Verification of Newly FDA-Approved Kappa and Lambda Free Light Chain Assays on a Previously Untested Platform

Penn Muluhngwi,[1†] Cierra N. Sharp,[1†] Nicole Pozzi,[2] Ronald J. Elin,[1] and Saeed A. Jortani[1*]

Background: κ and λ free light chains (FLCs) are monitored to aid in the diagnosis of plasma cell disorders. Our goal was to validate the Diazyme Human κ and λ assays on Beckman Coulter UniCel DxC 800 Synchron and compare to Freelite κ and λ assays on Roche Cobas Integra.
Methods: Linearity verification, within- and between-run precision, method comparison, and reference range (RR) verification were conducted using CLSI guidelines. Statistical analysis was performed using EP Evaluator®. Mean, SD, CV, and bias were determined.
Results: Diazyme κ FLC assay was linear within 0.00–191.00 mg/L. Diazyme λ FLC assay was linear within 0.00–205.30 mg/L. Diazyme κ FLC QC1 had a mean of 16.70 mg/L, CV of 7.0%. QC2 had a mean of 33.37 mg/L, CV of 2.6%. Diazyme λ FLC QC1 had a mean of 21.73 mg/L, CV of 2.3%. QC2 had a mean of 42.05 mg/L, CV of 1.5%. Bias of DxC-Diazyme FLCs compared to Integra-Freelite FLCs was −2.55 mg/L (κ FLC), and 4.54 mg/L (λ FLC). Qualitative comparison of κ FLC assays indicated 100% agreement for both normal and abnormal values. For λ FLC assay, agreement was 95% for normal values and 75% for abnormal values. For κ/λ ratio there was 50% agreement for normal values, and 100% for abnormal values. For RR verification, 1 sample was outside the Diazyme κ RR. For λ, all samples were within the manufacturer's RR.
Conclusions: Diazyme assays for FLCs have excellent precision and accuracy and are comparable to Freelite assays.

IMPACT STATEMENT

Patients with plasma cell disorders are diagnosed and monitored for disease response/progression using κ and λ and λ FLCs. Recently, Diazyme FLC assays were US Food and Drug Administration approved. However, these assays have never been validated for use on the Beckman Coulter DxC 800 on our automated line system. Thus, verification of the Diazyme assays on the DxC 800 provides an additional platform for FLC monitoring and reduces turnaround time. The work presented here indicates that the Diazyme FLC assays can used on the DxC 800 with excellent precision and accuracy and are comparable to Freelite assays on the Cobas Integra.

[1]Department of Pathology and Laboratory Medicine, University of Louisville, Louisville, KY; [2]University of Louisville Hospital, Louisville, KY
***Address correspondence to this author at:** Department of Pathology and Laboratory Medicine, University of Louisville, 511 S Floyd St., Louisville, KY 40202. E-mail saeed.jortani@louisville.edu.

Immunoglobulin heavy and light chains are produced in plasma cells and then assembled to form immunoglobulins. There are 2 types of light chains, κ and λ, which are commonly bound to the heavy chain of the immunoglobulin. However, in pathogenic states, there is often an excess production of these light chains in the unbound form, referred to as "free light chains" (FLCs).[3] Increased concentrations of either κ and/or λ FLCs are indicative of plasma cell disorders, namely, multiple myeloma (MM), Waldenstrom macroglobulinemia (WM), immunoglobulin light chain amyloidosis (AL amyloidosis), lymphocytic neoplasms, and systemic lupus erythematous (SLE) (*1*).

Monitoring FLC concentrations is important for assessing FLC-associated diseases. Typically, κ and λ FLCs are measured using nephelometric or turbidimetric methods (*1*). Both the Diazyme and Freelite κ and λ FLC assays use a latex-enhanced immunoturbidimetric technique. In these assays, either κ or λ FLC antigens in a serum sample will bind to the latex-coated particles labeled with either anti-λ or anti-κ FLC. As the antigen–antibody reactions proceed, immune complexes are formed (see product inserts for Diazyme and Freelite). The decrease in the intensity of the incident beam of transmitted light is then measured and is proportional to the amount of FLC in the sample. FLC concentration in the sample is then interpolated from a calibration curve of known concentrations.

The Diazyme κ and λ FLC assay were recently approved by the US Food and Drug Administration (FDA). Addition of the FLC method to our automated line in the laboratory was of interest owing to greater efficiency and better turnaround time. Therefore, we verified these new assays on the Beckman Coulter UniCel DxC 800 Synchron, in addition to comparing them to the Freelite assays performed on the Roche Cobas Integra 800 chemistry analyzer previously used in our laboratory.

METHODS

Analytical performance of the Diazyme Human κ and λ assays (Diazyme Laboratories) was evaluated on Beckman Coulter UniCel DxC 800 Synchron according to pertinent CLSI guidelines (EP 5-A3, 6-A, 9-A3, 12-A). After initial calibration, the calibrators (obtained from Diazyme Laboratories) were rerun for linearity studies. For within-run precision evaluation of FLC κ and λ assays, 20 measurements of QC levels 1 and 2 (obtained from Diazyme Laboratories) were obtained. For between-run precision evaluation of FLC κ and λ assays, QC Levels 1 and 2 (obtained from Diazyme Laboratories) were ran for a minimum of 20 days. Mean, SD, and CV were determined using EP Evaluator® Release 12.1.0.18 (Data Innovations LLC). Quality goals for performance characteristics were set based on evaluation of previous reports on FLC κ and λ assays (*2–4*). For the Diazyme FLC κ assay, a desirable total allowable error (TEa) was 10% and systematic error budget (SEa; bias) was 2.5% (25% of TEa). For the Diazyme FLC λ assay, TEa was 15% and SEa was 3.75% (25% of TEa).

Method comparison included quantitative evaluation by Deming regression between the Beckman Coulter DxC-Diazyme FLC platform and the Roche Cobas Integra-Freelite platform. Bias and percent bias plots were generated using EP Evaluator software. We were limited by the number of samples we could include in our method comparison because Freelite was no longer making reagents for our Roche Cobas Integra, and thus we

[†]P. Muluhngwi and C.N. Sharp contributed equally to this work.

DOI: 10.1373/jalm.2019.029215

[3] **Nonstandard abbreviations:** FLC, free light chain; MM, multiple myeloma; WM, Waldenstrom macroglobulinemia; AL amyloidosis, immunoglobulin light chain amyloidosis; SLE, systemic lupus erythematous; FDA, US Food and Drug Administration; TEa, allowable total error; SEa, allowable systematic error; RR, reference range; LOQ, limit of quantification; AMR, analytical measurement range.

could only use the reagents we had on hand. Qualitative evaluation (concordance analysis) of FLC κ, λ, and κ/λ ratio between both platforms was also performed. Samples were grouped as "Normal" if their values were within the manufacturer-reported reference range (RR) or "Abnormal" if their values were outside the specified RR.

For RR studies, samples were obtained from 20 ostensibly healthy individuals working in the laboratory and used for verification of manufacturer's suggested RR. These workers did not receive any formal medical evaluation of their health status before evaluation.

Statistical analysis was performed using EP Evaluator Release 12.1.0.18 (Data Innovations LLC).

RESULTS

Linearity

The Diazyme κ FLC assay was linear with an error of 1.3%, using a measured range of 0.00–191.00 mg/L (Fig. 1A and 1B). The Diazyme λ FLC assay was found to be linear using a measured range of 0.00–205.30 mg/L, with an error of 1.8% (Fig. 1C and 1D).

Within-run precision

The Control Levels 1 and 2 for Diazyme κ and λ FLC assays had observed means within manufacturer suggested 2SD (Table 1). For the Diazyme κ assay, the manufacturer's expected Control Level 1 was 13.1–19.7 mg/L (±2SD) with a mean of 16.4 mg/L and for Control Level 2, 26.5–39.7 mg/L with a mean of 33.1 mg/L. For the Diazyme λ FLC assay, the expected range for Control Level 1 was reported to be 16.6–25.0 mg/L (±2SD) with a mean of 20.8 mg/L. For Control Level 2, the expected range for this control level was reported to be 32.1–48.1 mg/L (±2SD) with a mean of 40.1 mg/L. Overall, the Diazyme κ and λ FLC assays had good within-run precision based on the parameter of 2SD as established by manufacturer.

Between-run precision

Control Levels for Diazyme κ and λ FLC assays had observed means within manufacturer suggested 2SD and observed CV of ≤7.1% (Table 1).

Method comparison

Quantitative comparison of the Beckman Coulter DxC-Diazyme FLC platform and the Roche Cobas Integra-Freelite platform indicated a −2.55 mg/L bias (95% CI, −6.91–1.81 mg/L) for κ FLC and a 4.54 mg/L bias (95% CI, 0.10–8.99 mg/L) for λ FLC (Fig. 2; Fig. 2 in the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol4/issue3).

Concordance analysis of the κ FLC assay (n = 32) on both platforms showed 100% agreement for both normal and abnormal values (Fig. 3A). "Normal" represents samples that had values within Diazyme's reported RR when the DxC-Diazyme FLC platform was used for analysis or had normal values within Freelite's manufacturer reference when the Integra-Freelite platform was used. Similarly, "Abnormal" represents samples that had values out of manufacturer's defined RR.

For the λ FLC assay (n = 28), comparison between platforms showed 95% (19/20) agreement for normal values and 75% (6/8) agreement for abnormal values (Fig. 3B).

A comparison of the κ/λ FLC ratio (n = 28) showed 100% concordance for normal values, and 50% (9/18) concordance for abnormal values (Fig. 3C).

Reference range verification

Of the 20 samples that were used for verifying Diazyme's κ RR, 1 sample was outside the upper limit of the Diazyme RR (RR, 2.37–20.73 mg/L; Table 2), and this sample was discordant between both platforms. For the λ RR verification, all samples were within the manufacturer's RR and concordant between the 2 platforms. For the κ/λ ratio RR verification, 5 values obtained from samples (3

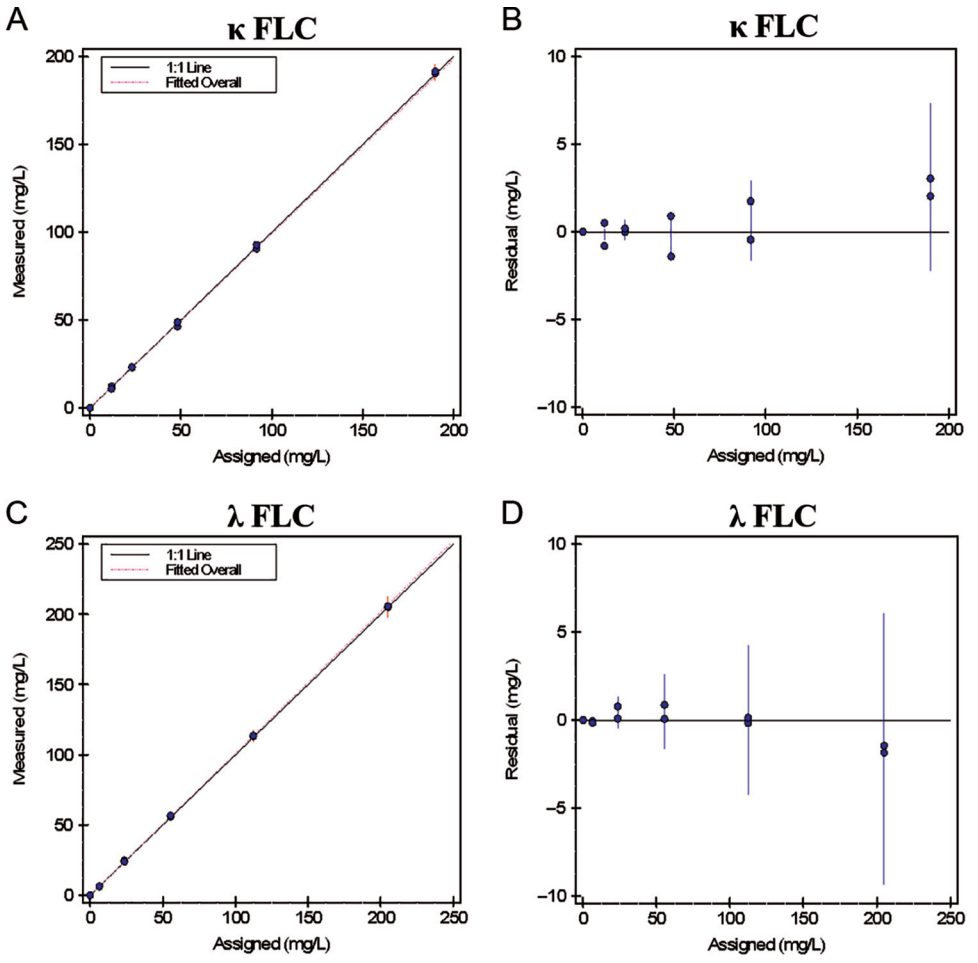

Fig. 1. Linear regression analysis of Diazyme kappa (A and B) and lambda (C and D) FLC calibrators performed on Beckman Coulter DxC 800.
Scatter plots (A and C) and residual plots (B and D) are indicated. A, slope = 0.992; intercept = 0.00; error = 1.3%. (B) slope, 1.010; intercept, 0.00; error, 1.8%.

Diazyme and 2 Freelite) were not within the manufacturer's suggested RRs and were discordant between the 2 platforms.

DISCUSSION

We assessed the accuracy and precision of Diazyme's κ and λ FLC assays on the Beckman Coulter DxC analyzer. In addition, we compared Beckman Coulter DxC-Diazyme assays to the Roche Cobas Integra-Freelite assays that have been retired in our laboratory (Table 1). For all linearity studies, the manufacturer used CLSI EP6-A guidelines. The reported analytical measurement range (AMR) was 4.5–150 mg/L for κ FLC, with a limit of quantification (LOQ) of 4.5 mg/L. We found κ FLC to be linear within a range of 0.00–191.00 mg/L. Thus, we can accept the manufacturer's AMR. For λ FLC, AMR was reported as 6.1–200 mg/L with a LOQ of 6.10 mg/L. We found λ FLC to

Table 1. Summary report of performance characteristics.

	λ	κ
Linearity	Measured range: 0.00–205.30 mg/L	Measured range: 0.00–191.00 mg/L
	Error: 1.8%	Error: 1.3%
Precision	*Within-run precision:*	*Within-run precision:*
	Observed mean (Control Level 1): 21.73 mg/L	Observed mean (Control Level 1): 16.70 mg/L
	Observed CV (Control Level 1): 2.3%	Observed CV (Control Level 1): 7.0%
	Observed 2 SD range (Control Level 1): 20.74–22.73 mg/L	Observed 2 SD range (Control Level 1): 16.15–17.25 mg/L
	Observed mean (Control Level 2): 42.05 mg/L	Observed mean (Control Level 2): 33.37 mg/L
	Observed CV (Control Level 2): 1.5%	Observed CV (Control Level 2): 2.6%
	Observed 2 SD range (Control Level 2): 40.83–43.28 mg/L	Observed 2 SD range (Control Level 2): 31.63–35.11 mg/L
	Between-run precision:	*Between-run precision:*
	Observed mean (Control Level 1): 22.22 mg/L	Observed mean (Control Level 1): 18.03 mg/L
	Observed CV (Control Level 1): 6.0%	Observed CV (Control Level 1): 12.0%
	Observed SD (Control Level 1): 1.34 mg/L	Observed SD (Control Level 1): 2.17 mg/L
	Observed mean (Control Level 2): 43.20 mg/L	Observed mean (Control Level 2): 35.33 mg/L
	Observed CV (Control Level 2): 7.1%	Observed CV (Control Level 2): 5.0%
	Observed SD (Control Level 2): 3.08 mg/L	Observed SD (Control Level 2): 1.76 mg/L
	Total imprecision:	*Total imprecision:*
	Total CV (Control Level 1): 6.43%	Total CV (Control Level 1): 13.89%
	Total SD (Control Level 1): 1.43 mg/L	Total SD (Control Level 1): 2.47 mg/L
	Total CV (Control Level 2): 7.26%	Total CV (Control Level 2): 5.64%
	Total SD (Control Level 2): 3.14 mg/L	Total SD (Control Level 2): 1.96 mg/L
Method Comparison	Slope (Deming): 1.573	Slope (Deming): 0.990
	Intercept: −5.85	Intercept: −2.18
	R: 0.75	*R*: 0.92
	Total bias: 4.54 mg/L (25.05%)	Total bias: −2.55 mg/L (−6.92%)

be linear within a range of 0.00–205.30 mg/L. Thus, we can accept the manufacturer's AMR.

For all precision studies, the manufacturer used the CLSI EP5-A protocol. For the Diazyme κ FLC assay, the manufacturer's established within-run CVs for Control Level 1 and Control Level 2 were 3.2% and 2.1%, respectively (see Diazyme Human κ FLC assay product insert). To validate the manufacturer's established precision claim, we performed a within-run study using 2 levels of control run 20 times each. We found Control Level 1's CV to be 7.0% and Control Level 2's CV was 2.6%. Our CV for Control Level 1 was higher than that established by the manufacturer. However, this is less than our preset 10% TEa. Furthermore, our 2 SD range is comparable to that established by the manufacturer. Using this same approach for the λ FLC assay, we found Control Level 1's CV to be 2.3% and Control Level 2's CV was 1.5%. Both CVs were comparable to manufacturer's reported precision for the λ assay (Control Level 1 CV, 3.9%; Control Level 2 CV, 1.3%; see Diazyme Human λ FLC assay product insert).

A quantitative comparison of the Beckman Coulter DxC-Diazyme platform to the Cobas Integra-Freelite platform indicated that there was a negative bias between the 2 κ assays (Fig. 2A), but using a qualitative approach to distinguish normal and abnormal values indicated 100% concordance (Fig. 3A). A large positive bias was observed

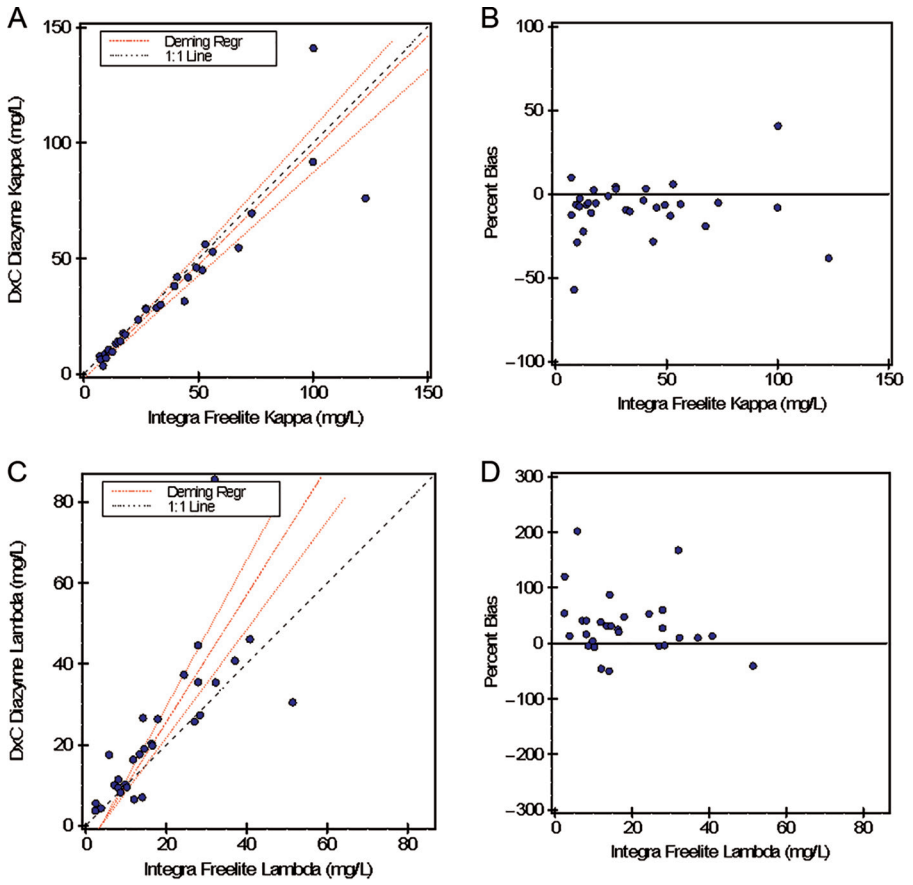

Fig. 2. Quantitative method comparison between DxC-Diazyme and Integra-Freelite platforms.
(A, B) FLC κ and (C, D) FLC λ. (A, C) scatter plot, (B, D) percent bias plot. (A), FLC κ Deming regression analysis, correlation coefficient R^2 = 0.9222, slope = 0.990, intercept = −2.18. (C), FLC λ Deming regression analysis, R^2 = 0.7458, slope = 1.573, intercept = −5.85.

between the 2 λ assays, and concordance for normal values was 95%, and 75% for abnormal values (Fig. 2C, Fig. 3B). The 2 discordant samples that were abnormal for Freelite but normal for Diazyme averaged 1.19 mg/L (4.3%) below the Diazyme upper RR cutoff. The 1 discordant sample that was normal for Freelite but abnormal for Diazyme was 1.9 mg/L (7.2%) below the Freelite upper RR cutoff. For the κ/λ FLC ratio, of the 9 negative samples that were not concordant during method comparison, 4 of them averaged 0.10 (6.1%) above the upper RR cutoff for the Freelite FLC ratio (RR, 0.26–1.65; Table 2). The other 5 discordant samples averaged 0.10 (5.7%) below the upper RR cutoff for the Diazyme FLC ratio (RR, 0.22–1.74; Table 2). The bias observed between the κ and λ assays on both platforms was expected, considering that we are making comparisons between 2 different assays designed by 2 different manufacturers, using 2 different antibodies, and running on 2 different platforms.

Recently, Smith and Wu (*5*) evaluated the use of the Diazyme assay on the Siemens Advia 1880 analyzer. In addition to reporting precision of the κ and λ assays, they performed a study to determine their RR. They reported a 99% concordance rate

A

K		Integra freelite		
		Normal	Abnormal	Total
DxC Diazyme FLC	Normal	13	0	13
	Abnormal	0	19	19
	Total	13	19	32

B

λ		Integra freelite		
		Normal	Abnormal	Total
DxC Diazyme FLC	Normal	19	2	21
	Abnormal	1	6	7
	Total	20	8	28

C

κ/λ		Integra freelite		
		Normal	Abnormal	Total
DxC Diazyme FLC	Normal	10	9	19
	Abnormal	0	9	9
	Total	10	18	28

Fig. 3. Qualitative method comparison for κ, λ, and κ/λ ratio.
Grey indicates concordant results, with values within the RR (Normal) or outside the RR (Abnormal). (A), FLC κ agreement = 100.0% for Normal and Abnormal. (B), FLC λ agreement = 89.3%, Normal= 75.0%, Abnormal= 95.0%. (C), FLC κ/λ agreement = 67.9%, Normal = 100.0%, Abnormal = 50.0%.

for κ FLC, and discordant samples were close to the upper limit of the RR, but within the 10% cutoff limit for assay imprecision. The concordance for λ FLC was 90% using the manufacturer's suggested RR. However, Smith and Wu opted for increasing the upper limit of the RR from 27.69 mg/L to 32.0 mg/L, which resulted in a 94% concordance rate. In our RR study, overall concordance for Diazyme κ FLC assay was 95%, and the only discordant sample was <10% from the upper cutoff limit for the assay. For λ FLC, concordance was 100%. For the κ/λ RR verification, 5 samples had discordant results between the different manufacturer's RRs. Two of the 5 samples had values averaging 0.12 below the Diazyme κ/λ upper RR cutoff (Table 2) and 3 samples had values averaging 0.13 below the Freelite κ/λ upper RR cutoff (Table 2). On the whole, these averaged <10% imprecision from the upper RR cutoff of both manufacturers' suggested κ/λ FLC RRs. Similar to Smith and Wu (*5*), all discordant samples were close to the upper limit of the RR. We suggest that further diagnostic workup including serum protein electrophoresis, serum immunofixation, hematological assessment, proteinuria, creatinine and eGFR evaluation, and biopsy is needed when monitoring a patient's condition, as a normal FLC ratio does not rule out the presence of other diseases such as AL amyloidosis (*6*). In MM, diagnosis usually relies on serum/urine protein electrophoresis, serum/urine immunofixation electrophoresis, bone marrow biopsy, and imaging in addition to FLC analysis (*7*).

FLCs can also be used to monitor progression of disease or treatment response (*8*). In this instance, intraindividual changes in FLC are more important than a population-based RR. It has been shown with the Freelite FLC assays that intraindividual variation is low in healthy subjects (CVs <2.5% for κ and λ FLCs, as well as κ/λ FLC ratio) (*4*). Thus, although we see differences in our RR verification results vs what has been suggested by Diazyme, intraindividual variation is low in healthy subjects.

We conclude the Diazyme κ and λ FLC assays when run on the Beckman-Coulter DxC analyzer exhibit excellent precision and accuracy and are comparable to the Freelite assays on the Roche Cobas Integra analyzer.

Table 2. Reference ranges.

	Freelite	Diazyme
κ FLC	3.30–19.40 mg/L	2.37–20.73 mg/L
λ FLC	5.71–26.30 mg/L	4.23–27.69 mg/L
κ/λ ratio	0.26–1.65	0.22–1.74 mg/L

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

Role of Sponsor: No sponsor was declared.

REFERENCES

1. Jenner EL, Evans JA, Harding SJ. Serum free light chain (FLC) analysis: a guiding light in monoclonal gammopathy management. J Appl Lab Med 2017;2:98–106.
2. Hansen CT, Munster AM, Nielsen L, Pedersen P, Abildgaard N. Clinical and preclinical validation of the serum free light chain assay: identification of the critical difference for optimized clinical use. Eur J Haematol 2012;89:458–68.
3. Messiaen AS, De Sloovere MM, Claus PE, Vercammen M, Van Hoovels L, Heylen O, et al. Performance evaluation of serum free light chain analysis: nephelometry vs turbidimetry, monoclonal vs polyclonal reagents. Am J Clin Pathol 2017;147:611–22.
4. Tate J, Bazeley S, Sykes S, Mollee P. Quantitative serum free light chain assay–analytical issues. Clin Biochem Rev 2009;30:131–40.
5. Smith A, Wu AH. Analytical and clinical concordance of free light chain assay. Pract Lab Med 2019;13: e00112.
6. Siegel D, Bilotti E, van Hoeven KH. Serum free light chain analysis for diagnosis, monitoring, and prognosis of monoclonal gammopathies. Lab Med 2009;40: 363–6.
7. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15: 70442–5.
8. Tosi P, Tomassetti S, Merli A, Polli V. Serum free light-chain assay for the detection and monitoring of multiple myeloma and related conditions. Ther Adv Hematol 2013;4:37–41.