SIEMENS

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Multicenter Evaluation of New Free Light Chain Methods

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Abstract

BACKGROUND: The International Myeloma Working Group has provided consensus guidelines for the use of immunoglobulin free light chain (FLC) determinations in the diagnosis and management of clonal plasma cell disorders. We describe preliminary repeatability, linearity, reference range, and method comparison data for two new immunoassays designed for the detection of free Ig light chains type kappa and lambda in serum and plasma. In addition, we investigated samples from patients with kidney impairment for their FLC content.

METHODS: Latex-enhanced mouse monoclonal antibody reagents from Siemens for free Ig light chain type kappa (N Latex FLC kappa)† and free Ig light chain type lambda (N Latex FLC lambda)† were assayed on three BN™ II systems† and three BN ProSpec® systems† using serum samples. Precision studies were carried out according to CLSI guideline EP5A-2 to estimate repeatability performance of three sample pools and two controls each for kappa and lambda, using up to three independent reagent and calibrator lots at the four test sites. Linearity was evaluated by calculating the recovery of repetitions performed at higher or lower dilutions compared to the results obtained with the default dilutions. Reference ranges were conducted using 397 clinical samples from apparently healthy adults (aged 16–89 years). Qualitative method-comparison studies using 314 samples from patients with diagnoses of multiple myeloma, amyloidosis, Waldenstroem's macroglobulinemia, monoclonal gammopathy of undetermined significance, polyclonal immunoglobulin stimulation, or renal disease were performed at two U.S. sites against commercially available FLC methods for the BN II system. Statistical analysis used concordance tables and the kappa statistic.

RESULTS: ANOVA studies for between-site, between-lot, and total precision for kappa on the BN II system were 1.1–2.1%, 2.0–3.1%, and 3.7–5.2%, respectively. On the BN ProSpec system, the corresponding kappa results were 2.6–5.2%, 2.4–4.8%, and 3.6–6.9%. Lambda results on the BN II system were 0.4–3.7%, 3.5–7.8%, and 6.2–9.1%; on the BN ProSpec system, lambda results were 1.8–2.5%, 1.2–5.6%, and 4.3–7.7%. In terms of linearity, there were 78 kappa and 117 lambda data sets available for analysis. For kappa 89% and for lambda 97% of the repeats recovered within ±20% of the initial value. Reference-range studies resulted in κ/λ ratios of 0.19/0.87/1.74 (min/median/max). Method-comparison studies resulted in the following: Site 1 analyzed 139 samples and revealed concordance rates of 89.9% for kappa, 77.0% for lambda, and 91.4% for the κ/λ ratio. The results at Site 2 based on 175 samples were 88.6%, 81.7%, and 89.1%, respectively. Combining the data revealed concordance rates of 89.2%, 79.6%, and 90.1%. N Latex FLC results for 57 patients with kidney impairments ranged from 14.1–208 mg/L for kappa and 15.1–228 mg/L for lambda, respectively, and resulted in a κ/λ ratio distribution of 0.43/0.78/1.46 (min/median/max).

CONCLUSION: The new FLC methods performed well under routine laboratory conditions on both BN platforms.

Introduction

FLC (free Ig-light chains) are secreted by immunoglobulin-producing plasma cells. FLC kappa circulates as a monomer of 25 kD, whereas FLC lambda usually is present in dimers of 59 kD. As small proteins, FLC are freely filtered by the kidney, the larger FLC lambda complexes somewhat less efficiently than the smaller FLC kappa molecules, resulting in a half-life time of 2–3 hours[5] for FLC kappa and 4–6 hours[5] for FLC lambda.

The short half-life time is important for monitoring therapy response in monoclonal gammopathies, as the complete immunoglobulin molecules show a much longer half-life, with the longest being three weeks for IgG.

Like other small proteins (e.g., cystatin C, α1-microglobulin), FLC are reabsorbed from urine by tubulus cells. Only when the reabsorption capacity is fully used are FLC excreted into the urine (= overflow proteinuria).

Precipitates in urine from myeloma patients were first described by Dr. Bence Jones as early as 1847; later these precipitates were identified as a proteinuria of FLC.

Material and Methods

Instruments

A total of three BN ProSpec and three BN II systems were used during the evaluation. Both analyzer platforms used pre-reaction software protocols to detect antigen excess issues.

Materials

The N Latex FLC reagents consist of individual reagent packs for FLC kappa and FLC lambda. A single calibrator and two levels of controls, each available separately, are used to calibrate and monitor both methods. In total, three different lot combinations of the Siemens reagents and calibrators were utilized. Random distribution of reagent combinations was used in order to mimic routine use.

Each evaluation site used individual reagent lots of other commercially available FLC methods from The Binding Site, Ltd. for the method-comparison studies.

Procedures

Reproducibility was estimated during a 10-day evaluation period with three reagent lot combinations using three BN II and three BN ProSpec systems. Three samples and two controls were analyzed in duplicate out of two separate vials twice a day.

Linearity between dilutions was evaluated by calculating the recovery of repetitions performed at higher or lower dilutions compared to the results obtained with the default dilutions. Since the data were derived out of the method-comparison studies, this procedure was applied to the Siemens methods and to the comparison methods.

Reference-range confirmation studies were conducted at two sites. The selected leftover samples either came from hospitalized patients or were referral samples from patients with diseases/illnesses that were unlikely to be linked with FLC-related disorders.

Samples were identified to cover age groups for which myeloma-related diseases are most prevalent (elderly population).

Method-comparison studies were conducted at two sites using 314 samples from patients with diagnoses of multiple myeloma, amyloidosis, Waldenstroem's macroglobulinemia, monoclonal gammopathy of undetermined significance (MGUS), polyclonal immunoglobulin stimulation, or renal disease. Each sample was analyzed in singlicate within each method using BN II analyzers.

Results

A graphical distribution of the reproducibility data is shown in Figure 1. Plotted are the minimum and maximum % CVs of the five samples analyzed on the three BN II and three BN ProSpec analyzers. They are grouped for between-site, between-lot, and total precision.

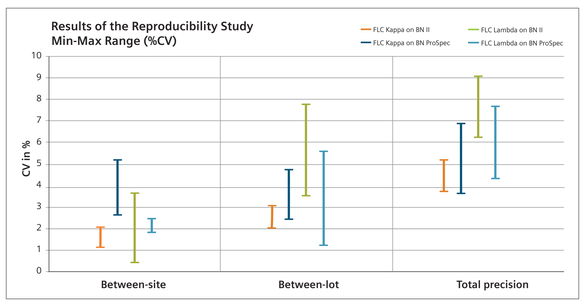

Figure 1. Results of the reproducibility study.

The overall precision performance of both methods on each analyzer and between analyzers was rated as being acceptable by all sites.

For each sample, the deviation of recovery compared to the results from the initial dilution was calculated and expressed as percent deviation. The obtained results were plotted as box plots and compared.

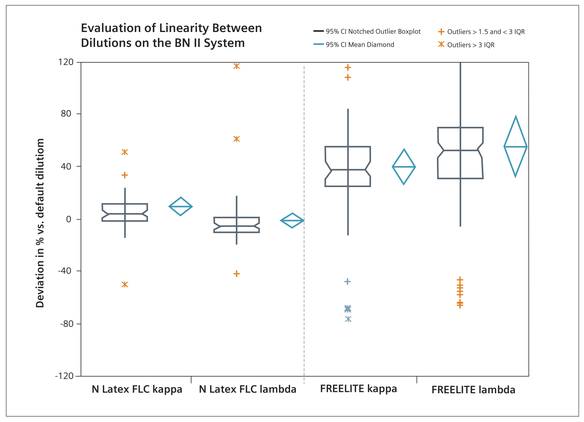

Figure 2. Evaluation of linearity between dilutions on the BN II system.

Both N Latex methods showed significantly better performance with respect to recovery of results between dilutions when using nephelometric BN II systems. Counting the outliers with recoveries outside of the ±50% range resulted in the following data:

Table 1. Recoveries between dilutions with deviations >±50%.

Number of outliers vs. total analyzed			
N Latex FLC Kappa	N Latex FLC Lambda	FREELITE Kappa	FREELITE Lambda
4/77	2/117	26/71	72/110

The distribution of results from the 397 samples from apparently healthy adults between the age of 16 and 89 years was calculated. Figures 3 and 4 contain the one-way analysis results of the data sets:

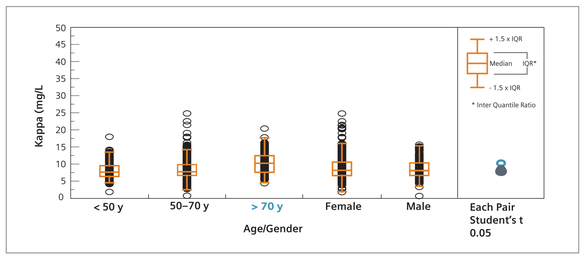

Figure 3. One-way analysis results, kappa.

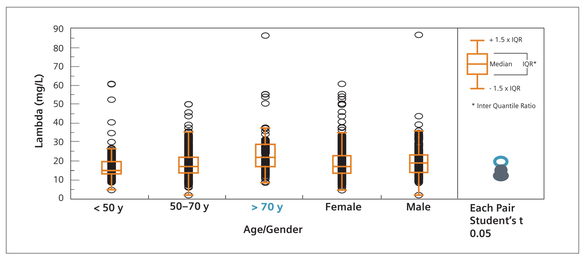

Figure 4. One-way analysis results, lambda.

Patients above the age of 70 revealed on average slightly higher values compared to all other age groups, but the κ/λ ratio remained unaffected. The results of the "Each Pair Student's t test" are an indicator of the significance of this observation. Gender-specific variations were not observed for either parameter.

Method-comparison results were evaluated using Passing-Bablok regression analysis as well as concordance tables. The regression results are plotted in a log/log format in order to spread data points more evenly and to allow inclusion of the individual reference-interval limits as dashed lines (Figures 5–7).

When applying the individual reference ranges of the methods to the data, Table 2 shows the agreement rates obtained for kappa, lambda, and the κ/λ ratio. For the κ/λ ratio values derived by the FREELITE0 methods, we applied the recommended renal range of 0.37–3.1 to the data of the kidney-disease group.

Table 2. Comparison agreement rates.

	Kappa	Lambda	κ/λ Ratio
Site 1	89.9%	77.0%	91.4%
Site 2	88.6%	81.7%	89.1%
Combined	89.2%	79.6%	90.1%

Conclusion

The N Latex FLC methods performed well in these studies. According to all evaluation sites, they have the potential to become a valuable tool as an aid in diagnosing and monitoring plasma cell proliferative diseases.

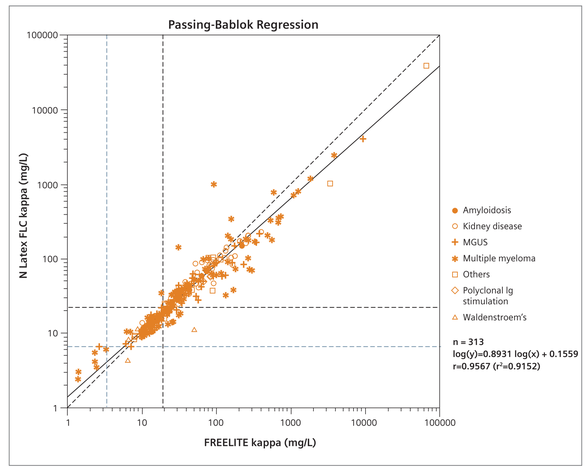

Figure 5.

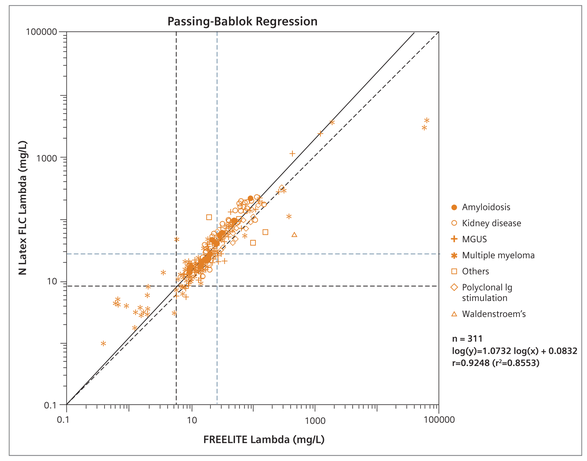

Figure 6.

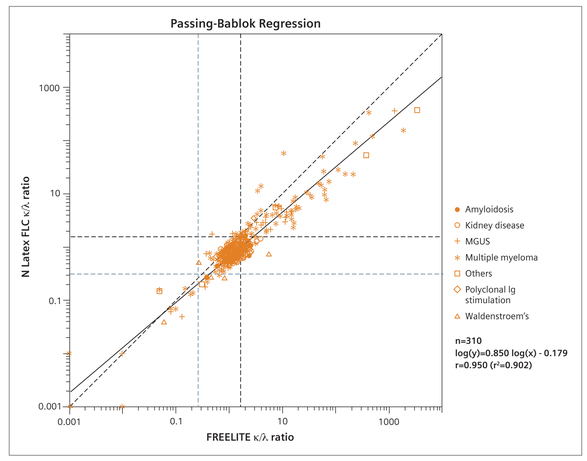

Figure 7.

References:

1. Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany
2. Foundation for Blood Research, Scarborough, ME, U.S.
3. Henry Ford Hospital, Detroit, MI, U.S.
4. University of Maryland School of Medicine, Baltimore, MD, U.S.
5. en.wikipedia.org/wiki/Serum_Free_Light_Chain_Measurement

† Not available for sale in the U.S. Not all product offerings are available in all countries.