ARTICLE

Serum Free Light Chain Quantification Testing: Comparison of Two Methods for Disease Monitoring

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Background: Levels of free immunoglobulin light chains in serum and urine are a sensitive measure of dysregulated immunoglobulin synthesis. The development of an assay for free light chains in serum was a major advance in laboratory testing for monoclonal gammopathies. The original assay by The Binding Site, called Freelite®, has been in common use in laboratory monitoring of monoclonal gammopathies. Two clinical entities, myeloma-defining condition and light chain-predominant multiple myeloma, rely on quantitative measurements of serum free light chains.

Methods: Using polyclonal antisera specific to free light chains, Diazyme Laboratories developed a latex immunoturbidimetric assay for quantification of human kappa and lambda serum free light chains. We evaluated the Diazyme assay by comparing the results of kappa and lambda free light chain quantification, and kappa/lambda ratio with the results on the same specimens by the Freelite method. We also compared the correlation of the 2 methods to evaluate response to treatment and to changes in clinical status of patients with multiple myeloma.

Results: The results of Freelite and Diazyme methods are comparable. There was no statistically significant difference in the performance of the 2 assays for quantification of light chains, kappa/lambda ratio, or correlation of clinical parameters from patients with multiple myeloma at various stages of monitoring the disease in 2 geographically diverse laboratory and clinical environments.

Conclusions: The Diazyme method is comparable to Freelite and provides an opportunity to add the test to front-end automation and improvement in efficiency of the assay.

INTRODUCTION

The general structure of immunoglobulins, the primary mediators of acquired humoral immunity, constitutes a tetrameric protein consisting of two heavy chains and two light chains. The heavy chains are alpha, gamma, mu, delta, and epsilon for IgA, IgG, IgM, IgD, and IgE, respectively. Light chains may be kappa or lambda. One immunoglobulin has only one type of heavy and one type of light chain. Through a combination of DNA rearrangement, permutation, and combination of

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IMPACT STATEMENT

Freelite® was the first assay on the market and has served as the industry standard for quantification of serum free light chains. Other assays offered by Sebia, Siemens, and Abingdon Health provide similar results. The latex-enhanced immunoturbidimetric assay offered by Diazyme provides results comparable to the industry standard Freelite assay. The Diazyme method can be deployed on multiple platforms linked to front-end automation, thereby improving the efficiency of operations and reducing need for personnel.

heavy and light chains and somatic hypermutation during maturation of antibody-producing cells, it is possible to generate over 10^{16} different types of immunoglobulins (1). In the process of immunoglobulin production by plasma cells and lymphocytes, light chains are generally produced in greater abundance than heavy chains (2). The excess light chains circulate in blood and, as small proteins of about 25 kDa, are filtered by the glomerulus and are detectable in urine. Lambda light chains also occur as dimers of 46 kDa size and the larger size may account for the longer half-life of lambda light chains as compared to kappa (3). In a normal healthy state, the free light chains in blood and urine are polyclonal. Multiple restricted-heterogeneity immunoglobulins are often produced as part of the normal immune response to pathogens and following stem cell transplantation (4, 5). During the progression of these events, a short-lived apparent monoclonal immunoglobulin may appear in serum.

In multiple disease states, monoclonal immunoglobulins are produced by neoplastic lymphocytes and plasma cells, namely lymphomas and plasma cell disorders. Non-neoplastic auto-immune disorders may be associated with monoclonal immunoglobulins, e.g., myasthenia gravis, cold agglutinin disease, Guillain Barré syndrome, systemic lupus erythematosus, and chronic inflammatory demyelinating polyneuropathy. A similar phenomenon accounts for the oligoclonal immunoglobulin pattern detected in cerebrospinal fluid of patients with multiple sclerosis. Additionally, monoclonal immunoglobulins are also noted in AL amyloidosis (light chain amyloid), diseases associated with deposition of light and heavy chains, POEMS (polyendocrinopathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes) syndrome, etc. (2).

Classical monoclonal gammopathies consist of monoclonal gammopathy of undetermined significance (MGUS), smoldering/asymptomatic myeloma (SMM), and multiple myeloma (MM). MGUS and SMM are premalignant dyscrasias and progress to the malignant state of MM at a frequency of 1% to 2%/year for MGUS and 10% to 25%/year for SMM (6–8).

The normal polyclonal as well as pathological monoclonal immunoglobulins can be tracked in serum and urine by electrophoresis followed by staining with antibodies specific to each heavy and light chain type. In intact immunoglobulins, some of the epitopes on light chains are inaccessible to conventional anti-immunoglobulin antibodies. Bradwell, in a paradigm-shifting study, demonstrated that antibodies to epitopes of light chains that are hidden in an intact immunoglobulin specifically detect free light chains (3). The Binding Site Company has marketed a turbidimetric assay, called Freelite®, based on latex particles coated with polyclonal antibodies specific to free light chains to measure serum levels of free light chains. Other vendors, namely Siemens—N-Latex FLC assay; Sebia—Sebia FLC; Abingdon

Health—Seralite®; and Diazyme, have also offered tests for quantification of free light chains using monoclonal or polyclonal antibodies. The results from tests offered by different vendors, and even using reagents from one vendor on different testing platforms, are not interchangeable. Nevertheless, the results are similar enough to be useful in clinical circumstances for monitoring disease (9–12).

Over the period of a few years, a variable amount of shift in the results for serum free light chains (SFLC) has been observed for the Freelite assay, especially towards higher kappa/lambda ratio; this has led to a risk of misdiagnosis (13). Different SFLC assays are also variably affected by renal failure; Freelite being affected more than the assay by Siemens (14).

The Freelite assay by The Binding Site Company was the first available SFLC assay, such that many of the studies and criteria for diagnosis, prognosis, and response levels in multiple myeloma are based on the results of this assay. The light chain criteria in the International Myeloma Working Group (IMWG) publications are predicated on the results by the Freelite method (15).

There is general agreement that quantification of SFLC is useful in the diagnosis and monitoring of light chain multiple myelomas (LCMM), though initial diagnosis of LCMM requires demonstration of monoclonal light chains in serum and/or urine by electrophoretic methods (16). It has been proposed that serum protein electrophoresis (SPEP), serum immunofixation electrophoresis (SIFE), and serum free light chain assay (SFLCA) are sufficient for screening for MM and urine protein electrophoresis (UPEP) and urine protein immunofixation electrophoresis (UIFE) may be omitted in the screening for MM (17, 18). This recommendation has been questioned due to the high incidence of abnormal kappa/lambda ratios in patients lacking monoclonal gammopathy and because SFLCA is not specific for monoclonal light chains, whereas presence of monoclonal light chains in UIFE is diagnostic for monoclonal gammopathy. The error rate may in part be due to the drift in Freelite result over time (13, 19, 20).

A normal kappa/lambda ratio is one of the criteria for stringent complete response of MM following treatment. This notion has also been challenged due to the high incidence of false-positive kappa/lambda ratio in patients who are status-post stem cell transplantation (2, 4, 20, 21).

It has been proposed that SFLCA may be used for early detection of response to treatment and relapse, however, clinical studies have not borne out the usefulness of this approach. SFLCA has also been promoted for detection of light chain escape; however, the validity of this phenomenon has been questioned (2).

SFLC quantification has been proposed for the identification of light chain-predominant subgroup of multiple myelomas (LCPMM) since the latter have been shown to have poorer prognosis. Similarly, LCMM patients with higher levels of SFLC are noted to have worse prognosis (22–24).

The IMWG has defined one criterion of myeloma-defining condition based on SFLCA results, i.e., the presence of a free light chain level of greater than 100 mg/L and ratio of involved to uninvolved light chain concentration of greater than 100 (15). The criterion in its current form has a low sensitivity of 16% and it has been proposed that separate criteria for kappa and lambda light chain pathologies should be established, since kappa chain-associated lesions produce about 4-times more free light chains than lambda light chain-associated lesions. In addition, the levels of uninvolved light chains are twice as high in lambda chain-associated lesions as levels seen in kappa chain-associated lesions (2, 25, 26).

Despite the shortcomings of SFLCA, there are sufficient cogent reasons to apply this test in the diagnosis and monitoring of monoclonal gammopathic lesions. In this study we evaluated a new test for SFLC levels using polyclonal antibodies in a turbidimetric assay, and the results were compared with the standard Freelite assay.

Moreover, the results were tested for correlation with clinical stages and progression as well as response to treatment in multiple myeloma.

MATERIALS AND METHODS

This study was a combined effort of 3 medical school-affiliated medical centers and Diazyme Laboratories, a division of General Atomics. The study protocol was approved by the Institutional Review Boards at the involved medical centers. Residual serum specimens from clinically indicated diagnostic and follow-up testing for monoclonal gammopathies were used for the study. No specimens were collected solely for the purpose of the study. The specimens were evaluated routinely for clinically indicated laboratory tests and medical records were reviewed for clinical status, therapy, and radiographic findings. The usual laboratory tests included complete blood counts including differential cell counts, SPEP SIFE, UPEP and UIFE, immunoglobulin quantification, and quantification of serum free light chains by using kits from The Binding Site Company on the Optilite analyzer platform. Serum creatinine and other analytes were measured by conventional automated chemistry analyzers procured from Beckman–Coulter. Bone marrow specimens were evaluated by morphology, cytogenetics, and flow cytometry. Additional investigations were carried out as indicated by clinical needs. All of the testing was driven by the clinical needs of the patients.

Residual serum specimens were stored at −20 °C. Specimens were shipped to Diazyme Laboratories on dry ice for serum free light chain quantification by the Diazyme method. Briefly, sera were analyzed by the Diazyme human kappa and lambda free light chain assay (DSFLCA) with a Roche Cobas c501 analyzer. The validation data for use of the Roche Cobas c501 analyzer is documented in the CLIA submission. DSFLCA is based on a latex-enhanced immunoturbidimetric assay. Kappa FLC/lambda FLC in the sample binds to a specific anti-kappa FLC/anti-lambda FLC antibody, which is coated onto latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of kappa FLC/lambda FLC in the sample. The instrument calculates the kappa FLC/lambda FLC concentration by interpolation of a 6-point calibration curve prepared from calibrators of known concentrations. The assays have been validated according to Clinical and Laboratory Standards Institute protocols using multiple lots of reagents on multiple clinical analyzers with precision, reproducibility, linearity, assay reportable range, stability, detection limit, analytical specificity, method comparison with predicate device, reference interval establishment, and high dose "hook effect" (27).

The patient inclusion and exclusion criteria were as follows:

Inclusion criteria included: (*a*) confirmed diagnosis of MM; (*b*) at least 3 serum specimens collected at different phases of illness and treatment; and (*c*) availability of clinical information to assess response level. Patients were excluded from consideration: (*a*) if fewer than 3 serum specimens were available and (*b*) patients had MGUS and SMM but a diagnosis of MM was not established.

In this study, a total of 541 specimens were collected from 169 subjects at 3 sites, namely, Augusta University-Medical College of Georgia (AU), San Francisco General Hospital (SFGH), and University of California San Francisco Health (UCSF). Three to 9 specimens per patient at the time points: baseline (BL), timepoint 1 (TP1), and timepoint 2 (TP2) up to timepoint 8 (TP8) were analyzed. All samples were frozen after collection and transported on dry ice, and were subjected to a single freeze/thaw before the kappa lambda FLC quantification test at Diazyme headquarters' laboratory.

The clinical status of patients was classified by criteria defined by the IMWG and Version 1.2020/ National Comprehensive Cancer Network (NCCN)

Table 1. Explanation of clinical response criteria.[a]

Response category	Response criteria based on KL FLC, SPE, and IFE results	Clinical assessment based on IMWG
Good response	FLC ratio normal, M protein not detectable and IFE negative[b]	sCR CR
Moderate response	≥ 90% reduction of rd_dFLC[c] and ≥ 90% reduction of M protein	VGPR
	≥ 50% reduction of rd_dFLC[c] and ≥ 50% reduction of M protein	PR
Stable disease	≤ 49% reduction to ≤25% increase of rd_dFLC and ≤ 49% reduction to ≤25% increase of M protein	MR SD
Progressive disease	>25% increase of rd_dFLC and >25% increase of M protein and increase of d_dFLC[d] ≥100 mg/L *and* increase of M protein ≥ 0.5 g/dL	PD

[a]Response to treatment with respect to SFLC concentrations determined by the Binding Site and Diazyme methods were calculated by taking into account the change in SFLC concentration and change in concentration of monoclonal immunoglobulin. The criteria for changes in SFLC concentrations are listed in Table 1.
[b]Applies only to MM population.
[c]rd_dFLC–relative difference = ((dFLC t2–dFLC t1)/dFLC t1) ×100.
[d]d_dFLC = dFLC t2–dFLC t1 (with 'dFLC = iFLC–niFLC').
t1, time point 1; t2, time point 2; iFLC, involved FLC; niFLC, not involved FLC; dFLC, iFLC–ni FLC (involved SFLC minus uninvolved SFLC); sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PR, partial response; MR, moderate response; SD, stable disease; PD, progressive disease.

Clinical Practice Guidelines in Oncology on Multiple Myeloma. A simplified version of the criteria was developed from the IMWG and NCCN guidelines as shown in Table 1. Criteria for response to treatment based on SFLC concentration are also illustrated in Table 1. Response to treatment with respect to SFLC concentrations determined by the Binding Site and Diazyme methods were calculated by taking into account the change in SFLC concentration and change in concentration of monoclonal immunoglobulin. The criteria for changes in SFLC concentrations are listed in Table 1.

The determination of progression or no progression, based on SFLC concentrations and interval changes, as shown in Table 2 was based on criteria listed in Table 1. Good response, moderate response, and stable disease all qualified as no progression, and progressive disease as progression was the basis for the terminology used in other tables.

Change in SFLC concentrations by both methods was also compared to progression of disease or lack of progression of disease based on IMWG and NCCN criteria (21, 28). Sensitivity and specificity of the change in SFLC concentration with respect to progression and lack of progression were calculated.

Pearson correlation coefficients were calculated to explore the correlation of the measures from the 2 methods, Binding Site/Freelite and Diazyme. The kappa statistic was used to test the reliability of the 2 different methods. The chi-squared test was used to compare the clinical disease response categories from Freelite and Diazyme methods. All statistical analyses were carried out using SAS 9.4 (SAS Institute, 2012).

RESULTS

Demographic information and the types of lesions in the patient population are displayed in online Supplemental Table S1 in the Supplementary Material.

Results from the Binding Site/Freelite and Diazyme DSFLCAs were compared for kappa and lambda light chain concentration results and for kappa/lambda ratio by assessing the correlation coefficients. The Pearson correlation coefficients for kappa free light chain concentration (FKCC),

Table 2. Criteria for progression and non-progression of MM disease.[a]

Patient		Diazyme FLC results						Response criteria	Progression/no progression
		KFLC mg/L	LFLC mg/L	K/L ratio	dFLC	d_dFLC	rd_dFLC		
A	BL	50.1	19.7	2.54	30.4				
	t1	13.0	14.7	0.88	−1.7	−32.10	−106%	good	no progression
	t2	9.8	9.9	0.99	−0.1	1.60	−94%	good	no progression
B	BL	49.4	6.1	8.10	43.3				
	t1	61.9	6.1	10.15	55.8	12.50	29%	stable	no progression
	t2	71.0	6.1	11.64	64.9	9.10	16%	stable	no progression
	t3	747.8	6.1	122.59	741.7	676.80	1043%	progressive	progression

[a]Representative examples of the calculations and terms are shown below. No progression in one patient "A" with 3 observations. No progression on the first 3 observations and progression on the fourth observation in patient "B."
BL, Base line; t1, time point 1; t2, time point 2, t3, time point 3; iFLC, involved FLC; niFLC, not involved FLC; dFLC, iFLC−ni FLC (involved SFLC minus uninvolved SFLC); d_dFLC, dFLC t2−dFLC t1 (with 'dFLC, iFLC−niFLC'); rd_dFLC−relative difference, ((dFLC t2−dFLC t1)/dFLC t1)*100.

Table 3. Clinical response per IMWG/NCCN criteria compared to response designation based on DSFLCA and Freelite data.[a]

	IMWG/NCCN clinical assessment				
Response based on Diazyme FLC	**Good response**	**Moderate response**	**Stable disease**	**Progressive disease**	**Total**
Good response	44	6	8	0	58
Moderate response	2	27	27	6	62
Stable disease	25	12	173	20	230
Progressive disease	0	0	5	17	22
Total	71	45	213	43	372
Agreement	62%	60%	81%	40%	70%
	IMWG/NCCN Clinical Assessment				
Response based on Freelite	**Good Response**	**Moderate Response**	**Stable Disease**	**Progressive Disease**	**Total**
Good response	50	3	5	0	58
Moderate response	4	23	36	5	68
Stable disease	16	19	164	19	218
Progressive disease	1	0	8	19	28
Total	71	45	213	43	372
Agreement	70%	51%	77%	44%	69%

[a]To compare the Diazyme and Freelite observations, we counted the total agreement and disagreement in in the upper and lower parts of the table. There are 261 (70.16%) total agreements in the upper part—Diazyme with clinical assessment and 256 (68.82%) total agreements in lower part—Freelite with clinical assessment. The chi-square test *P*-value of 0.69 indicates that there is no difference in the agreement rates between the 2 methods.

lambda free light chain concentration (FLCC), and kappa/lambda ratio between the Binding Site Freelite and DSFLCA results were 0.89, 0.85, and 0.98 respectively for the 541 specimens tested by both methods. The strength of positive correlation is supported by a *P*-value of <0.0001.

Table 4. Comparison of responses based on DSFLCA and Freelite assay.[a]

	Response based on Binding site/Freelite results				
Response based on Diazyme FLC	**Good response**	**Moderate response**	**Stable disease**	**Progressive disease**	**Total**
Good response	49	2	7	0	58
Moderate response	0	41	21	0	62
Stable disease	9	25	187	9	230
Progressive disease	0	0	3	19	22
Total	58	68	218	28	372
Agreement	84%	60%	86%	68%	80%

[a]Column labeled "Response based on Diazyme FLC" displays data from testing with the Diazyme method. The next 4 columns show data from the Freelite method and exhibit the distribution of different response categories for each of the response category from the Diazyme method. There is overall 80% agreement between the 2 methods in determination of clinical response.

Table 5. Comparison of Diazyme and Freelite serum free light chain quantification results with respect to clinical assessment determinations.[a]

		Clinical assessment		
		Progression	**No progression**	**Total**
Change in Diazyme FLC	Positive	17	5	22
	Negative	26	324	350
	Total	43	329	372
Clinical sensitivity: 40% (17/43; 95% CI, 25.0%–55.6%)				
Clinical specificity: 98% (324/329; 95% CI, 96.5%–99.5%)				
		Clinical Assessment		
		Progression	**No progression**	**Total**
Change in Binding Site/Freelite FLC	Positive	19	9	28
	Negative	24	320	344
	Total	43	329	372
Clinical sensitivity: 44% (19/43; 95% CI, 29.1%–60.1%)				
Clinical specificity: 97% (320/329; 95% CI, 94.9% –98.7%)				

[a]The performance of the 2 methods in terms of clinical sensitivity and specificity with respect to disease progrssion and no progression is similar. We compared the sensitivities of the 2 methods by the chi-square test. A *P*-value of 0.662 indicates there is no significant difference between the 2 methods. Positive represents increase in serum free light chain levels and negative a decrease in levels. Progression and no progression are clinical response measures based on International Myeloma Working Group and National Comprehensive Cancer Network criteria.

The types of responses identified from analysis of Diazyme FLC were compared to the response categories garnered from IMWG/NCCN clinical assessment. The results are shown in the upper part of Table 3. Corresponding data for Binding Site FLC and IMWG/NCCN clinical assessment are displayed in the lower part of Table 3. Overall, both methods had good agreement between the FLC change and the IMWG/NCCN clinical assessments.

Table 6. Comparison of the 2 methods based on clinical response over the time of multiple observations.[a]

Kappa 12 D vs B	0.84813
Lambda 12 D vs B	0.79499
Ratio 12 D vs B	0.79258
Kappa 13 D vs B	0.94538
Lambda 13 D vs B	0.81718
Ratio 13 D vs B	0.99071
Kappa 23 D vs B	0.99291
Lambda 23 D vs B	0.81718
Ratio 23 D vs B	0.99536

[a]Results from the 2 methods are comparable, the *P*-value of <0.0001 attests to the lack of difference in the results from the 2 methods.
B Kappa—Binding Site kappa concentration.
D Kappa—Diazyme kappa concentration.
B Lambda—Binding Site lambda concentration.
D Lambda—Diazyme lambda concentration.
B Ratio—Binding Site kappa/lambda ratio.
D Ratio—Diazyme kappa/lambda ratio.
BL—Baseline.
12—Difference of time point BL and time point 1.
13—Difference of time point BL and time point 2.
23—Difference of time point 1 and 2.

Table 4 displays a direct comparison of the FLC-based responses between the Diazyme and Binding Site results and there is 80% agreement between the 2 methods. The 80% agreement is similar to the agreement of individual methods with the IMWG/NCCN Clinical Assessment.

The response criteria established by clinicians are often based on multiple factors besides FLC results. Therefore, FLC results evaluated alone might lead to errant response designations. The clinical disease response categories were condensed into 2 clinical status categories: "Progression" and "No progression." Subjects with "Progression" consist of those with monitoring events defined as "Progressive Disease". Subjects with "No Progression" consist of those with monitoring events defined as "Good Response, Moderate Response, and Stable Disease Response" categories. The performance of Diazyme Human Kappa and Lambda FLC results and those from Binding Site/Freelite, compared to the clinical status are summarized in Table 5: The weighted kappa statistic for Table 6 is 0.65 with 95% confidence interval between 0.57 and 0.73, which indicates good agreement between the 2 methods. The symmetry test chi-square value is 5.60 with a *P*-value of 0.4697. Thus, there is no significant difference in the results based on the 2 methods.

The performance of the 2 methods in terms of clinical sensitivity and specificity with respect to disease progression and no progression is similar. To further evaluate Table 6, we compared the sensitivities of the 2 methods, and the chi-squared test *P*-value of 0.662 indicates there is no significant difference between the 2 methods.

There were 169 subjects who had FLC measurements with at least 3 time points. A representative plot of baseline level of the involved light chain concentration and the 2 time points in the course of disease is shown in Fig. 1, A while Fig. 1, B displays the kappa/lambda ratios at the various time points.

The results of clinical response over multiple observations between the Diazyme (D) and Binding Site (B) methods were also compared and the data are shown in Table 6. The results from the 2 methods are comparable. The Pearson correlation coefficients for the comparisons are shown in Table 6. The *P*-value of <0.0001 attests to the lack of difference in the results from the 2 methods as shown in Table 6.

DISCUSSION

Testing for SFLC concentration in diagnosis and monitoring of MM in particular, and monoclonal gammopathies in general, is recommended by the IMWG (21). The various recommended uses of SFLC analysis were addressed in the introductory section. Quantification of FLCs is particularly useful in monitoring light chain myelomas and light chain MGUS, light chain SMM, and light chain-predominant MM (2, 22–25, 29). Accurate SFLC

Patient#	date	Binding Site KFLC mg/L	Binding Site LFLC mg/L	Binding Site K/L	Diazyme KFLC mg/L	Diazyme LFLC mg/L	Diazyme K/L	M-spike g/dL	Initial IFE	Clinical Assessment	Response Criteria
6	04/2018 BL	22.48	93.39	0.24	24.5	140.7	0.17	1.62	IgG-L		
	10/2018 Point 1	17.00	17.15	0.99	18.3	16.1	1.14	0.66		PR	Moderate
	11/2018 Point 2	18.74	17.10	1.10	20.4	14.3	1.43	0.51		SD	Stable

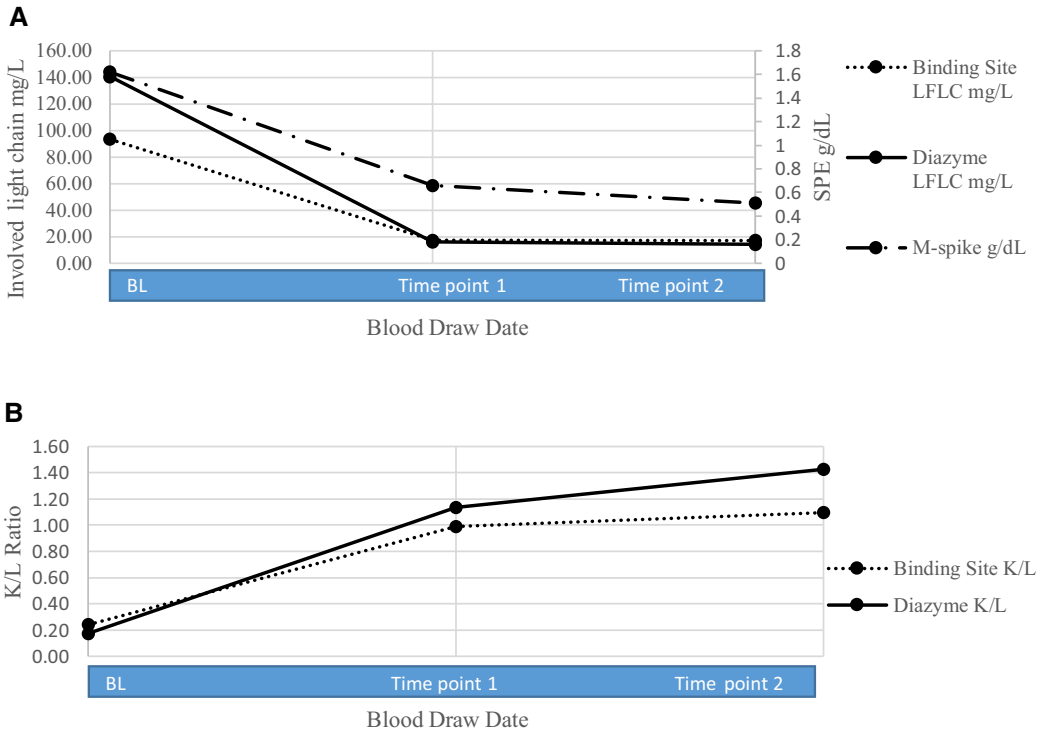

Fig. 1. Graphical representation of changes in laboratory and clinical response assessments. We calculated differences of 2 time points (timepoint 1 vs baseline timepoint [BL], timepoint 2 vs timepoint BL, and timepoint 2 vs timepoint 1). The Pearson correlation coefficients for the comparisons are shown in Table 6. The *P* value of <0.0001 attests to the lack of difference in the results from the 2 methods as shown in Table 6. Curve A represents the concentration of involved serum free light chain and curve B represents the kappa/lambda ratio.

quantification is essential for the diagnosis of light chain-predominant MMs secreting intact immunoglobulins and may be useful in monitoring this subtype of MM (22, 23). Monitoring the SFLC concentration may be useful in monitoring patients with AL amyloidosis and has been suggested for monitoring patients with non-secretory myelomas (30).

The Binding Site Company introduced the Freelite assay for quantification of serum free light chains about 20 years ago. This method has been used in most of the clinical trials and comprises the standard underlying the various light chain-based criteria promulgated by the IMWG. Siemens markets an assay for SFLC using monoclonal antibodies and Sebia

provides an assay based on polyclonal antibodies. These, and other assays marketed by Diazyme and Seralite, are more or less comparable (10, 31). The results of free kappa and lambda light chains in patients with MM, SMM, and MGUS were compared between Diazyme and Freelite by Smith and Wu and they found the results by the 2 methods to be comparable (12). In this report we compared the results between the 2 methods, with respect to clinical outcomes, and found the results to be comparable. To date, the test has not undergone industry-wide harmonization, and using the same reagent on different testing platforms can yield somewhat different results (32). With the exception of the light chain-based criteria for myeloma-defining condition and diagnosis of light chain-predominant MM, the exact concentration of the free light chain is not as important as the trend or change in SFLC concentration with response to treatment or disease progression. Free light chains have a narrow within-individual biological variation and a wide between-individual variation. This indicates that reference intervals are of limited value and serial samples should be collected and tested (33).

A frequent and partly justified criticism of the SFLCAs is that the test measures all free light chains and is not specific to monoclonal light chains. The usual SIFE test is not particulaly sensitive for detection of monoclonal light chains. This lack of sensitivity is due to two main reasons: (*a*) routine SIFE uses 10-fold diluted serum for gamma heavy chain and kappa light chain, and 5-fold diluted specimen for staining for alpha and mu heavy chains and lambda light chains (in accordance with the protocol provided by the vendor of the equipment, Helena Laboratories); (*b*) unless the free monoclonal light chain has a substantially different mobility by electrophoresis than the intact monoclonal immunoglobulin, the light chain bands are obscured by the much higher concentration of intact monoclonal immunoglobulin. Serum free light chain modified SIFE (FLC modified SIFE) and MASS-FIX MALDI have been proposed for detection of monoclonal light chains with a greater sensitivity. Both methods could be used for detecting minimal residual disease, although FLC modified SIFE has been shown to be more sensitive and specific (34, 35). While direct measurement is problematic, it is possible to obtain an approximate concentration of monoclonal light chains by electrophoresis, densitometry scanning, and extrapolation from total FLC concentration as has been established for the QUIET (quantification by ultrafiltration and immunofixation electrophoresis testing) assay (29).

In this study we compared the performance of the industry standard Binding Site Freelite assay with the Diazyme Human Kappa and Lambda FLC assay. The newer assay from Diazyme allows the method to be used on many chemistry analyzers from Roche, Abbott, Beckman, Ortho Vitros, Siemens, and Diazyme and affords the option of putting the assay on their front-end automation lines, thus improving the efficiency of operations. In contrast, the Binding Site Freelite assay is only available on the Optilite and Spa Plus and support for other analyzers is limited. Conventional methods for comparing the raw concentrations by the 2 methods showed good agreement. In assessing the relative equivalence of these assays, even the lowest correlation coefficient at 0.85 for lambda FLC concentration was an acceptable level of performance to adopt this new method. The correlation coefficient for kappa/lambda ratio was excellent at 0.98. More importantly, the SFLC results by the 2 methods displayed virtually identical performance in tracking the clinical course of disease. Specimens from only patients with a diagnosis of MM were used in this comparison. Lack of inclusion of specimens from patients with MGUS and SMM constitutes a limitation of the study. However, because patients with MGUS and SMM are usually not treated, we could not have used data from the patients to compare the clinical course with reference to the

2 methods for quantification of SFLCs. Additionally, an earlier publication documented comparability of the 2 methods in patients with MM, SMM, and MGUS (12).

CONCLUSION

The results of the Diazyme Human Kappa and Lambda FLC assay showed good concordance with IMWG/NCCN clinical assessment and the results of the industry standard, Binding Site Freelite assay.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

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REFERENCES

1. Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol 2010;125 (Suppl 2):S41–52.
2. Singh G. Serum and urine protein electrophoresis and serum-free light chain assays in the diagnosis and monitoring of monoclonal gammopathies. J Appl Lab Med 2020;5:1358–71.
3. Bradwell AR. Serum free light chain analysis plus Hevylite. 7th Ed. Birmingham, (UK): The Binding Site Group Ltd.; 2015.
4. Singh G. Oligoclonal pattern/abnormal protein bands in post-treatment plasma cell myeloma patients: implications for protein electrophoresis and serum free light chain assay results. J Clin Med Res 2017;9:671–79.
5. Drouet E, Chapuis-Cellier C, Bosshard S, Verniol C, Niveleau A, Touraine JL, Garnier JL. Oligo-monoclonal immunoglobulins frequently develop during concurrent cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections in patients after renal transplantation. Clin Exp Immunol 1999;118:465–72.
6. Kyle RA, Durie BG, Rajkumar SV, Landgren O, Blade J, Merlini G, Boccadoro M. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. Leukemia 2010;24: 1121–27.
7. Kyle RA, Remstein ED, Therneau TM, Dispenzieri A, Kurtin PJ, Hodnefield JM, Rajkumar SV. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. N Engl J Med 2007;356:2582–90.
8. Korbet SM, Schwartz MM. Multiple myeloma. J Am Soc Nephrol 2006;17:2533–45.
9. Fleming CKA, Swarttouw T, de Kat Angelino CM, Jacobs JFM, Russcher H. Method comparison of four clinically available assays for serum free light chain analysis. Clin Chem Lab Med 2019;58:85–94.
10. Muluhngwi P, Sharp CN, Pozzi N, Elin RJ, Jortani SA. Verification of newly FDA-approved Kappa and Lambda free light chain assays on a previously untested platform. J Appl Lab Med 2019;4:323–30.
11. Jacobs JF, Tate JR, Merlini G. Is accuracy of serum free light chain measurement achievable? Clin Chem Lab Med 2016;54:1021–30.
12. Smith A, Wu AHB. Analytical and clinical concordance of free light chain assay. Pract Lab Med 2019;13:e00112.

13. Murray D, Dispenzieri A, Kumar S, Gill H, Vachon C, Snyder M, Willrich M. Free light chain assay drift: potential for misdiagnosis? J Appl Lab Med 2020;5:1411–13.
14. Rindlisbacher B, Schild C, Egger F, Bacher VU, Pabst T, Leichtle A, Sédille-Mostafaie N. Serum free light chain assay: shift toward a higher κ/λ ratio. J Appl Lab Med 2020; 5:114–25.
15. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, Miguel JF. International myeloma working group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15:e538–48.
16. Singh G. Concentrations of serum free light chains in kappa and lambda lesions in light-chain myelomas. Lab Med 2019;50:189–93.
17. Dejoie T, Corre J, Caillon H, Hulin C, Perrot A, Caillot D, Avet-Loiseau H. Serum free light chains, not urine specimens, should be used to evaluate response in light-chain multiple myeloma. Blood 2016;128: 2941–48.
18. Katzmann JA, Dispenzieri A, Kyle RA, Snyder MR, Plevak MF, Larson DR, Rajkumar SV. Elimination of the need for urine studies in the screening algorithm for monoclonal gammopathies by using serum immunofixation and free light chain assays. Mayo Clin Proc 2006;81:1575–78.
19. Singh G. Serum free light chain assay and κ/λ ratio performance in patients without monoclonal gammopathies: high false-positive rate. Am J Clin Pathol 2016;146:207–14.
20. Singh G. Serum free light chain assay and κ/λ ratio: performance in patients with monoclonal gammopathy-high false negative rate for κ/λ ratio. J Clin Med Res 2017;9:46–57.
21. Dimopoulos M, Kyle R, Fermand JP, Rajkumar SV, San Miguel J, Chanan-Khan A, Jagannath S. Consensus recommendations for standard investigative workup: report of the International Myeloma Workshop Consensus Panel 3. Blood 2011;117:4701–05.
22. Singh G, Savage NM, Jillella AP, Bollag RJ. Light chain-predominant multiple myeloma subgroup: impaired renal function correlates with decreased survival. Lab Med 2022;53:145–48.
23. Singh G, Xu H. Light chain predominant intact immunoglobulin monoclonal gammopathy disorders: shorter survival in light chain predominant multiple myelomas. Lab Med 2021;52:390–8.
24. Jin Y, Savage NM, Bollag RJ, Xu H, Singh G. Light chain multiple myeloma: high serum free light chain concentrations portend renal damage and poorer survival. J Appl Lab Med 2021;6:1592–600.
25. Lee WS, Singh G. Serum free light chains in neoplastic monoclonal gammopathies: relative under-detection of lambda dominant kappa/lambda ratio, and underproduction of free lambda light chains, as compared to kappa light chains, in patients with neoplastic monoclonal gammopathies. J Clin Med Res 2018;10:562–9.
26. Lee WS, Singh G. Serum free light chain assay in monoclonal gammopathic manifestations. Lab Med 2019;50:381–9.
27. U.S. Food and Drug Administration. 510(k) substantial equivalence determination decision summary. https://www.accessdata.fda.gov/cdrh_docs/reviews/K153394.pdf (Accessed August 2022).
28. Callander NS, Baljevic M, Adekola K, Anderson LD, Campagnaro E, Castillo JJ, Kumar SK. NCCN guidelines® insights: multiple myeloma, version 3.2022. J Natl Compr Canc Netw 2022;20:8–19.
29. Singh G, Bollag R. Quantification by ultrafiltration and immunofixation electrophoresis testing for monoclonal serum free light chains. Lab Med 2020;51: 592–600.
30. Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, Durie BG. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia 2009;23: 215–24.
31. Moreau C, Lefevre CR, Decaux O. How to quantify monoclonal free light chains in plasma cell disorders: which mass spectrometry technology? Ann Transl Med 2020:8:973–9.
32. Messiaen AS, De Sloovere MMW, Claus PE, Vercammen M, Van Hoovels L, Heylen O, De Smet D. Performance evaluation of serum free light chain analysis: nephelometry vs turbidimetry, monoclonal vs polyclonal reagents. Am J Clin Pathol 2017;147:611–22.
33. Braga F, Infusino I, Dolci A, Panteghini M. Biological variation of free light chains in serum. Clin Chim Acta 2013;415:10–11.
34. Sepiashvili L, Kohlhagen MC, Snyder MR, Willrich MAV, Mills JR, Dispenzieri A, Murray DL. Direct detection of monoclonal free light chains in serum by use of immunoenrichment-coupled MALDI-TOF mass spectrometry. Clin Chem 2019;65: 1015–22.
35. Wilhite D, Arfa A, Cotter T, Savage NM, Bollag RJ, Singh G. Multiple myeloma: detection of free monoclonal light chains by modified immunofixation electrophoresis with antisera against free light chains. Pract Lab Med 2021;27:e00256.