

The Analytical Performance Evaluation of Freelite™ Human Kappa Free and Human Lambda Free on the SPAPLUS™ Immunoturbidimetric Analyzer

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Background: SPAPLUS™ is a turbidimetric immunoassay analyzer for detection of excess free light chain (FLC) antigens in serum. Here, we evaluated the analytical performance of Freelite™ Human Kappa Free and Lambda Free on a SPAPLUS™ instrument. **Methods:** We evaluated the precision, linearity, sample carryover, and drift of the SPAPLUS™ instrument and compared it with Hitachi 7600 and BN™ II instruments. We evaluated the detection of antigen excess for 12 specimens from patients with monoclonal gammopathy. **Results:** The coefficients of variations of κFLC and λFLC were below 5.0%. Linearity was shown in the range of 9.68–152.25 mg/l for κFLC and 4.96–171.09 mg/l for λFLC,

and no drift was observed. The κFLC sample carryover was statistically significant, but much smaller than the optimum allowable bias. Agreement rates with the two comparative methods were 87.1, 87.1, and 97.1% or higher for κFLC, λFLC, and the κ/λ ratio, respectively. Antigen excess signals were observed for all 12 antigen excess specimens. **Conclusions:** The Freelite™ on the SPAPLUS™ exhibited appropriate precision, linearity, and relative comparability to the reagents on the other instruments. It was good at detecting specimens that had previously demonstrated the hook effect due to antigen excess. J. Clin. Lab. Anal. 28:229–236, 2014. © 2014 Wiley Periodicals, Inc.

Key words: analytical performance; evaluation; free light chain; immunoassay; turbidimetry

INTRODUCTION

In conditions characterized by plasma cell dyscrasias such as multiple myeloma and amyloidosis, the serum concentration of the light chain increases because the excess monoclonal light chains generated by the abnormal proliferation of plasma cells cannot combine with heavy chains and, therefore, exist as free light chains (FLCs) in the blood. Quantifying serum FLC is useful in the diagnosis of monoclonal gammopathy and monitoring the therapeutic effects of treatment (1–3). The International Myeloma Working Group recommended the addition of a serum FLC test to serum protein electrophoresis and serum immunofixation electrophoresis (1, 4, 5). A recent study that examined around 2,500 patients with multiple myeloma, who were undergoing treatment, re-

vealed that the prevalence of monoclonal gammopathy of undetermined significance, which can develop into multiple myeloma, has reached 3.3% in Korea (6). Because of this, since 2005, more than 20 clinical laboratories in Korea have performed the serum FLC test in patients with monoclonal gammopathy or kidney disease by means of nephelometric or turbidimetric

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TABLE 1. Precision of the Freelite™ FLC Assay on the SPAPLUS™ Using Two Concentration Levels of Control Material (n = 20)

Analyte (mg/l)	Test result				Claim of manufacturer				Allowable imprecision ^a			
	Within-run imprecision		Total imprecision		Within-run imprecision		Total imprecision		SD	%CV		
	SD	95% CI	%CV	95% CI	SD	%CV	SD	%CV				
κFLC	0.51	0.358-0.898	3.40	0.505-1.275	0.72	4.80	0.319	2.11	1.584	10.49	0.50	3.30
	0.99	0.694-1.742	3.40	0.922-1.850	1.23	4.20	0.483	1.64	2.764	9.40	0.97	
λFLC	0.40	0.279-0.701	1.30	0.866-2.823	1.33	4.40	0.754	2.51	1.495	4.98	1.58	5.25
	1.13	0.791-1.988	1.90	1.872-6.945	2.96	4.90	1.356	2.26	2.941	4.89	3.16	

^aMinimum specifications for imprecision derived from biologic variation of κFLC and λFLC.

immunoassays (7–9). Generally, serum FLC can be quantitatively measured by latex particle immunoassays using monospecific polyclonal anti-FLC antibodies that uniquely detect unbound, free-form light chains (10). Immunoassay analyzers are usually designed to enter the measurement step after initial standard dilution of specimens with a specific dilution fold because the range of FLC concentrations encountered in the clinical laboratory is broad. However, nonlinearity or the hook effect can be observed due to antigen excess when monoclonal FLC levels are very high (4, 9, 11–19).

Here, we evaluated the analytical performance of Freelite™ Human Kappa Free and Freelite™ Human Lambda Free (The Binding Site Ltd., Birmingham, UK) on a SPAPLUS™ instrument (The Binding Site Ltd.), which was developed as a dedicated immunoturbidimetric analyzer in 2007 and has been introduced in Korea recently. The precision, linearity, sample carryover, and drift were evaluated in the current study. Method comparisons were performed by additionally testing the Freelite™ Human Kappa Free and Freelite™ Human Lambda Free on the Hitachi 7600 Modular P (Hitachi High-Tech Co., Tokyo, Japan) and Siemens BN™ II (Siemens Healthcare Diagnostics Inc., Marburg, Germany) instruments. The rate of detection of FLC antigen excess was also evaluated using specimens known to exhibit the hook effect.

MATERIALS AND METHODS

Analytical Systems

Kappa FLC (κFLC) and lambda FLC (λFLC) in the serum were measured using Freelite™ Human Kappa Free and Freelite™ Human Lambda Free on the SPAPLUS™ analyzer (the test method). Method comparison was performed by testing the same reagents, that is, Freelite™ Human Kappa Free and Freelite™ Human Lambda Free on different analyzers, on the Hitachi 7600 P-module (for immunoturbidimetry) and BN™ II (for immunonephelometry). The initial fold value for the standard dilutions for each of analyzers was set according to the manufacturer's instructions at 1:10 for both κFLC and λFLC for SPAPLUS™ and at 1:100 for those of BN™ II. In the case of the Hitachi 7600 P-module, the ratios were 1:5 for κFLC and 1:8 for λFLC.

Analytical Performance Evaluation

Precision

κFLC and λFLC were measured in duplicate in each run, and two runs per day for 5 days were run for two concentrations of control materials included in the Freelite™ reagent kits. The calculated total coefficients of variations (CVs) were compared with manufacturer's claims about

TABLE 2. Linearity of the Freelite™ FLC Assay on the SPAPLUS™ Using Multiple Dilutions of Patient Serum Pools

Analyte (mg/l)	Measuring interval claimed by manufacturer		Allowable nonlinearity		Tested range		Linear fit		
	Lower limit	Upper limit	Lower goal for levels 1 and 2	Upper goal for levels 3–5			Slope	y Intercept	SE of fit
κFLC	4.0	180.0	<9.7 mg/l 3.60 mg/l	>9.7 mg/l 7.95%	9.68	152.25	1.020	1.000	3.512
λFLC	4.5	165.0	<5 mg/l 5.30 mg/l	>5 mg/l 11.4%	4.96	171.09	0.996	–5.120	5.833

precision in the reagent package insert sheets and the minimum specifications for imprecision derived from biologic variations (3.3% for κFLC and 5.25% for λFLC, calculated on the basis of desirable specifications reported by Hansen et al. (20)). The manufacturer’s claim for three concentration levels was presented, and they differed from the averages yielded by this study. Thus, using the linear regression equation derived from the means and SDs among the three concentrations specified on the package insert sheet, we calculated the manufacturer’s claimed imprecision corresponding to the mean obtained from this study.

Linearity

Available specimens from patients that had concentrations as close as possible to the upper and lower limits of the measuring interval claimed by the manufacturer were selected. Specimens having the lowest and highest concentrations were mixed at ratios of 4:0, 3:1, 2:2, 1:3, and 0:4 into five concentration levels and were measured in quadruplicate. The minimum specifications for bias derived from biologic variations of 7.95% for κFLC and 11.40% for λFLC, which were calculated as described above, were used as allowable nonlinearities in data analysis. The non-linearity limit in the absolute concentration unit for the specimen with the second lowest concentration among the five concentration levels examined was also applied to that with lowest concentration.

Sample carryover and drift

Clinical and Laboratory Standards Institute (CLSI) EP10 guidelines were referred to in the evaluation of sam-

ple carryover and drift (21). With specimens from patients having concentrations as close as possible to the upper and low limits of the measuring interval claimed by the manufacturer, low and high concentration pools were prepared, and a middle concentration pool was made by mixing the two pools in equal volumes. Aliquots of the pools for these three concentrations were measured in the sequence specified in the CLSI document for five days. We evaluated the existence of carryover and drift through statistical analysis to determine the significance of the regression coefficients.

Method comparison

We analyzed the 80 specimens accepted for κFLC and λFLC tests by the Department of Laboratory Medicine at Seoul St. Mary’s Hospital (Seoul, Korea) over a period of 6 months, beginning in May 2011. All the specimens were measured twice by the test method, SPAPLUS™. The upper limits of the reference intervals presented by the manufacturers, that is, 19.40 mg/l for κFLC and 26.30 mg/l for λFLC, were considered to represent the medical decision level (MDL). Estimated differences with 95% confidence intervals (CIs) were derived from a regression analysis of the test and comparative methods and compared with the allowable differences for each analyte. The minimum specifications for total errors derived from biologic variations, that is, 13.35% for κFLC and 20.10% for λFLC, which were calculated from the desirable specification (20), were used as allowable differences. In addition, we calculated the agreement rates when subject specimens were classified on the basis of the references intervals of κFLC, λFLC, and the κ/λ ratio (3.30–19.40, 5.71–26.30, and 0.26–1.65 mg/dl, respectively).

TABLE 3. Carryover and Drift From Multifactor Regression Showing Components of Measurement Error in Preliminary Evaluation of the Freelite™ FLC Assay on the SPAPLUS™

Analyte (mg/l)	Components of measurement error	Mean of five runs	Sign statistic	P ^a
κFLC	Carryover	1.2%	5	0.0625
	Drift	–0.018	2	1.0000
λFLC	Carryover	0.0%	2	1.0000
	Drift	–0.230	1	0.3750

^aThe sign test was statistically significant at 6.25% significance level.

TABLE 4. Method Comparison of FLC Measured by the Freelite™ Assay on the SPAPLUS™ Against That on the Hitachi 7600 and BN™ II

Analyte (unit)	Comparative Method	N	Concentration range from analyzed samples	r	Slope	95% CI	y Intercept	95% CI	S _{y.x}	MDL	Estimated difference	Allowable total error ^a		
												(mg/l)	%	
κFLC (mg/l)	Hitachi 7600	79	4.78–1,703.50	0.996	0.890	0.875–0.902	-3.420	-6.408 to -0.425	18.050	19.40	-5.59	-8.505 to -2.667	2.59	13.35
	BN II	80	3.43–1,680.00	0.990	0.990	0.968–1.012	-3.160	-8.036–1.719	29.680		-3.35	-8.108–1.416		
λFLC (mg/l)	Hitachi 7600	78	4.27–2,685.50	0.996	1.030	1.014–1.042	-2.860	-8.962–3.237	36.640	26.30	-2.14	-8.129–3.860	5.29	20.10
	BN II	77	6.94–2,935.00	0.999	0.930	0.925–0.940	-2.120	-5.631–1.401	21.110		-3.90	-7.358 to -0.432		
κ/λ ratio	Hitachi 7600	70	0.02–478.64	0.997	0.697	0.688–0.705	0.961	0.4754–1.447	2.870	0.26	0.88	0.397–1.368		
	BN II	70	0.02–214.32	1.000	1.551	1.547–1.556	-0.410	-0.5177 to -0.307	0.618	1.65	0.46	-0.023–0.945		
										0.26	-0.27	-0.374 to -0.164		
										1.65	0.50	0.393–0.602		

^aMinimum specifications for total error derived from biologic variation.

Antigen excess detection capabilities and comparison of the measured values

Eleven specimens from two patients with κFLC monoclonal gammopathy and one specimen from a patient with λFLC monoclonal gammopathy were used. All specimens, following analyses with a Hitachi 7600, had been found to have hook effects due to antigen excess and had been stored at -70°C from March 2009 to May 2011. Before analysis, specimens were thawed at room temperature and then measured once more with a Hitachi 7600 and twice with SPAPLUS™. Spearman's correlation analysis and Passing-Bablok regression were performed for κFLC estimation in specimens from patients with a monoclonal gammopathy.

Statistical data analysis

Microsoft Excel 2007 (Microsoft, Redmond, WA), StatisPro (CLSI, Wayne, PA), and MedCalc statistical software version 12.2.1.0 (Mariakerke, Belgium) were used for data processing and analysis. This study was approved by the Institutional Review Board (IRB) of the Catholic Medical Center at The Catholic University of Korea (XC11DIM10006H).

RESULTS

Precision

The total CVs for κFLC values were 4.20–4.80%, while those of λFLC were 4.40–4.90%. Although the analyses did not meet the minimum specification for imprecision of 3.3% for κFLC, that for λFLC (5.25%) was met. Moreover, the imprecision was approximately equal to or less than the imprecision claimed by the manufacturer (Table 1).

Linearity

Linearity was observed in the concentration ranges of 9.68–152.25 mg/l for κFLC and 4.96–171.09 mg/l for λFLC (Table 2).

Sample Carryover and Drift

In the analysis of the κFLC values, there was no significant drift among the three pools (concentrations of 3.99 ± 0.33 , 96.62 ± 2.12 , and 162.05 ± 3.20 mg/l). However, a statistically significant sample carryover of 1.2% was observed among these samples. In λFLC analysis, there was no significant sample carryover or drift in specimens with concentrations of 11.89 ± 0.25 , 69.28 ± 1.66 , and 134.65 ± 3.27 mg/l (Table 3).

TABLE 5. Comparison of Groups Classified by the Reference Intervals of κFLC, λFLC, and the κ/λ FLC Ratio Between SPAPLUS™ and the Other Two Comparative Methods

Comparative method	SPAPLUS™			Agreement rate		
Hitachi 7600	κFLC	<3.30	3.30–19.40	>19.40	97.1%	
	<3.30	0.0% (0/70)	0.0% (0/70)	0.0% (0/70)		
	3.30–19.40	0.0% (0/70)	68.6% (48/70)	0.0% (0/70)		
	>19.40	0.0% (0/70)	2.9% (2/70)	28.6% (20/70)		
	λFLC	<5.71	5.71–26.30	>26.30		92.9%
	<5.71	0.0% (0/70)	0.0% (0/70)	0.0% (0/70)		
	5.71–26.30	2.9% (2/70)	70.0% (49/70)	0.0% (0/70)		
	>26.30	0.0% (0/70)	4.3% (3/70)	22.9% (16/70)		
	κ/λ FLC ratio	<0.26	0.26–1.65	>1.65	97.1%	
	<0.26	14.3% (10/70)	1.4% (1/70)	0.0% (0/70)		
	0.26–1.65	0.0% (0/70)	68.6% (48/70)	1.4% (1/70)		
	>1.65	0.0% (0/70)	0.0% (0/70)	14.3% (10/70)		
BN™ II	κFLC	<3.30	3.30–19.40	>19.40		87.1%
	<3.30	0.0% (0/70)	0.0% (0/70)	0.0% (0/70)		
	3.30–19.40	0.0% (0/70)	58.6% (41/70)	0.0% (0/70)		
	>19.40	0.0% (0/70)	12.9% (9/70)	28.6% (20/70)		
	λFLC	<5.71	5.71–26.30	>26.30	87.1%	
	<5.71	0.0% (0/70)	0.0% (0/70)	0.0% (0/70)		
	5.71–26.30	2.9% (2/70)	64.3% (45/70)	0.0% (0/70)		
	>26.30	0.0% (0/70)	10.0% (7/70)	22.9% (16/70)		
	κ/λ FLC ratio	<0.26	0.26–1.65	>1.65		97.1%
	<0.26	14.3% (10/70)	1.4% (1/70)	0.0% (0/70)		
	0.26–1.65	0.0% (0/70)	68.6% (48/70)	1.4% (1/70)		
	>1.65	0.0% (0/70)	0.0% (0/70)	14.3% (10/70)		

Method Comparison

The mean estimated difference (95% CI) between κFLC measurements using SPAPLUS™ and BN™ II at the MDL of 19.40 mg/l was -3.35 mg/l (-8.108 to -1.416), which was not statistically significant considering the allowable difference of 2.59 mg/l. When comparing the SPAPLUS™ results to those from Hitachi 7600, a significant difference over the allowable limit was observed at the MDL of 19.40 mg/l, and the κFLC values obtained by SPAPLUS™ were lower than those obtained by Hitachi 7600 by 5.59 mg/l (2.667–8.505, Table 4). By classifying the specimens on the basis of the reference intervals of κFLC, we achieved a 97.1% agreement between Hitachi 7600 and SPAPLUS™, and a 87.1% agreement between BN™ II and the test method (Table 5). The estimated difference for λFLC measurement at the MDL of 26.30 mg/l, using either Hitachi 7600 or BN™ II as a comparative method, was not significantly different from the allowable difference of 5.29 mg/l (Table 4). Classifying the specimens by the reference intervals of λFLC, we achieved agreement rates of 92.9% between Hitachi 7600 and SPAPLUS™, and 87.1% between BN™ II and the test method (Table 5). The κ/λ ratio of SPAPLUS™ compared with Hitachi 7600 was statistically significantly

higher by 0.88 (0.3969–1.3681) at the MDL of 0.26 mg/l; however, there were no significant differences at the MDL of 1.65. The κ/λ ratio of the test method compared with BN™ II was significantly lower, by 0.27 (0.1640–0.3741), at the MDL of 0.26, but was significantly higher, by 0.50 (0.3933–0.6017), at the MDL of 1.65 (Table 4). In the classification of specimens according to the reference intervals, we achieved a 97.1% agreement between Hitachi 7600 and SPAPLUS™, and between BN™ II and SPAPLUS™ (Table 5).

Capability of Antigen Excess Detection and Comparison of the Measured Values

An antigen excess signal was flagged by SPAPLUS™ for all 11 specimens for κFLC and for a single specimen for λFLC; all of these samples were previously confirmed to contain high levels of the antigen. The pre- and postdilutions results from Hitachi 7600 and SPAPLUS™ are shown in Table 6. A significant correlation between the κFLC values measured by Hitachi 7600 (x) and SPAPLUS™ (y) was obtained, with a Spearman's coefficient of 0.922 (0.722–0.980; P = 0.0001), and the regression equation was $y = -513.0625 + 1.4392x$. The 95%

TABLE 6. κ FLC and λ FLC Measured by Hitachi 7600 and SPAPLUS™ From Specimens With the Hook Effect Caused by Antigen Excess

Patient ID	Sex/age	Analyte	Specimen ID	Hitachi 7600		SPAPLUS™	
				Predilution	Postdilution	Predilution	Postdilution
A	M/58	κ FLC	A1	26.7	838.0	21.4	892.0
			A2	29.8	874.0	18.2	744.8
			A3	14.4	1,094.0	14.5	1,333.0
			A4	23.0	1,167.0	19.2	1,124.0
B	F/66	κ FLC	B1	21.7	4,547.0	24.1	6,230.0
			B2	12.7	4,628.0	9.9	4,031.0
			B3	12.7	4,628.0	11.8	3,285.0
			B4	13.7	6,451.0	18.6	9,114.0
			B5	6.1	10,320.0	8.0	15,890.5
			B6	4.7	13,986.0	<3.8	10,951.0
			B7	4.7	13,986.0	5.5	10,541.0
C	F/61	λ FLC	C1	58.5	14,709.0	21.5	23,400.0

CI of the y intercept was $-1887.31-516.83$, and that of the slope was $0.746-1.723$ (Fig. 1).

DISCUSSION

To evaluate the analytical performance of Freelite™ Human Kappa Free and Freelite™ Human Lambda Free on SPAPLUS™, we analyzed the precision, linearity, sample carryover, and drift of the samples on the test instrument, and compared the results obtained with those from the Hitachi 7600 Modular P and Siemens BN™ II instruments. Additionally, we examined the capacity to detect antigen excess in specimens known to exhibit the hook effect. Although the number of measurements was relatively small ($n = 20$), measurement of both κ FLC and λ FLC by the Freelite™ assay on the SPAPLUS™ instrument met the manufacturer's claims. The observed imprecision of λ FLC met the minimum specification for the allowable imprecision, while that of κ FLC did not. The desirable specification for imprecision was not fulfilled by either κ FLC or λ FLC, as described in a previous report testing the Freelite™ FLC assay on the BN™ II instrument (20). Though κ FLC showed a statistically significant sample carryover of 1.2% in the concentration range of 3.99–162.05 mg/l, this difference was not thought to be significant when considering the optimum allowable bias of 2.65% for κ FLC. Although the estimated difference at the MDL of 19.40 mg/l between the κ FLC measured by the test method and Hitachi 7600 was significantly larger than the minimum specification for the allowable total error of 13.35%, the difference from BN™ II was not. For λ FLC, the estimated differences at the MDL of 26.30 mg/l between the test method and the two comparative methods were not significantly larger than the minimum specification for total error of 20.10%. Although the Freelite™ Human Kappa and Lambda

Free was commonly used on all three analyzers, a certain degree of difference among them was observed. In the comparison of quantitative results, SPAPLUS™, an immunoturbidimetry instrument, seemed to be more comparable with the immunonephelometry instrument, BN™ II, than with another immunoturbidimetry analyzer, Hitachi 7600 Modular P. When comparing the agreement rates in the classification according to the reference intervals, SPAPLUS™ agreed in 92.9% or more of specimens with the Hitachi 7600 analyzer and in 87.1% or more of specimens with the BN™ II analyzer. Here, agreement rates between the two immunoturbidimetry analyzers were higher than those between immunoturbidimetry and immunonephelometry instrument. The agreement rates in the classification of κ/λ ratios for SPAPLUS™ and the Hitachi 7600 or BN™ II analyzers were both 97.1%. The reason for the differences in measured values between analyzers using the same reagent may be due to the fact that the initial standard sample dilution ratios were different, that is, 1/5, 1/100, and 1/10 in Freelite™ Human Kappa Free, and 1/8, 1/100, and 1/10 in Freelite™ Human Lambda Free for Hitachi 7600, BN™ II, and SPAPLUS™, respectively, according to the package insert sheet. The dilution ratios for the subsequent steps were also different among these three analyzers. Previous studies have commented on the poor linearity of data from these analyzers after sample dilution, especially for the immunonephelometry analyzer (22–24). However, a thorough evaluation of the dilution effect of Freelite™ assays on SPAPLUS™, for example, comparisons among concentration from more than two dilutions, was not performed in the current study. SPAPLUS™ showed antigen excess signals in all 12 specimens already known to have antigen excess. The κ FLC showed a high correlation of 0.922 (Spearman's correlation coefficient) between the postdilution results from Hitachi 7600 and

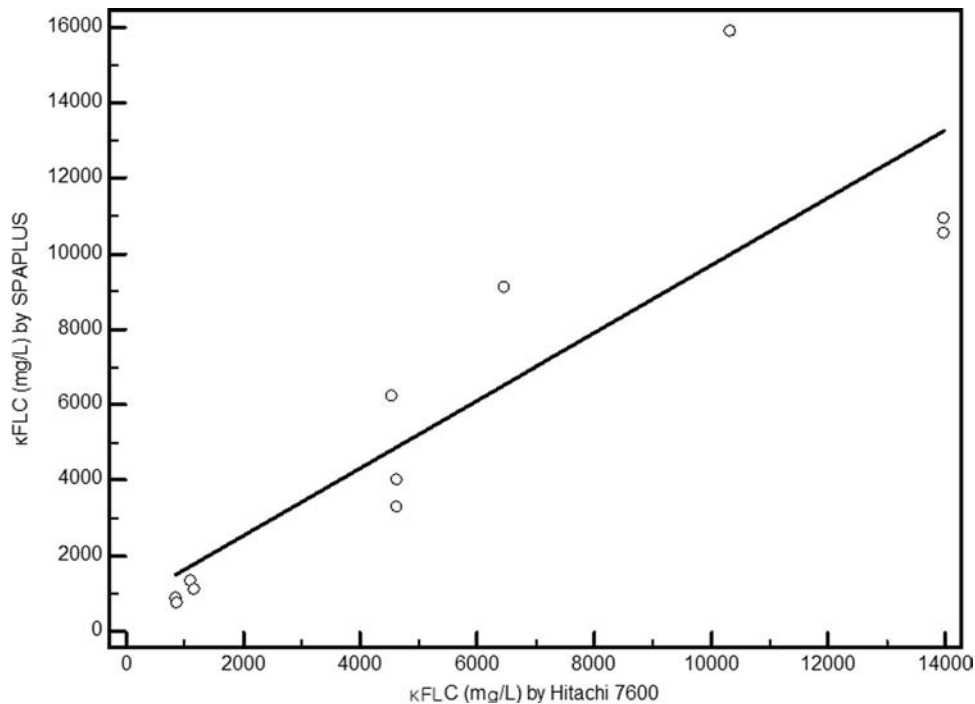


Fig. 1. κ FLC measured by Hitachi 7600 and SPAPLUS™ from specimens with known hook effects due to antigen excess.

SPAPLUS™ analyzers. Although numerous researchers have reported cases of the hook effect caused by antigen excess in serum FLC measurements (9, 12–16), the built-in algorithm in the SPAPLUS™ instrument seemed to detect the hook effect well. Moreover, this algorithm may reduce turnaround time and the chance of missing the need for additional dilution steps because the measuring intervals of SPAPLUS™ (4.0–180 mg/l for κ FLC and 4.5–165 mg/l for λ FLC) are as wide as those of BN™ II (5.9–190 mg/l for κ FLC and 5.0–160 mg/l for λ FLC) and wider than those of Hitachi 7600 (3.7–56.2 mg/l for κ FLC and 5.6–74.8 mg/l for λ FLC).

In conclusion, measurement using the Freelite™ Human Kappa and Lambda Free kit on a SPAPLUS™ analyzer seemed to have appropriate precision and linearity for clinical use and relative comparability with the same reagent on Hitachi 7600 and BN™ II analyzers, which are commonly used in the clinical setting. However, it is necessary to be cautious when determining κ FLC and λ FLC levels using different analyzers, even if the same reagents are used (i.e., the Freelite™ assay). The SPAPLUS™ instrument also showed excellent performance in detecting specimens exhibiting hook effects due to antigen excess.

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