A Comparative Study of Sysmex Latex Test BL-2 P-FDP and LIAS AUTO D-Dimer NEO with Similar Assay Reagents of Two Other Companies on the Fully Automated Blood Coagulation Analyzer CS-5100

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The fully automated blood coagulation analyzer CS-5100 (CS-5100; Sysmex Corporation, Kobe, Japan) is capable of a high throughput, of up to 400 tests per hour, and its automatic reagent barcode reading and cap piercing capabilities provides enhanced user friendliness. We evaluated the basic performance of Latex Test BL-2 P-FDP (Sysmex Corporation), LIAS AUTO D-Dimer NEO (Sysmex Corporation) and assay reagents from two other companies (Nanopia® P-FDP and Nanopia® D-dimer; SEKISUI MEDICAL Co., Ltd., Tokyo, Japan, and LPIA FDP-P and LPIA Ace D-D dimer II; LSI Medience Corporation: previsously Mitsubishi Chemical Medience Corporation, Tokyo, Japan) in the measurement of plasma FDP and D-dimer with our newly introduced CS-5100 system.

Latex Test BL-2 P-FDP and LIAS AUTO D-Dimer NEO showed good performance on the CS-5100 for all the performance parameters tested; within-run reproducibility, onboard reagent stability, linearity and hook effect. Moreover, there was a good correlation with the results of current reagents, Nanopia® P-FDP and Nanopia® D-dimer that were obtained with the current blood coagulation analyzer in use, Coagrex-800 (CR-800; SEKISUI MEDICAL Co., Ltd.). Nanopia® P-FDP and Nanopia® D-dimer generally gave good results on the CS-5100 for all the performance parameters tested. However, Nanopia® D-dimer showed a slight variation in the within-run reproducibility, and the linearity of Nanopia® P-FDP was only up to 25 μg/mL. LPIA FDP-P and LPIA Ace D-D dimer II showed slight variations in the within-run reproducibility.

Among the three companies' plasma FDP and D-dimer assay reagents that were evaluated on the CS-5100, Latex Test BL2 P-FDP and LIAS AUTO D-Dimer NEO demonstrated good basic performance necessary for a satisfactory reagent for routine testing.

Key Words CS-5100, Latex Test BL2 P-FDP, LIAS AUTO D-Dimer NEO

INTRODUCTION

The fully automated blood coagulation analyzer CS-5100 (CS-5100; Sysmex Corporation, Kobe, Japan) has detectors capable of measurements by four different principles, i.e., by the clotting, chromogenic, immunoturbidimetric and aggregation methods, and can assay a wide range of parameters at high throughput. Besides this, its user friendliness has now been improved by the addition of automatic reagent barcode reading and cap-piercing functions.

At our hospital, we had been using the fully automated blood coagulation analyzer Coagrex-800 (CR-800; SEKISUI MEDICAL Co., Ltd., Tokyo, Japan) until recently. When we acquired the CS-5100 to replace the CR-800, we carried out a basic performance evaluation of FDP and D-dimer reagents with the new analyzer, the results of which are reported here.

SPECIMENS AND REAGENTS

1. Specimens

For assessment of within-run reproducibility, dilution linearity, hook effect and correlation with measurements made by the conventional method, we used patients' plasma (3.8% citrated) sent to our laboratory for hemostasis tests. Commercially obtained controls were used for evaluating within-run reproducibility and onboard reagent stability.

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2. Analyzers

The CS-5100 was used for the performance evaluation and the CR-800 as the reference analyzer for examining the correlation of measured values.

3. Reagents and controls

The reagents evaluated, reference reagents, and controls used are listed in ***Table 1***.

METHODS

1. Within-run reproducibility

Ten replicate measurements were made on two concentrations of control that matched each reagent and one concentration of pooled 3.8% citrated plasma as the sample.

2. Onboard reagent stability

Each reagent bottle was loaded in the cap opened condition in the analyzer, and two concentrations of controls that matched each reagent were analyzed daily for 12 consecutive days.

3. Dilution linearity

Two patients' specimens with high concentration of the target substance were respectively diluted with a diluent in 10 steps (Sample1) and 6 steps (Sample2), then assayed.

4. Assessment of hook effect and dilution linearity in automatic dilution (1/8) measurements with the CS-5100

One specimen from a patient with high concentration of the target substance was diluted in 7 steps to prepare samples for measurement. These samples were assayed in the normal way and also after 1/8 automatic dilution.

5. Correlation

Correlation between assay results for FDP and D-dimer obtained in different combinations of the conventional method (the CR-800 with Nanopia® P-FDP and Nanopia® D-dimer; SEKISUI MEDICAL Co., Ltd.) and the CS-5100 with Latex Test BL-2 P-FDP (Sysmex Corporation), LPIA FDP-P (LSI Medience Corporation; previsously Mitsubishi Chemical Medience Corporation, Tokyo, Japan), Nanopia® P-FDP, LIAS AUTO D-Dimer NEO (Sysmex Corporation), LPIA Ace D-D dimer II (LSI Medience Corporation) and Nanopia® D-dimer was examined (***Table 2***).
Patient plasmas with FDP (n=40) and D-dimer (n=97) were used as the assay samples.

Table 1 *Reagents and controls*

	FDP			D-dimer		
Particulars	Reagent	Control	Supplier	Reagent	Control	Supplier
Evaluated reagents	Latex Test BL-2 P-FDP	FDP CONTROL NEO	Sysmex	LIAS AUTO D-Dimer NEO	D-Dimer CONTROL NEO	Sysmex
Reference reagents	Nanopia® P-FDP	FDP Control	Sekisui Medical	Nanopia® D-dimer	FDP Control	Sekisui Medical
	LPIA FDP-P	Iatrosera® TH level I, Iatrosera® TH level II	LSI Medience	LPIA Ace D-D dimer II	Iatrosera® TH level I, Iatrosera® TH level II	LSI Medience

Table 2 *Combinations used for examining correlation*

FDP			D-dimer		
Latex Test BL-2 P-FDP CS-5100	vs	Nanopia® P-FDP CR-800	LIAS AUTO D-Dimer NEO CS-5100	vs	Nanopia® D-dimer CR-800
LPIA FDP-P CS-5100	vs	Nanopia® P-FDP CR-800	LPIA Ace D-D dimer II CS-5100	vs	Nanopia® D-dimer CR-800
Nanopia® P-FDP CS-5100	vs	Nanopia® P-FDP CR-800	Nanopia® D-dimer CS-5100	vs	Nanopia® D-dimer CR-800

RESULTS

1. Within-run reproducibility

The results are shown in ***Table 3***. The coefficient of variation (CV) was satisfactory at 1.01 - 4.07% with Latex Test BL-2 P-FDP and 0.77 - 1.99% with LIAS AUTO D-Dimer NEO. The CV was 1.14 - 2.65% with Nanopia® P-FDP. With Nanopia® D-dimer, it was 1.97 - 7.84%, being high in the low D-dimer concentration range. The CV was 2.31 - 13.79% with LPIA FDP-P and 1.48 - 8.38% with LPIA Ace D-D dimer II. For both FDP and D-dimer, the CV was large for measurements on the Iatrosera® TH level I (LSI Medience Corporation).

Table 3 *Within-run reproducibility*

Reagent	Latex Test BL-2 P-FDP		
Sample	FDP CONTROL NEO-L	FDP CONTROL NEO-H	Pooled plasma
MEAN	8.50	27.62	8.42
SD	0.21	0.28	0.34
CV	2.5%	1.0%	4.1%
MAX	8.9	28.0	8.9
MIN	8.3	27.2	7.9
RANGE	0.6	0.8	1.0

Reagent	LIAS AUTO D-Dimer NEO		
Sample	D-Dimer CONTROL NEO-L	D-Dimer CONTROL NEO-H	Pooled plasma
MEAN	2.12	11.39	3.98
SD	0.04	0.09	0.04
CV	2.0%	0.8%	1.1%
MAX	2.2	11.5	4.0
MIN	2.1	11.3	3.9
RANGE	0.1	0.2	0.1

Reagent	Nanopia® P-FDP		
Sample	FDP Control LOW	FDP Control HIGH	Pooled plasma
MEAN	9.848	30.385	5.014
SD	0.261	0.347	0.112
CV	2.7%	1.1%	2.2%
MAX	10.19	30.87	5.11
MIN	9.47	29.79	4.79
RANGE	0.72	1.08	0.32

Reagent	Nanopia® D-dimer		
Sample	FDP Control LOW	FDP Control HIGH	Pooled plasma
MEAN	3.230	9.628	2.841
SD	0.087	0.189	0.223
CV	2.7%	2.0%	7.8%
MAX	3.34	9.94	3.44
MIN	3.10	9.36	2.71
RANGE	0.24	0.58	0.73

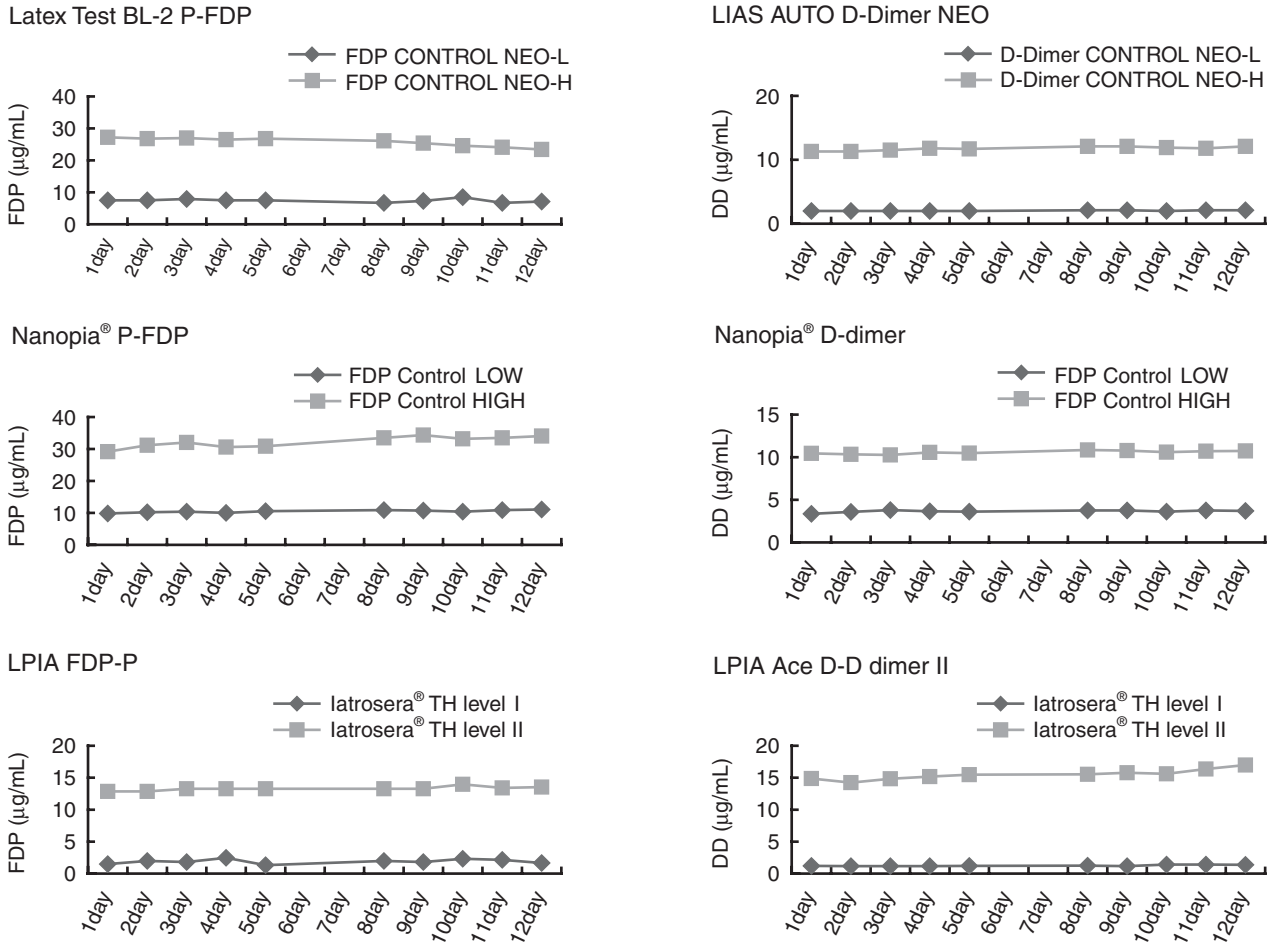
Reagent	LPIA FDP-P		
Sample	Iatrosera® TH level I	Iatrosera® TH level II	Pooled plasma
MEAN	1.994	13.224	7.175
SD	0.275	0.306	0.546
CV	13.8%	2.3%	7.6%
MAX	2.31	13.68	8.08
MIN	1.48	12.71	6.10
RANGE	0.83	0.97	1.98

Reagent	LPIA Ace D-D dimer II		
Sample	Iatrosera® TH level I	Iatrosera® TH level II	Pooled plasma
MEAN	1.174	14.576	3.441
SD	0.098	0.225	0.051
CV	8.4%	1.5%	1.5%
MAX	1.31	14.93	3.50
MIN	1.02	14.23	3.37
RANGE	0.29	0.70	0.13

Unit (μg/mL)

2. Onboard reagent stability

The results are shown in ***Fig. 1***. The variation of the measured values from the value on Day1 was within ±10% for high concentration controls and within ±20% for the low concentration controls up to Day10 with Latex Test BL-2 P-FDP, Day12 with LIAS AUTO D-Dimer NEO, Day5 with Nanopia® P-FDP, Day12 with Nanopia® D-dimer, and Day9 with LPIA Ace D-D dimer II. The CV was 18.9% in the assay of Iatrosera® TH Level I with the LPIA FDP-P reagent.



Parameter	Reagent	Sample	MEAN	SD	CV
FDP	Latex Test BL-2 P-FDP	FDP CONTROL NEO-L	7.42	0.54	7.2%
		FDP CONTROL NEO-H	25.79	1.34	5.2%
	Nanopia® P-FDP	FDP Control LOW	10.491	0.412	3.9%
		FDP Control HIGH	32.237	1.732	5.4%
	LPIA FDP-P	Iatrosera® TH level I	1.895	0.358	18.9%
		Iatrosera® TH level II	13.295	0.318	2.4%
D-dimer	LIAS AUTO D-Dimer NEO	D-Dimer CONTROL NEO-L	2.04	0.05	2.5%
		D-Dimer CONTROL NEO-H	11.76	0.31	2.6%
	Nanopia® D-dimer	FDP Control LOW	3.658	0.130	3.6%
		FDP Control HIGH	10.575	0.194	1.8%
	LPIA Ace D-D dimer II	Iatrosera® TH level I	1.250	0.112	8.9%
		Iatrosera® TH level II	15.481	0.787	5.1%

Unit (μg/mL)

Fig. 1 *Onboard reagent stability*

3. Dilution linearity

The results are shown in ***Fig. 2A-1*** and ***Fig. 2A-2***. Linearity was confirmed up to about 100 µg/mL with Latex Test BL-2 P-FDP and up to about 150 µg/mL with LIAS AUTO D-Dimer NEO. Dilution linearity was not seen with Nanopia® P-FDP and there was linearity with Nanopia® D-dimer up to about 110 µg/mL, although this slightly varied with the samples.
LPIA FDP-P showed linearity up to about 80 µg/mL, although this varied with the samples. LPIA Ace D-D dimer II showed linearity up to about 30 µg/mL.
To find out the reason for lack of dilution linearity with Nanopia® P-FDP, we checked the linearity in terms of change in optical density (dOD), which confirmed linearity (***Fig. 2B***).

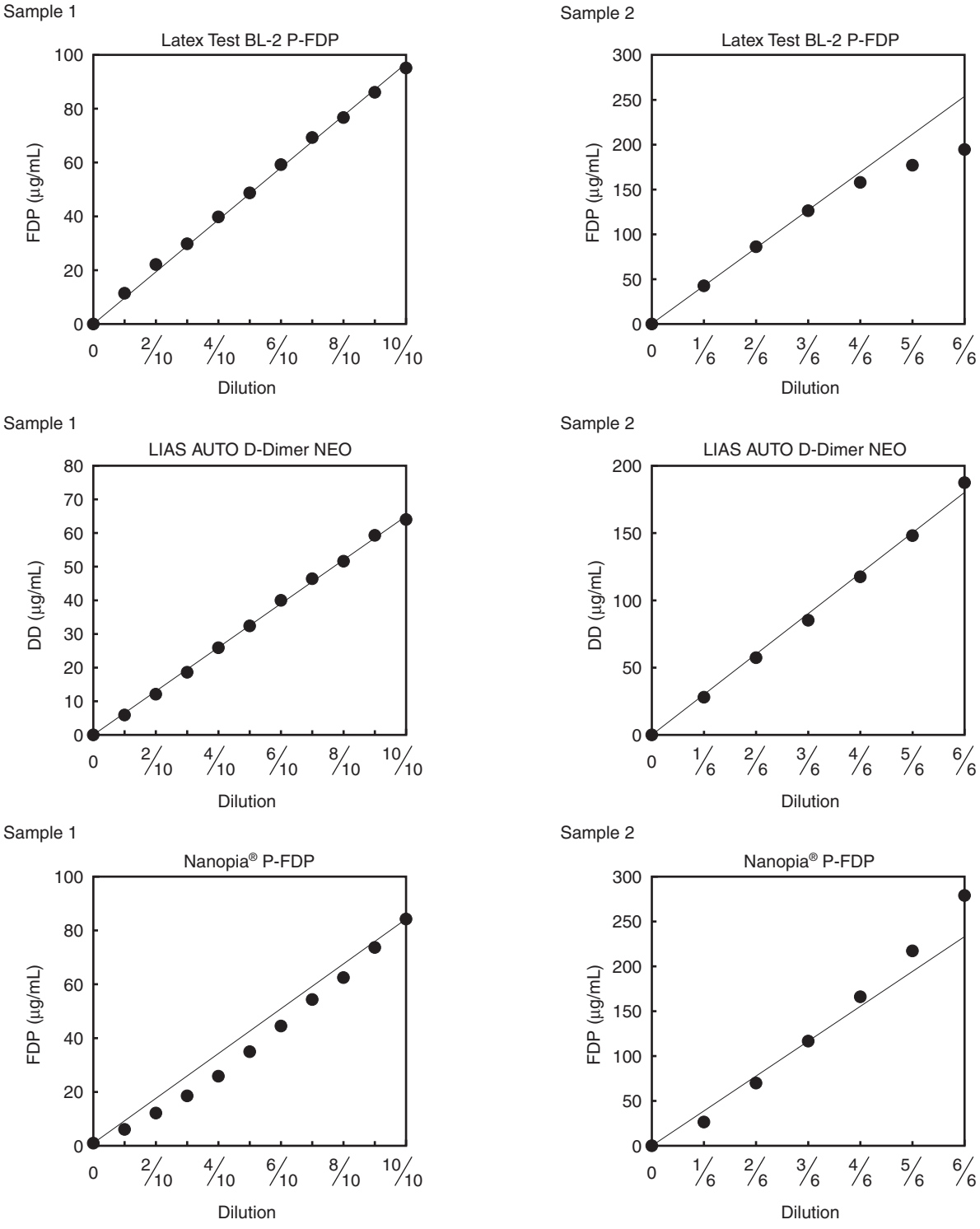


Fig. 2A-1 *Dilution linearity*

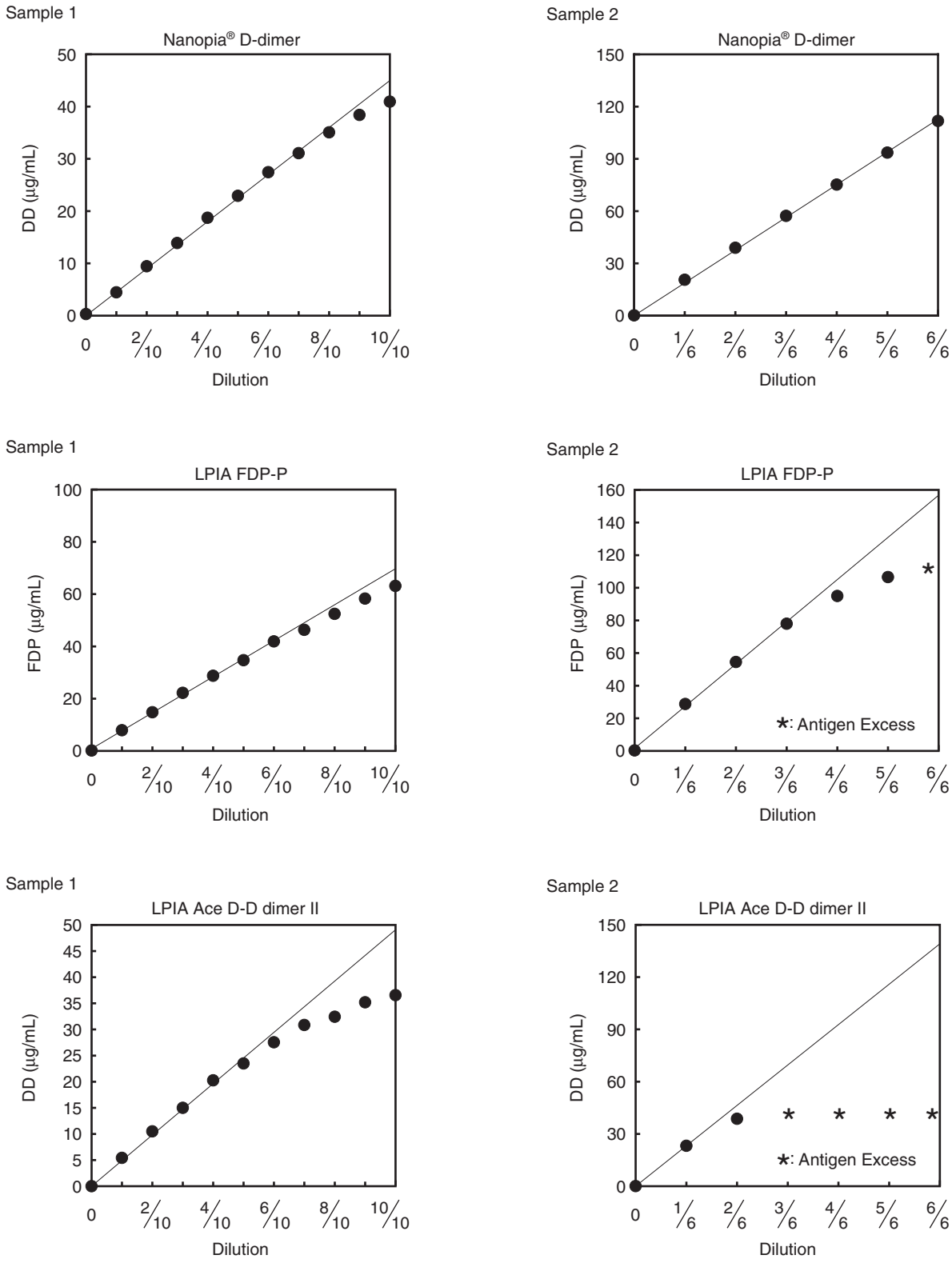


Fig. 2A-2 *Dilution linearity*

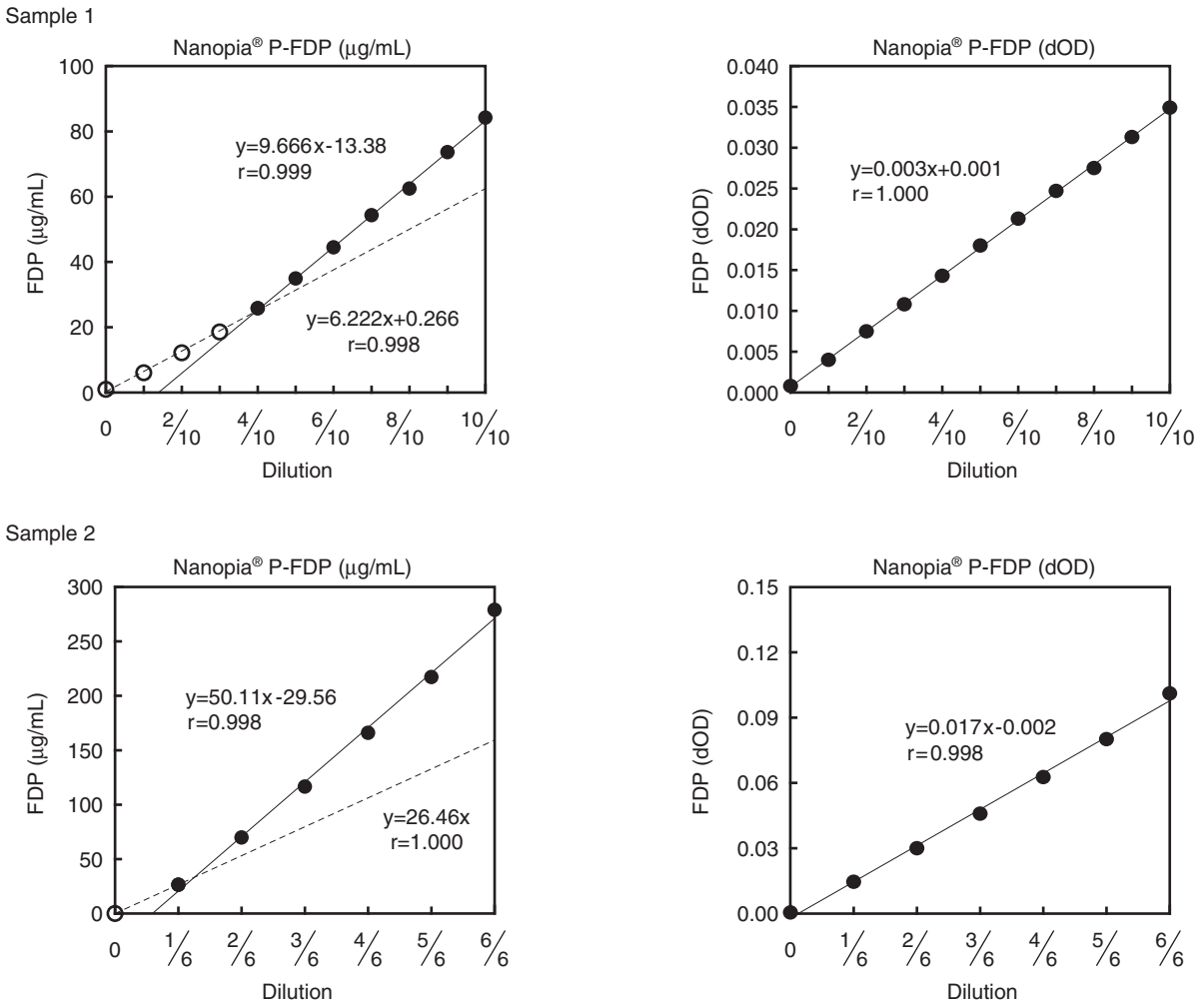


Fig. 2B *Verification of dilution linearity with Nanopia® P-FDP*

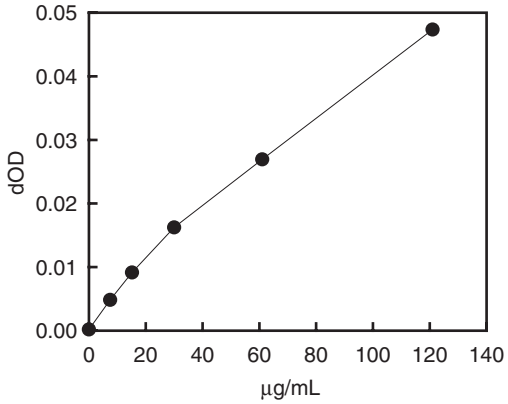


Fig. 2C *Calibration curve for Nanopia® P-FDP*

4. Assessment of hook effect and dilution linearity in automatic dilution (1/8) measurements with the CS-5100

Fig. 3 shows the results. In the normal measurement mode, no hook effect was seen in the specified range of measurements with any of the reagents. There was linearity in measurements made by automatic 1/8 dilution with the 5 reagents other than Nanopia® P-FDP. No linearity was seen with Nanopia® P-FDP here also, as in the dilution linearity measurements.

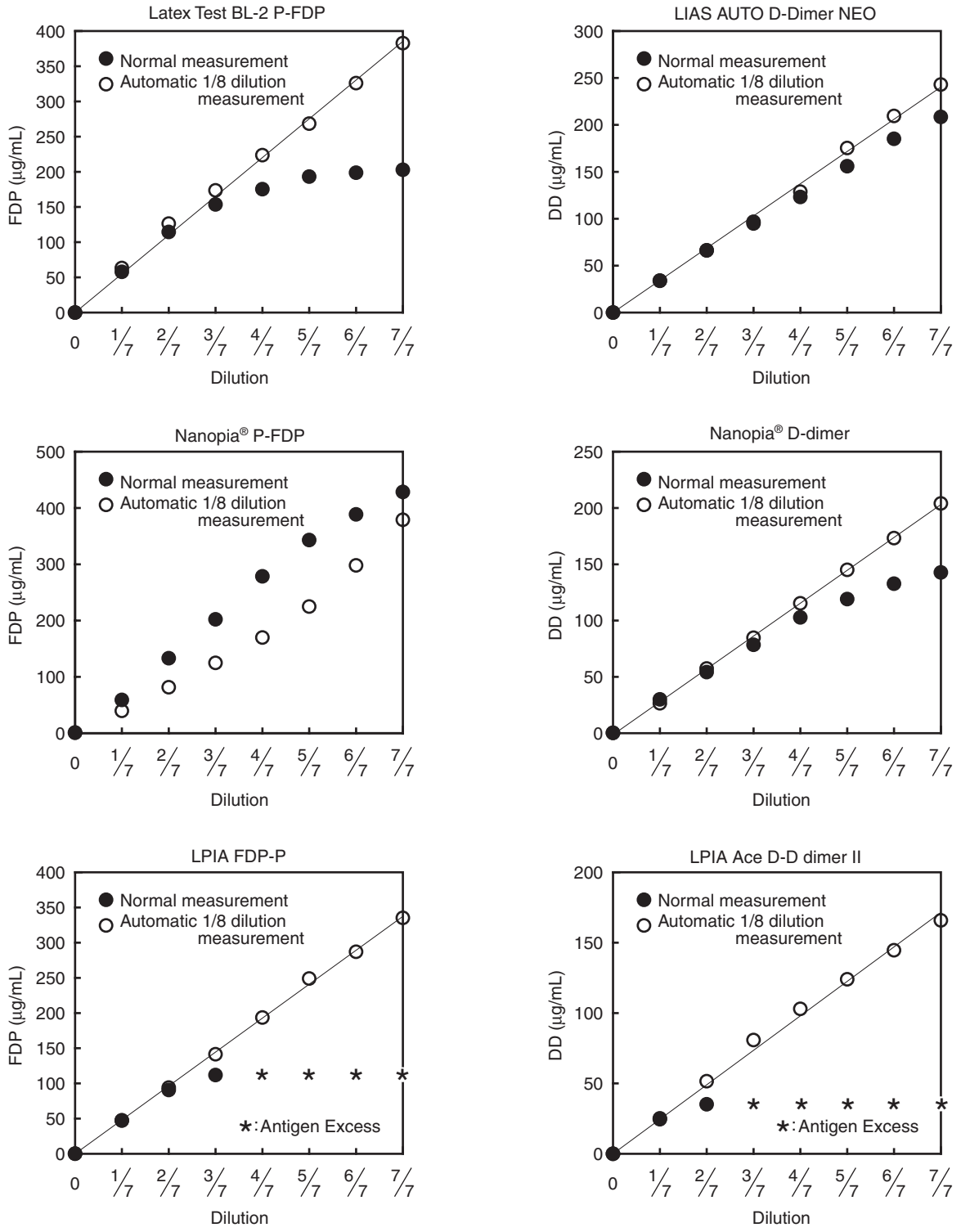


Fig. 3 *Assessment of hook effect and dilution linearity in automatic dilution (1/8) measurements with the CS-5100*

5. Correlation

Correlation with the results obtained using the conventional method is shown in ***Fig. 4***. The correlation was good for FDP, with the correlation coefficient r in the range 0.981 - 0.997 in all the cases. The regression equations were y=1.165x - 1.168, y=0.744x+2.290 and y=0.853x - 0.383 respectively for Latex Test BL-2 P-FDP, LPIA FDP-P and Nanopia® P-FDP. The correlation was good for D-dimer also, with *r* in the range 0.977 - 0.998 in all the cases. The regression equations were y=1.299x - 0.590, y=1.071x - 0.354 and y=0.997x - 0.248 respectively for LIAS AUTO D-Dimer NEO, LPIA Ace D-D dimer II and Nanopia® D-dimer.

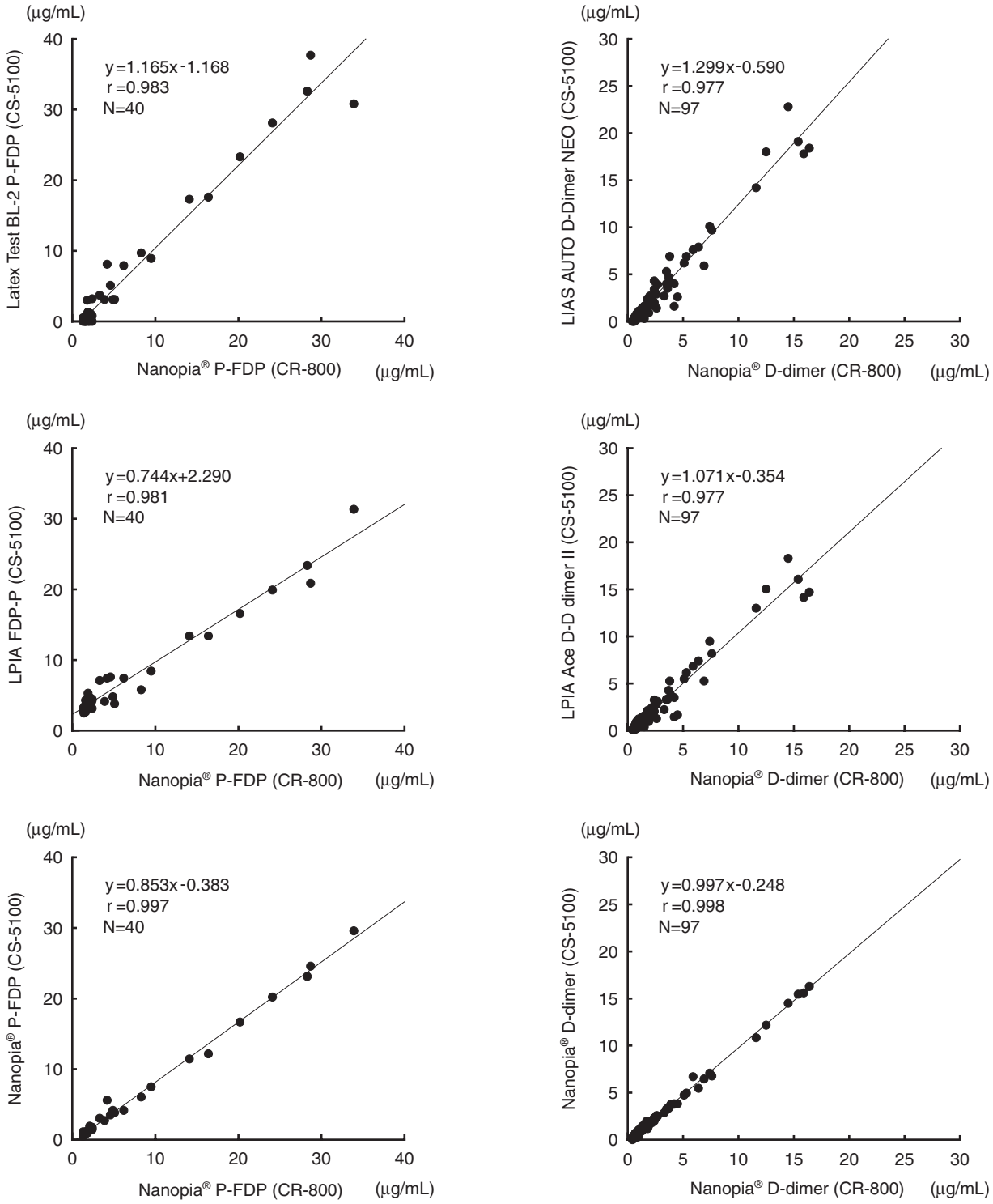


Fig. 4 *Correlation*

DISCUSSION

Latex Test BL-2 P-FDP and LIAS AUTO D-Dimer NEO showed good performance in within-run reproducibility and onboard reagent stability. They gave dilution linearity up to 100 µg/mL for FDP and 150 µg/mL for D-dimer, which satisfied the 80 µg/mL and 100 µg/mL upper limits specified respectively for FDP and D-dimer in the package inserts. No hook effect was seen with both these reagents within the specified ranges of measurements and there was linearity of values measured with the CS-5100 using automatic 1/8 dilution. More or less satisfactory correlation with the conventional method was observed for FDP, with a regression line slope of 1.165. The slope was slightly larger at 1.299 for D-dimer. This deviation seems to arise from the reactivity of the reagents, especially in the high molecular weight range of D-dimer. There are some reports that claim that the D-dimer in DIC patients is mainly of the high molecular weight fraction [1] and that they almost never have DD/E, the smallest D-dimer unit, in their blood [2]. These results seem to suggest that reagents that can sensitively measure high molecular weight D-dimers are the ones that can gauge the patient's condition better. LIAS AUTO D-Dimer NEO evaluated in the present study is a reagent that is highly reactive with high molecular weight D-dimers [2] and 72% of the D-dimer present in D-Dimer CALIBRATOR NEO (Sysmex Corporation) is of the high molecular weight fraction, which is about the same proportion as in DIC patients [1]. Therefore, the present findings suggest that LIAS AUTO D-Dimer NEO which has high reactivity with high molecular weight D-dimers would be useful for early diagnosis of thrombosis such as in DIC and for gauging the clinical condition of the patients.

Nanopia® P-FDP and Nanopia® D-dimer, which were evaluated as reference reagents, had onboard stability for 5 and 12 days respectively. The within-run reproducibility was satisfactory for FDP, but the CV was 7.84%, suggesting large data scatter, in the low D-dimer concentration range (about 2.8 µg/mL) tested. The normal reference level of D-dimer is 1.0 µg/mL and as the diagnosis of thrombosis requires accurate measurements in the low concentration range, the use of Nanopia® D-dimer in routine testing with the CS-5100 is likely to have some issues. Dilution linearity was seen for D-dimer up to about 110 µg/mL. However, FDP measurements did not show dilution linearity, the plot showing an upward bend. The calibration curve of Nanopia® P-FDP (***Fig. 2C***) had a smaller slope in the range above 30 µg/mL or so than in the range below this. In spite of this, there was dilution linearity when examined in terms of change in optical density (dOD) of the sample. This suggests a possible mismatch between the reactivity of the sample and that of the calibrator. In the dilution linearity expressed in the terms of sample concentration (µg/mL) also the slope of the line changed with about 30 µg/mL as the boundary. A similar phenomenon was seen in the automatic 1/8 dilution measurements with the CS-5100. This calls for special attention when using Nanopia® P-FDP for routine testing.

LPIA FDP-P and LPIA Ace D-D dimer II respectively had CV 13.79% and 8.38%, suggesting large variation, in the within-run reproducibility test for FDP and D-dimer using the exclusive control Iatrosera® TH level I. In the onboard reagent stability test using Iatrosera® TH level I also LPIA FDP-P gave highly varied results. The large data variation seen here was probably because Iatrosera® TH level I is a control with concentration below the lower limit of the measurement range of LPIA FDP-P, which is 2.5 µg/mL. As for D-dimer however, the concentration in the control was within the assay range of LPIA Ace D-D dimer II and the high CV of 8.38% could become an issue in using it for routine testing. Dilution linearity was confirmed up to about 80 µg/mL for FDP and 30 µg/mL for D-dimer. Moreover, as the "Antigen excess" error message appeared with high concentration samples, there seems to be no problem. In the study of correlation of FDP and D-dimer values measured using these reagents with those measured by the conventional method, the slope of the regression line was high at 0.744 for FDP and good at 1.071 for D-dimer. This deviation in the slope in the case of FDP appears to have arisen from differences in the reactivity of the FDP reagents used in the two methods.

CONCLUSION

We evaluated plasma FDP and D-dimer reagents from three companies using the CS-5100 analyzer. Latex Test BL-2 P-FDP and LIAS AUTO D-Dimer NEO gave satisfactory results and were assessed to have performance levels sufficient for routine testing.

References

1) *Abe Y et al. Reaction characteristics of a latex D-dimer reagent and analysis of D-dimer fractions in specimens from DIC patients. Journal of the Japanese Society for Laboratory Hematology. 2006; 7 (2): 294-302. (Japanese).*

2) *Kawasumi I et al. Antibody characteristics of LIAS AUTO D-Dimer NEO. Journal of Analytical Bio-Science. 2006; 29 (4): 315-320. (Japanese).*