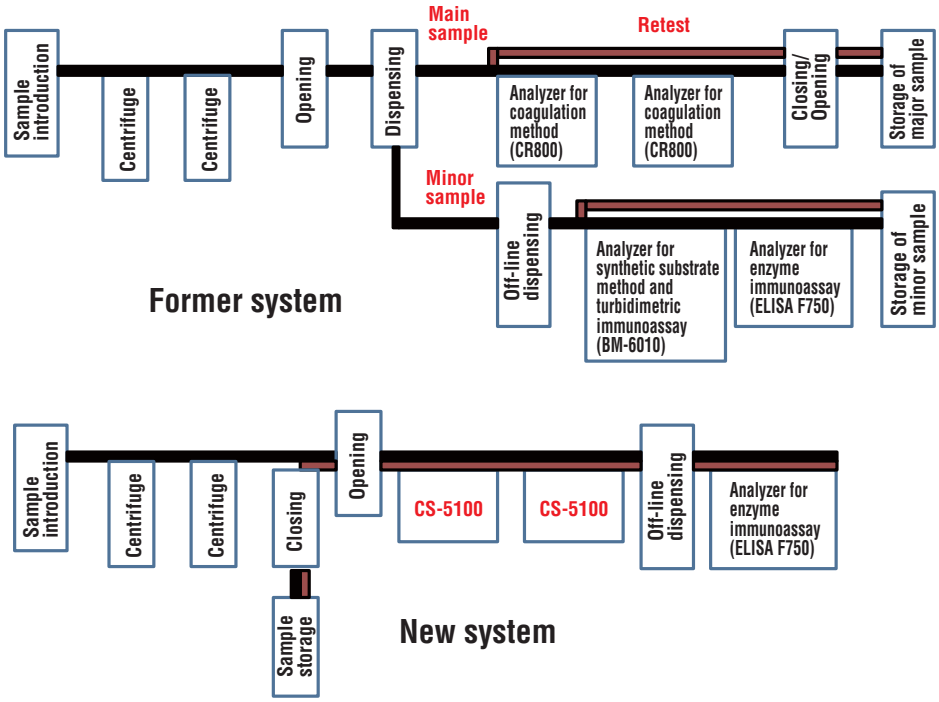


Fig. 5 *New/Former delivery systems for hemostatic tests*

delivered to the laboratory (on the Monday following the Monday that was a substitute holiday). During the sample delivery period (8:30-16:00), 278 samples had been delivered to the laboratory and each sample required 3.2 tests. The time from introduction of a sample to output of the initial results was about 39 minutes on average, while TAT, from introduction of a sample to reporting of all results, including results from retests, was about 44 minutes on average. TAT required by the CS-5100 was found to be no shorter than that required by conventional analyzers. In our laboratory, however, we make efforts to report the final results of routine tests within one hour after receipt of a sample. Therefore, the new system that incorporates the CS-5100 was determined to have high processing ability. Our former delivery system consisted of two analyzer units used exclusively for the items required by the coagulation time method (CR800), one analyzer unit for the items required by the synthetic substrate method/turbidimetric immunoassay (BM-6010) and one analyzer unit for off-line dispensing and items required by enzyme immunoassay method (ELSIA-F750, fully automated immunoassay system). To increase efficiency and save time during the conduction of measurements, we adopted a two line connection system by dividing the sample into small tubes. As a result of introduction of the CS-5100, we were able to eliminate the dispensing operation altogether and integrate the lines into a single line. By doing this, we were able to streamline the analyzers and ensure sufficient work space (***Fig. 5***).

During the conduction of routine laboratory tests, preparation of analyzers for measurement of samples (start-up settings, loading reagents, maintenance, etc.) requires much time and labor. The CS-5100, however, requires few maintenance operations. The device can start up quickly within five minutes if the reagents are stored in the refrigerated cabinet, which allows for the immediate analysis of samples. Although the pipette needs to be washed once daily, this operation can be completed during shut-down. Additionally, there is no need to select a specific function for washing. Because the refrigerated reagent cabinet is structured such that it is not affected by the external environment, reagents are able to be stored stably at 10°C. Reagents can be loaded at any time since they are in the refrigerated cabinet even during shut-down. Thus, time for loading reagents can be saved. During 24-hour operation, the user only needs to start up and shut down the analyzer to assure safe data storage. Additionally, because no specialized operations are needed, staff from other departments can use the CS-5100 easily in emergency situations during holidays. Consequently, introduction of the CS-5100 contributes immensely to reduction of burden on medical technologists. The CS-5100 enables online operations utilizing the measurement devices, delivery system and superior computer system. A medical technologist simply needs to set a sample on the delivery line. The results are then displayed on the system screen after completion of automated retesting. The technologist simply confirms the results and reports them. The aforementioned advantages enable increased efficiency of laboratory testing. As the results show, the CS-5100 is remarkably useful for the conduction of routine laboratory tests; however, the introduction of new measurements requires setting many items and engaging in complicated operations. Additionally, additional operations are required when reagents are introduced without bar codes that are compatible with the CS-5100. In the case of measurement of calibration curves, the reference substance should be set as a reagent in the cabinet.

Some laboratories, including this one, are equipped with multiple systems and the use of the reference substance as a sampler for measurement serves the convenience of such laboratories. We have reported these requests to Sysmex and would appreciate Sysmex's upgrade of the CS-5100 to fulfill our requests.

CONCLUSION

We performed an evaluation of the CS-5100 prior to its introduction and were able to confirm excellent basic performance of this analyzer. Improvement in the ability of processing multiple items/samples allowed the integration of hemostatic testing systems. Thus, the space occupied by the delivery system, including analyzers, was able to be reduced successfully.

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