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The Basic Evaluation of Light Transmission Platelet Aggregation Method on an Automated Coagulation Analyzer CN-6000

Mai EGASHIRA[*1] Mai KONO[*2], Kyoko NAKAJIMA[*2], Keisuke KITANO[*1] and Tasuku SAKAYORI[*1]

[*1] *Protein Technology, Engineering 1, Sysmex Corporation, 4-4-4, Takatsukadai, Nishi-ku, Kobe, 651-2271, Japan*
[*2] *IVD Clinical Development, Engineering 1, Sysmex Corporation*

Light transmission aggregometry (LTA) is known as a gold standard method for assessing platelet function. The fully automated coagulation analyzer CS-5100 (Sysmex Corporation, Kobe, Japan) is already enabled to measure platelet aggregation using LTA. It means that a semi-automated aggregometer, which is required only for testing platelet aggregation, and an automated coagulation analyzer for routine testing have been integrated. Moreover, the accuracy of measurement has been improved with the introduction of automated dispensing. Recently, a new automated coagulation analyzer called the CN-6000 (Sysmex Corporation) was launched.

In this report, we have evaluated performance of the CN-6000 for platelet aggregation function using 2.0 µM adenosine diphosphate (ADP), 2.0 µg/mL of collagen, 5.0 µM epinephrine, 1.0 mM arachidonic acid and 1.2 mg/mL ristocetin. We evaluated within-run precision and onboard stability as compared with the CS-5100 study and reference intervals. Platelet-rich plasma (PRP) and Platelet-poor plasma (PPP) from healthy volunteers were used as normal samples, and PPP from healthy volunteers, PRP spiked with anti-platelet drugs or artificial PRP were used as abnormal samples.

Within-run precision was measured using 30 replicates of analysis. The coefficient of variation (CV) with all agonists tested with normal and abnormal samples was less than 5% and 13%, respectively. The onboard stability evaluation indicated favorable stability up to 10 hours with all agonists. A comparative study between the CN-6000 and the CS-5100 was performed using 130 PRP samples. The CS-5100 showed remarkably high correlation, the correlation coefficient (r) with all agonists was more than 0.97. Reference intervals were 59.1%–98.3% for ADP, 80.8%–100% for collagen, 68.8%–99.8% for epinephrine, 63.2%–100% for arachidonic acid, and 77.7%–100% for ristocetin.

The CN-6000 has a reduced footprint by approximately half as compared to the CS-5100, with a reduced processing speed of 12.5% (450 tests per 1 hour) for the PT test. Our results demonstrated that the CN-6000 is a robust automated coagulation analyzer for platelet aggregation testing. It has automatic reagent dilution, which reduces errors in reagent preparation and can be expected to further help standardize testing.

Key Words Platelet aggregation, Light Transmission Aggregometry, CN-6000, Revohem, Automation

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INTRODUCTION

Light transmission aggregometry (LTA) is a standard platelet aggregation test method developed by Born in 1962.[1)] Since then, it has been used as a gold standard to test platelet function for diagnosis of congenital platelet dysfunctions, such as thrombasthenia or von Willebrand disease.[2,3)] This method has also been used for management of antithrombotic therapy in recent years to verify the effectiveness of antiplatelet agents, such as COX-1 inhibitors (e.g., acetylsalicylic acid [aspirin]) and P2Y12 receptor inhibitors (e.g., clopidogrel and prasugrel).[2-4)] The pharmacological actions of antiplatelet agents vary among different individuals.[5)] Therefore, platelet aggregation testing may be clinically important for assessing unresponsiveness to antiplatelet agents or deciding whether or not to continue the administration.

Notes: This article is based on current regulatory requirements in Japan. (as of May 2020)
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LTA requires two materials obtained from different centrifugal conditions, PRP and PPP. Conventionally, the semi-automated coagulation analyzer has been primarily used. However, this method requires a considerable amount of time to prepare reagents or dispense samples and reagents by experienced technologists. Automated Coagulation Analyzers CS-5100, CS-2400, CS-2500, CS-2000*i*, and CS-2100*i* (Sysmex Corporation) have been used for routine testing, such as PT, APTT, Fbg, AT, and D-dimer, and these analyzers have recently become available for platelet aggregation testing. Subsequently, several studies compared existing semi-automated coagulation analyzers.[6-12)]

Recently, an automated coagulation analyzer CN-6000 (Sysmex Corporation) has been released. The CN-6000 inherited the features of the previous model (CS-5100), including multi-wave detection, HIL check, and wave analysis technologies. It improves the processing speed of the PT test by 12.5% (450 tests per 1 hour) and reduces the footprint by approximately half when compared with the CS-5100. Thus, the CN-6000 has excellent potential to further improve operational workflow in laboratories. The CN-6000 has a new feature of automated serial dilution after reagent dissolution to a concentration of use, whereas the CS-5100 is manually operated. With this feature, reagents are automatically prepared after the serial dilution by entering the number of tests and desired concentrations and by setting up the stock solutions of reagent and diluent to the device. This method can enhance the efficiency of the platelet aggregometry workflow (***Table 1***, ***Fig. 1***).

Table 1 *Comparison of platelet aggregation testing work flow*

Work Flow	Semi-automated	CS-5100	CN-6000
Blood sampling	Manual	Manual	Manual
Prepare PPP and PRP	Manual	Manual	Manual
Dilute to a concentration of use	Manual	Manual	Auto
Place stirring bar into cuvette	Manual	No need	No need
Dispense PPP and PRP to sample cuvette	Manual	Auto	Auto
Add agonist to cuvette	Manual	Auto	Auto
Detection	Auto	Auto	Auto
Results	Auto	Auto	Auto

Changes from “semi-automated” are shown in red.

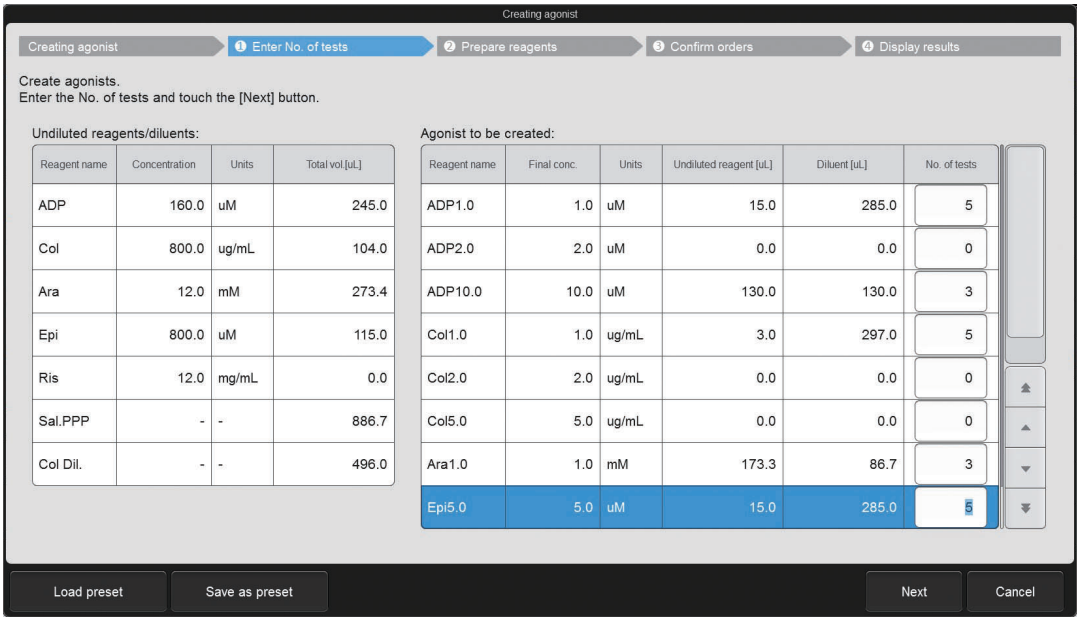

Fig. 1 *Example of automated serial dilution ordering displayed on the instrument*

In this report, we evaluated the performance of platelet aggregation tests with the new automated coagulation analyzer CN-6000, including within-run precision,[13] onboard stability of the agonists,[14] comparative study[7,15], and reference intervals.[16]

EXPERIMENTAL MATERIALS AND METHODS

1. Subjects

This study was conducted with the approval of the ethics committee of Sysmex Corporation. Blood samples were collected from in-house healthy volunteers by using blood collection tubes with 3.2% sodium citrate. The samples were centrifuged at 200 × g for 10 min, and the supernatant was collected to obtain PRP. PPP was obtained from the residual blood by centrifuging at 1500 × g for 15 min and collecting the supernatant.

The PRP with no additives after the collection served as the normal samples. For ADP, abnormal samples were prepared from the normal samples: cangrelor (AdooQ Bioscience)-spiked samples. For collagen and epinephrine, two types of abnormal samples were prepared from the normal samples: acetylsalicylic acid (FUJIFILM Wako Pure Chemical Corporation)-spiked samples and cangrelor -spiked samples. For arachidonic acid, abnormal samples were prepared from the normal sample acetylsalicylic acid -spiked samples. Abnormal samples for ristocetin were prepared by combining the PPP of healthy volunteers, vWF Deficient Plasma (HYPHEN BioMed), and lyophilized platelets (HYPHEN BioMed) (***Table 2***). Normal and abnormal samples for a comparative study were also prepared with the same methods to cover the measurement range.

2. Analyzers and reagents for measurement

The CN-6000 was used for within-run precision, onboard stability, and comparative studies with the CS-5100 as a control device in the comparative studies. The CS-5100, CS-2400, CS-2500, CS-2000*i*, and CS-2100*i* were used for calculating the reference ranges.

For reagents for measurement, *in vitro* diagnostics including Revohem™ ADP, Revohem collagen, Revohem epinephrine, Revohem arachidonic acid and Revohem ristocetin (Sysmex Corporation) were used. Final concentrations of reagents used for this study were determined in accordance with the recommendation of the Standardization Committee of the International Society of Thrombosis and Hemostasis (ISTH) (ADP: 2.0 μM, collagen: 2.0 μg/mL, arachidonic acid: 1.0 mM, epinephrine: 5.0 μM, and ristocetin: 1.2 mg/mL).

1) Within-run precision
For within-run precision, the coefficient of variation (CV%) was obtained from the maximal aggregation rate (%) that was acquired by 30 consecutive measurements of normal and abnormal samples.[13]

Table 2 *Abnormal samples preparation*

	Within-run precision / Onboard stability	Comparative study
ADP	PRP + cangrelor (Final concentration 0.050 μM)	PRP + cangrelor (Final concentration 0.025–5.0 μM)
Collagen	PRP + acetylsalicylic acid (Final concentration 0.50–1.0 mM)	PRP + acetylsalicylic acid (Final concentration 0.10–5.0 mM) PRP + acetylsalicylic acid + cangrelor (Final concentration 1.0–5.0 μM)
Arachidonic acid	PRP + acetylsalicylic acid (Final concentration 1.0 mM)	PRP + acetylsalicylic acid (Final concentration 0.10–5.0 mM)
Epinephrine	PRP + acetylsalicylic acid (Final concentration 1.0 mM)	PRP + acetylsalicylic acid (Final concentration 0.10–5.0 mM) PRP + acetylsalicylic acid + cangrelor (Final concentration 1.0–5.0 μM)
Ristocetin	PPP + vWF Deficient Plasma + lyophilized platelets	PPP + vWF Deficient Plasma + lyophilized platelets

2) Onboard stability
Onboard stability was evaluated as the difference of maximal aggregation rate (%) between 0 hour (h) sample and each time point sample using the maximal aggregation rate (%) of four time points at 0, 4, 8, and 10 h. Considering that the activities of samples for platelet aggregation change over time, we prepared specific sets of reagents (agonists) to measure the normal and abnormal samples simultaneously at the predetermined time points. The reagents were prepared 4, 8, and 10 h before measurement and placed on the analyzer (agonists onboard for 4, 8, and 10 h), and the control agents were prepared and placed at the time of measurement (agonists onboard for 0 h).[14]

3) Comparative study
For each batch of 130 samples, a measurement was performed on the CN-6000 and the CS-5100 to obtain a regression equation and coefficient of correlation by using the maximal aggregation rate (%).[7,15]

4) Reference intervals
From the measurement results obtained from a sample size of 120 or more healthy volunteers in accordance with the recommendation of the Standardization Committee of the International Society on Thrombosis and Hemostasis (ISTH), outliers (mean ± 2SD) were excluded once. Then, reference intervals (95% confidence interval) were obtained using Analyse-it (Analyse-it Software, Ltd.).[16]

RESULTS

1) Within-run precision
The CVs (%) for within-run precision with normal/abnormal samples were 1.9/10.7 for ADP, 3.1/7.2 for collagen, 3.0/10.4 for epinephrine, 2.0/12.1 for arachidonic acid, and 4.5/6.9 for ristocetin (***Table 3***).

2) Onboard stability
The changes in maximal aggregation rates at 0 h and up to 10 h were within 5% with all agonists, which indicated favorable stability (***Table 4***, ***Fig. 2***).

3) Comparative study
The CN-6000 and the CS-5100 showed high correlation, the correlation coefficient (r) with all agonists (ADP, collagen, epinephrine, arachidonic acid, and ristocetin) was more than 0.97. (***Fig. 3***)

Table 3 *Results of within-run precision*

[Maximal aggregation(%)]

	Normal sample		Abnormal sample	
	Average	CV (%)	Average	CV (%)
ADP	92.8	1.9	28.1	10.7
Collagen	84.8	3.1	27.4	7.2
Arachidonic acid	93.6	2.0	17.6	12.1
Epinephrine	89.7	3.0	41.8	10.4
Ristocetin	79.0	4.5	45.6	6.9

Table 4 *Results of on-board stability*

A. ADP [Maximal aggregation(%)]

	Normal sample				Abnormal sample			
	0 hour	4 hours	8 hours	10 hours	0 hour	4 hours	8 hours	10 hours
1	88.1	89.1	87.0	90.9	27.5	29.4	30.7	29.6
2	89.6	85.9	87.2	82.9	26.3	29.3	28.5	32.9
Average	88.9	87.5	87.1	86.9	26.9	29.4	29.6	31.3
Difference (0 hour - each time point)	—	1.3	1.8	1.9	—	−2.5	−2.7	−4.4

B. Collagen [Maximal aggregation(%)]

	Normal sample				Abnormal sample			
	0 hour	4 hours	8 hours	10 hours	0 hour	4 hours	8 hours	10 hours
1	85.5	91.7	91.5	85.3	24.9	25.5	25.2	27.4
2	92.2	87.9	90.0	89.2	23.4	24.7	25.2	24.9
Average	88.9	89.8	90.8	87.3	24.2	25.1	25.2	26.2
Difference (0 hour - each time point)	—	−1.0	−1.9	1.6	—	−1.0	−1.1	−2.0

C. Arachidonic acid [Maximal aggregation(%)]

	Normal sample				Abnormal sample			
	0 hour	4 hours	8 hours	10 hours	0 hour	4 hours	8 hours	10 hours
1	94.0	95.8	98.4	99.6	3.5	3.0	2.7	4.6
2	89.7	96.0	93.7	95.2	2.3	4.1	3.4	4.7
Average	91.9	95.9	96.1	97.4	2.9	3.6	3.1	4.7
Difference (0 hour - each time point)	—	−4.1	−4.2	−5.6	—	−0.7	−0.2	−1.8

D. Epinephrine [Maximal aggregation(%)]

	Normal sample				Abnormal sample			
	0 hour	4 hours	8 hours	10 hours	0 hour	4 hours	8 hours	10 hours
1	88.6	91.0	91.6	90.1	35.3	34.5	36.4	34.6
2	91.0	89.7	89.4	85.7	37.8	35.5	37.9	37.9
Average	89.8	90.4	90.5	87.9	36.6	35.0	37.2	36.3
Difference (0 hour - each time point)	—	−0.5	−0.7	1.9	—	1.6	−0.6	0.3

E. Ristocetin [Maximal aggregation(%)]

	Normal sample				Abnormal sample			
	0 hour	4 hours	8 hours	10 hours	0 hour	4 hours	8 hours	10 hours
1	95.9	97.5	94.8	96.8	39.1	35.5	40.5	40.4
2	93.7	95.4	94.4	93.9	36.2	38.7	40.1	39.8
Average	94.8	96.5	94.6	95.4	37.7	37.1	40.3	40.1
Difference (0 hour - each time point)	—	−1.6	0.2	−0.5	—	0.6	−2.6	−2.4

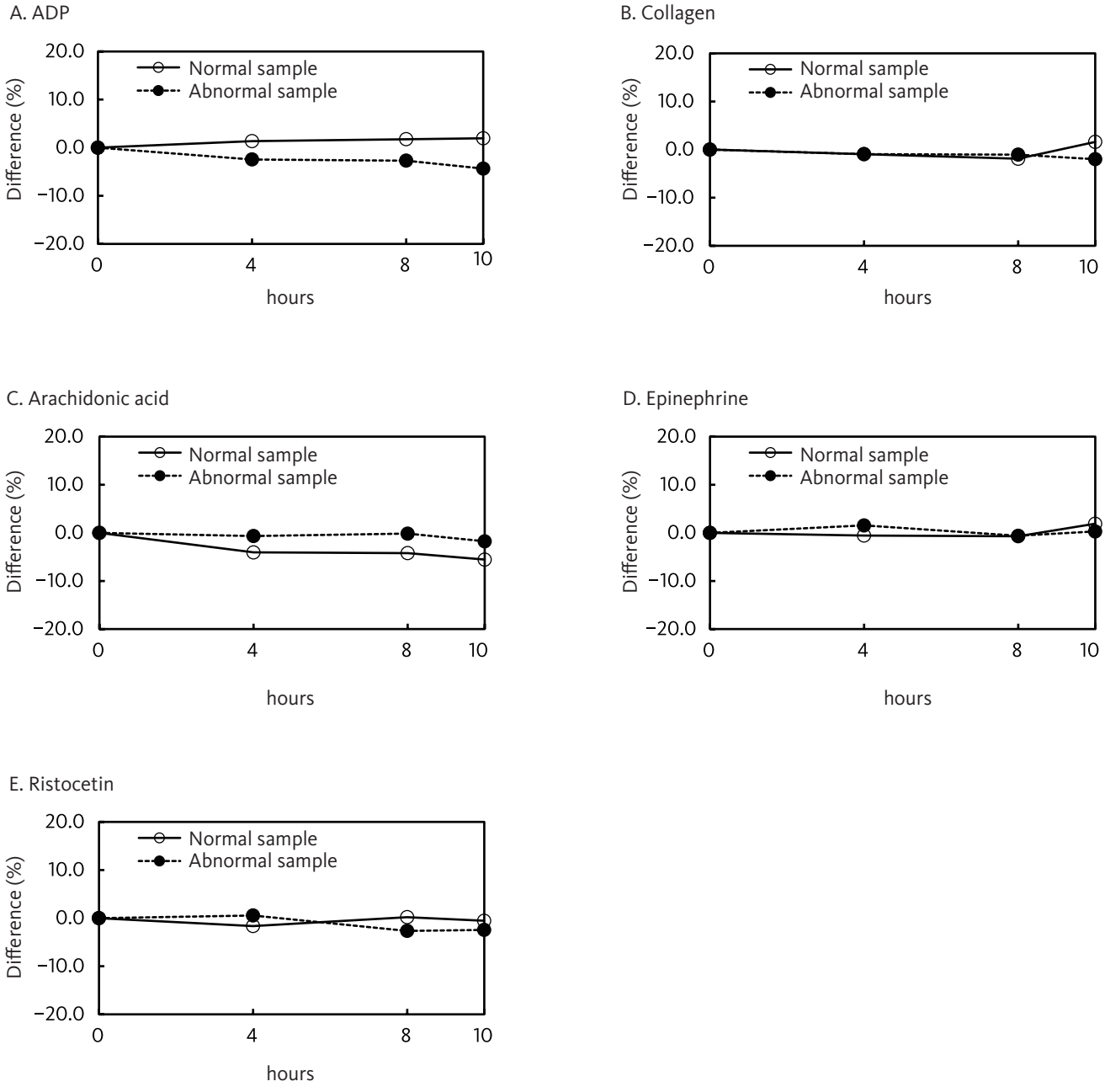

Fig. 2 *Results of onboard stability*

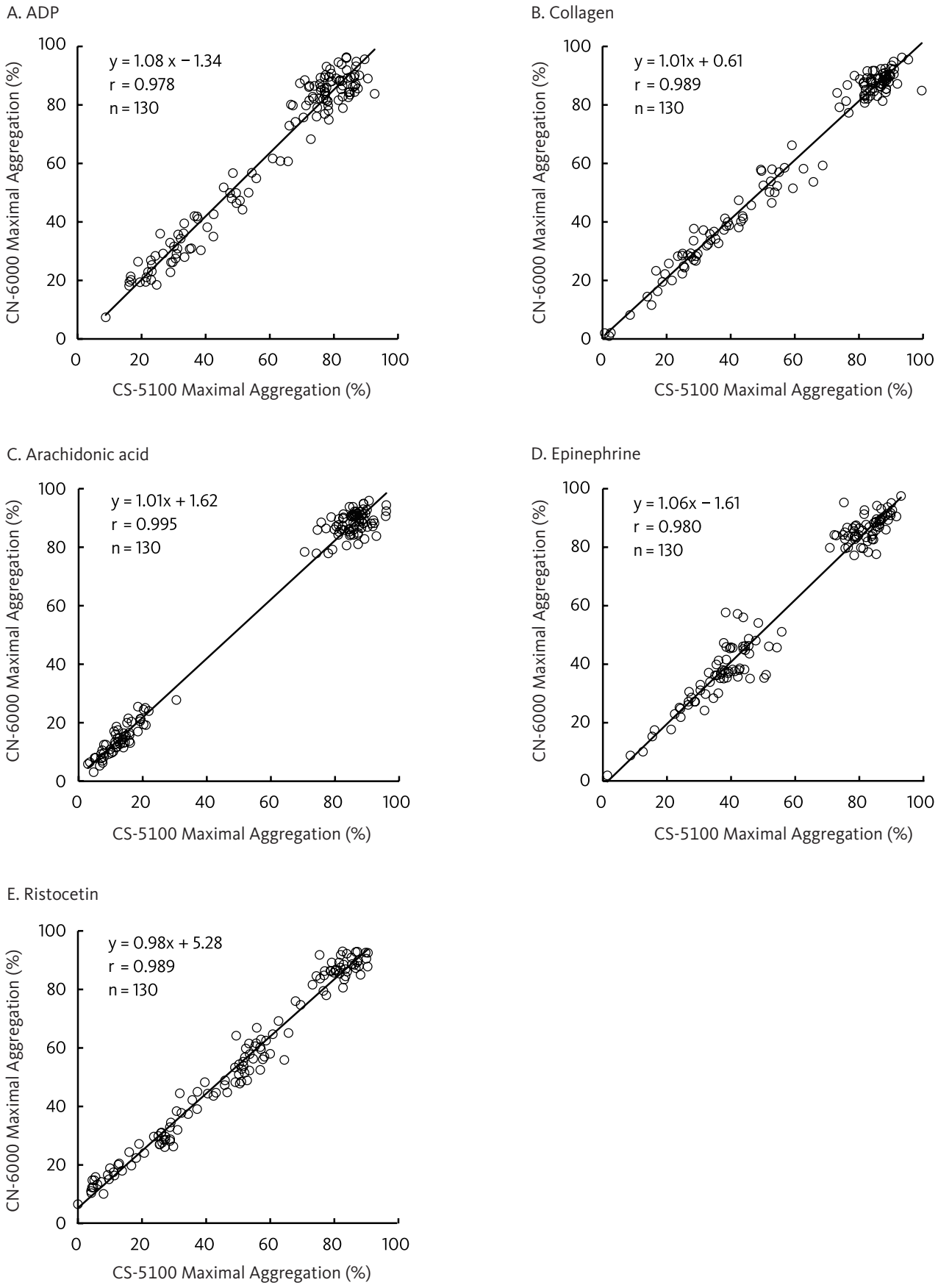

Fig. 3 *Results of comparative study*

4) Reference intervals
Reference intervals were 59.1%–98.3% (n = 141) for ADP, 80.8%–100% (n = 133) for collagen, 68.8%–99.8% (n = 150) for epinephrine, 63.2%–100% (n = 126) for arachidonic acid, and 77.7%–100% (n = 123) for ristocetin (***Table 5***).

DISCUSSION

The automated coagulation analyzer routinely used in clinical laboratories has become available for platelet aggregation testing. This device offers cost savings because it does not require dedicated devices for particular testing. It also allows high-quality testing because it decreases the measurement errors and inter-operator differences associated with the complex procedures of existing devices. A differential diagnosis of thrombasthenia was achieved by initiating the platelet aggregation testing with the automated coagulation analyzer in a hospital where platelet aggregation had not been measured.[17] Thus, incorporation of platelet aggregation testing into the routinely used device can provide a remarkable contribution to medical services.

In the current study, we investigated the basic performance of the platelet aggregometry built into the new automated coagulation analyzer CN-6000 and correlation with an existing device (CS-5100). The reference intervals (concentrations recommended by the Standardization Committee of the ISTH) of the automated coagulation analyzer with five platelet aggregation agonists were also investigated.

For the within-run precision, the results with the CN-6000 were comparable with those with the CS-2000*i*/CS-2100*i*,[9,14] as evidenced by the CV of 5% or less in the normal samples and 13% or less in the abnormal samples (***Table 3***). Considering that the CS-2000*i*/2100*i* have demonstrated higher reproducibility than semi-automated analyzers,[9] we can expect a similarly high reproducibility with the CN-6000.

The results of onboard stability with the CN-6000 demonstrated that the measurement of the five investigated agonists were stable after 10 h. Given that the CS-5100 also shows 10-hour stability,[14] the CN-6000 can also be used for measurement within the day without any issues.

The CS-5100 showed significantly high correlation. With ristocetin, y = 0.98x + 5.28 and approximately 5% of intercepts were observed. The major purposes of platelet aggregation testing with ristocetin include aiding the diagnosis of von Willebrand disease or Bernard–Soulier syndrome.[18] Existing reports on semi- and full-automated coagulation analyzers also showed approximately 5% deviation in patients with von Willebrand disease;[12] therefore, the difference in results from the current study did not affect the interpretation of the measurement values. This study demonstrated the correlation between automated coagulation analyzers. Thus, the reference intervals calculated in this study can be applicable to the CN-6000.

For the reference intervals, no substantial fluctuations were observed in most parameters compared with the previously reported small study.[14] However, the arachidonic acid results showed approximately 10% wider interval on the lower value side from 75%–105% (n = 43) to 63.2%–100% (n = 126). While the current study investigated a Japanese population, a European study has reported the reference interval of 70%–105% (n = 42).[19] Since major differences based on race may be absent, further investigation is required.

For the CS-5100, the CS-2400, and the CS-2500, a research-use-only scoring system (APAL and CPAL) was installed to score ADP- and collagen-induced results in two concentrations. This system is useful for interpretation of platelet aggregation results, which is often challenging.[20] These indicators captured the reactions that could not be detected by the maximal aggregation rate with one concentration, which has been conventionally used in clinical settings.[15] In consideration that the same indicators were installed, the use of the CN-6000 is also expected to facilitate the interpretation of the platelet aggregation results.

Table 5 *Results of reference intervals*

	n	Reference intervals [Maximal Aggregation (%)]
ADP	141	59.1 – 98.3
Collagen	133	80.8 – 100.0
Arachidonic acid	126	63.2 – 100.0
Epinephrine	150	68.8 – 99.8
Ristocetin	123	77.7 – 100.0

Due to integration of platelet aggregometry with the automated coagulation analyzer, variation of the results is expected to decrease depending on the operators' experience/technical levels and frequency of human errors. The latest platelet aggregometry with the CN-6000 features automated reagent dilution. The automated dilution preparation reduces errors in reagent preparation and the number of processes with manual dilution, thereby improving usability.

Platelet aggregation testing has numerous factors that may cause variability, such as differences in reagent preparation methods, concentrations, and measuring devices. The reagent concentrations for congenital dysfunction are expected to be converged in accordance with the recommendation of the Standardization Committee of the ISTH.[2,3] In the present study, the difference in measured values among the automated coagulation analyzers was small. Therefore, the convergence of platelet aggregation testing results is expected. Meanwhile, issues such as unifying the centrifugation conditions for reagent preparation remain unsolved. As for centrifugation conditions, despite the recommendation by the Standardization Committee of the ISTH,[2,3] gravity on the PRP during centrifugation varied depending on the length of the blood collection tube or other factors. Therefore, the centrifugation conditions differ depending on the facilities, and it requires further effort to standardize. Integrating the methods of platelet aggregation testing, including length of the blood collection tube, centrifugation conditions, and use of automated coagulation analyzer, is necessary to improve the quality of testing. Accordingly, new knowledge could be obtained by resolving those variabilities.

CONCLUSION

Platelet aggregation testing with the CN-6000 yielded favorable results for within-run precision, onboard stability, and correlation with the existing device CS-5100. The simplified process of reagent preparation could decrease human errors and contribute to the standardization of platelet aggregation testing in the future.

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