Clinical Biochemistry 111 (2023) 47–53

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Accuracy of determination of free light chains (Kappa and Lambda) in plasma and serum by Swedish laboratories as monitored by external quality assessment

Niclas Rollborn [a], Jenny Jakobsson [a], Andrew Campbell [b], Gunnar Nordin [c], Mathias Karlsson [a,c], Anders Larsson [a], Kim Kultima [a,*]

[a] Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden
[b] NABAS AS, Ås, Norway
[c] Equalis AB, Uppsala, Sweden

ARTICLE INFO

Keywords:
External quality assessment scheme
Free light chains
Kappa
Lambda

ABSTRACT

Background: Free light chain (FLC) measurements are important in diagnosing monoclonal gammopathies. As FLC are heterogeneous, different reagents and instruments for measuring FLC concentrations may give diverging results that affect assessment of patients with monoclonal gammopathies. Here we investigated agreement between different FLC methods using data from the Swedish external quality assurance (EQA) programme.
Methods: The two main FLC assays, N Latex FLC (Siemens) and Serum Freelite (The Binding Site), using four nephelometric or turbidimetric instrument platforms, were compared. Results from 27 EQA rounds distributed to 11–16 Swedish hospital laboratories during 2015–2020 were investigated.
Results: The kappa (κ) FLC measurements deviated significantly over time, but when only nephelometry was used, deviation from the mean was lower (median ranges: −5% to 13 %). The CV was significantly higher for the Freelite assay (mean CV = 8.7) than for the N latex assay (mean CV = 5.7) ($p < 0.0001$). The coefficient of determination between all combinations of reagents and instrument platforms used was generally good (r^2 = 0.76–0.87), and the correlation slope acceptable (0.81–1.2). For lambda (λ) FLC measurements, no concordance between combinations of instruments and reagents is apparent, deviating between −40 % to + 48 % from the mean. The CV was significantly higher for the combination with nephelometry and the Freelite assay (CV mean = 13.9 %) than nephelometry and the N latex assay (CV mean = 9.9 %) ($p < 0.001$). The coefficient of determination varied between combinations of reagents and instrument platforms (r^2 = 0.59–0.89) and the slope ranged between 0.48 and 1.5. Significant differences between the two reagents used were sometimes noted.
Conclusions: Imprecision in λFLC affects the κFLC/λFLC ratio. This may be important in clinical assessment of patients, especially differentiating between monoclonal and polyclonal gammopathies.

1. Introduction

Monoclonal gammopathies are conditions characterised by clonal proliferation of plasma cells and the production of immunoglobulins and/or antibody fragments [1]. As these antibodies and antibody fragments are derived from the same cell clone, they will be identical, thus the definition monoclonal. Monitoring of monoclonal proteins is an essential part of the care of patients with plasma cell disorders [2]. Traditionally this has been performed by protein electrophoresis of serum or urine samples, often combined with immunofixation [1]. The effect of this two-step approach is that the test-turnaround time (TAT) usually is a few days.

Human antibodies are composed of two heavy chains and two light chains. The light chains are of either kappa (κ) or lambda (λ) type and are usually produced in excess compared to the heavy chains. The excess light chains are secreted into the blood and are usually referred to as free light chains (FLC). It is generally considered that λFLC are released as dimers and κFLC as either monomers or dimers [3,4]. FLCs in the bloodstream have a typical half-life of 2–6 h [5]. Normally, FLCs are freely filtered by kidney glomeruli and are reabsorbed from cells of the

* Corresponding author.
E-mail address: kim.kultima@medsci.uu.se (K. Kultima).

https://doi.org/10.1016/j.clinbiochem.2022.10.003
Received 30 June 2022; Received in revised form 7 September 2022; Accepted 7 October 2022
Available online 12 October 2022

proximal convoluted tubule.

Usually, we consider FLC as a monoclonal protein without a specific function. However, there are several biological attributes of FLCs including complement activation [6], mast cell activation [7] and as part of circulating immune complexes [8]. Today, methods to measure the concentration of FLCs are part of the assay panel of most Swedish hospital laboratories. Serum FLC assays are recommended by the International Myeloma Working Group and the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for initial diagnostic workup of multiple myeloma (MM) and other gammopathies including monoclonal gammopathies of undetermined significance (MGUS) [9].

Automated immunoassays for FLC measurements from the Binding Site became available approximately 20 years ago [10]. The assay time for these types of particle enhanced turbidimetric assays are usually around 10 min. These types of FLC methods can thus shorten the TAT in comparison with traditional serum/plasma protein electrophoresis for the detection of monoclonal gammopathies.

The aim of the present study was to report the method variations between different reagents and instrumentations used by routine laboratories. The study was based on the external quality materials sent out from the Swedish external quality assurance organisation Equalis (Uppsala, Sweden) during the time-period 2015–2020.

2. Materials and methods

2.1. EQA material

A total of 27 external quality assessment (EQA) rounds were distributed from Equalis to Swedish hospital laboratories during the period 2015–2020. Each EQA round consisted of one sample for the period 2015–2017 and two samples for the period 2018–2020. The EQA materials were mixtures of pooled plasma samples with elevated levels of C-reactive protein mixed with serum samples from healthy blood donors that were generated for the Equalis EQA scheme for plasma proteins. All donors were anonymised.

All samples were prepared at five different Clinical Chemistry Departments in hospitals in Sweden (Stockholm, Göteborg, Uppsala, Malmö, and Gävle). The mixed preparations were stored at −20 °C before being sent to Equalis. At Equalis, the samples were thawed, and 1 mL pipetted into 2 mL tubes with one 1 mL sample per tube. These EQA materials were then stored at −20 °C until distribution to participating laboratories, with distribution by post. A standard operating procedure, developed at Equalis, was sent with the EQA materials to the participating laboratories. The samples were stored refrigerated until analysis. These EQA materials were handled exactly as routine samples for analysis, and were analysed within five days from distribution.

2.2. Ethical approvement

The use of unidentified pooled plasma samples was approved by the ethical committee at Uppsala University (Dnr 01–367). Ethical approval regarding preparing EQA materials from surplus unidentified pooled blood donor plasma was not required.

2.3. Reagents and instruments

Freelite reagents from the Binding Site ltd., (Birmingham, UK) (BS) and N latex reagents from Siemens Healthineers AG., (Erlangen, DE) (S) were used by the study participants. Different instrument platforms from three separate suppliers, Beckman Coulter (BC), Roche Diagnostics (RD), Siemens Healthineers (SH) were used. The BC and RD platforms use turbidimetry and the SH platform uses nephelometry (Table 1.). The participating laboratories were anonymised during the 27 EQA rounds. In each round the number of participating laboratories ranged between 11 and 16. The combinations of instrument and reagent supplier for different participants are presented in Table 1.

2.4. Statistical analysis

Data from the different laboratories were combined then grouped for reagent type and instrument platform. Results that were generated following an automatic dilution step were removed from the statistical analyses. Statistical analyses were performed with the R software [11], using R packages ggplot2 [12] and tidyverse [13]. For all tests $p < 0.05$ was considered to indicate statistically significant differences.

Robust mean value and coefficient of variation (CV) were calculated according to ISO 13528 [14]. The CV for Siemens/Siemens and Siemens/Binding Site were compared using paired t-tests with test statistic t= (average difference)/(standard deviation). The deviations from the total mean according to time-period and instrument/reagent combinations were explored by two-way ANOVA. This was done for SH/S and SH/BS λFLC in the time period 2016:01–2017:06 and 2019:01–2021:01.

If the time period was not associated with a significant difference, then instruments/reagents were assumed to be responsible for significant differences identified. A pairwise Pearson correlation analysis was used to compare all methods and reagents (i.e., six combinations) using the ggplot2 package in R. Bland Altman plots were used for comparison of all combinations of methods and reagents (i.e., six combinations) by calculating the mean concentration obtained by each method and comparing it with the difference between concentrations in percentage.

3. Results

3.1. Kappa Chain, free (κFLC)

The overall robust mean concentration (±SD) of κFLC over the study period was 22.6 ± 10.6 mg/L, (range: 4.7–48 mg/L). The κFLC results of the EQA materials varied widely between different methods and reagents over time. At the end of 2015 (2015:06) the deviation was largest (−58 % to + 52 %), with a single result at 2016–04 (+98 %), excluded as this result was generated following an automatic instrument dilution (Fig. 1). From 2017:03 an apparent concordance between all methods/reagents was observed up until 2017:06 (−10 % to + 20 %). When two EQA materials were sent out per round starting 2018:01, the results reported deviated again (−59 % to + 35 %). Overall, the largest deviation was found for the BC/BS combination, consisting of only one or two laboratories (Fig. 1). Supplemental figure S1 shows results when only nephelometry had been used. From 2017:03 and onwards the deviation was low (median ranges: −5% to + 13 %), although high

Table 1
Instrument and reagent supplier combinations used for analysis.

Instrument platform		Reagents		Max no. participant labs per platform/reagent
Supplier	Method	Supplier	Type	
Beckman Coulter	Turbimetric	Binding site	Freelite	BC/BS = 2
Roche Diagnostics	Turbimetric	Binding site	Freelite	RD/BS = 2
Siemens Healthineers	Nephelometric	Binding site	Freelite	SH/BS = 7
Siemens Healthineers	Nephelometric	Siemens Healthineers	N Latex	SH/S = 7

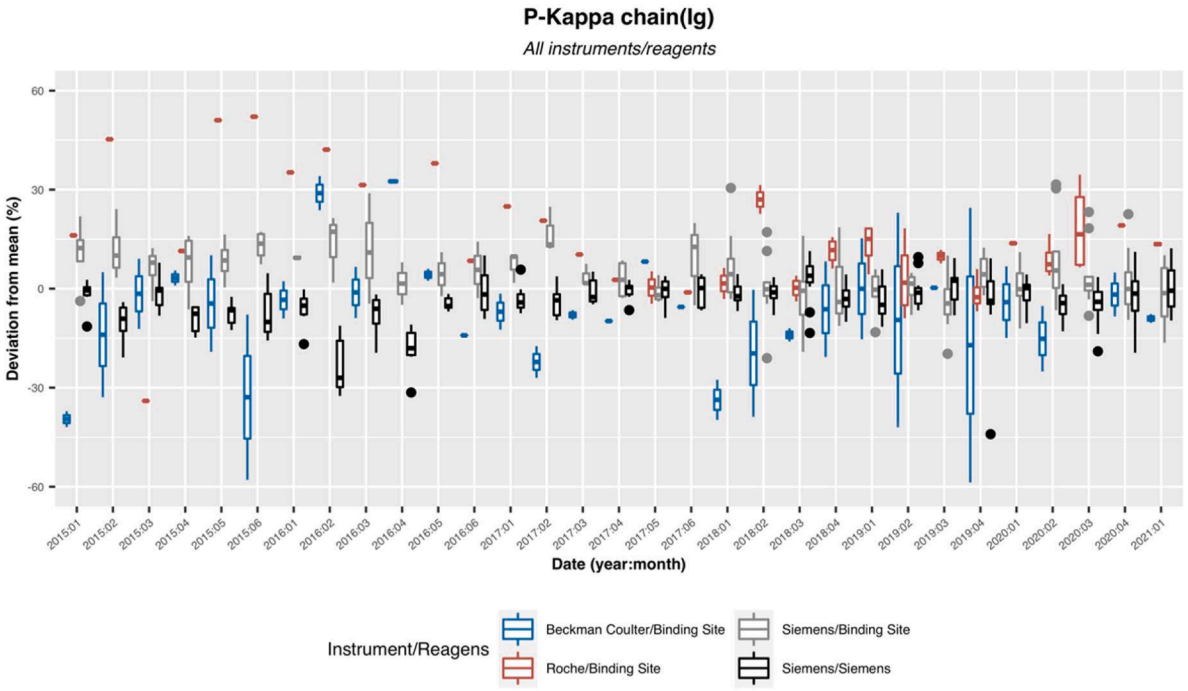

Fig. 1. Illustrates the deviation from the mean for the κFLC results of the EQA materials. The results varied widely between different methods and reagents over time. At the end of 2015 (2015:06) the deviation was largest (-58 % to + 52 %).

deviations occurred for single observations (−44 % to + 31 %). The CV was statistically significantly higher for SH/BS (mean CV = 8.7) than SH/S (mean CV = 5.7) ($p < 0.0001$) (Fig. 4A).

Generally, the coefficient of determination was good between all combinations of reagents and instrument platforms used for κFLC (r^2 = 0.76–0.87), and the correlation slope was acceptable, ranging between 0.81 and 1.2 (Supplemental Fig. S4A-S4F). The largest deviation of the correlation slope was found for the RD/BS combination. Corresponding Bland Altman plots are found in Supplemental Figure S7A-S7F.

3.2. *Lambda Chain, free (λFLC)*

The overall robust mean concentration of λFLC over the study period was 27.3 ± 10.04 mg/L, (range: 7.2–80.5 mg/L). Up until early 2016:01 the deviation was largest (−40 % to + 48 %), with a single result at 2016–01 (+109 %), excluded as this result was generated following an

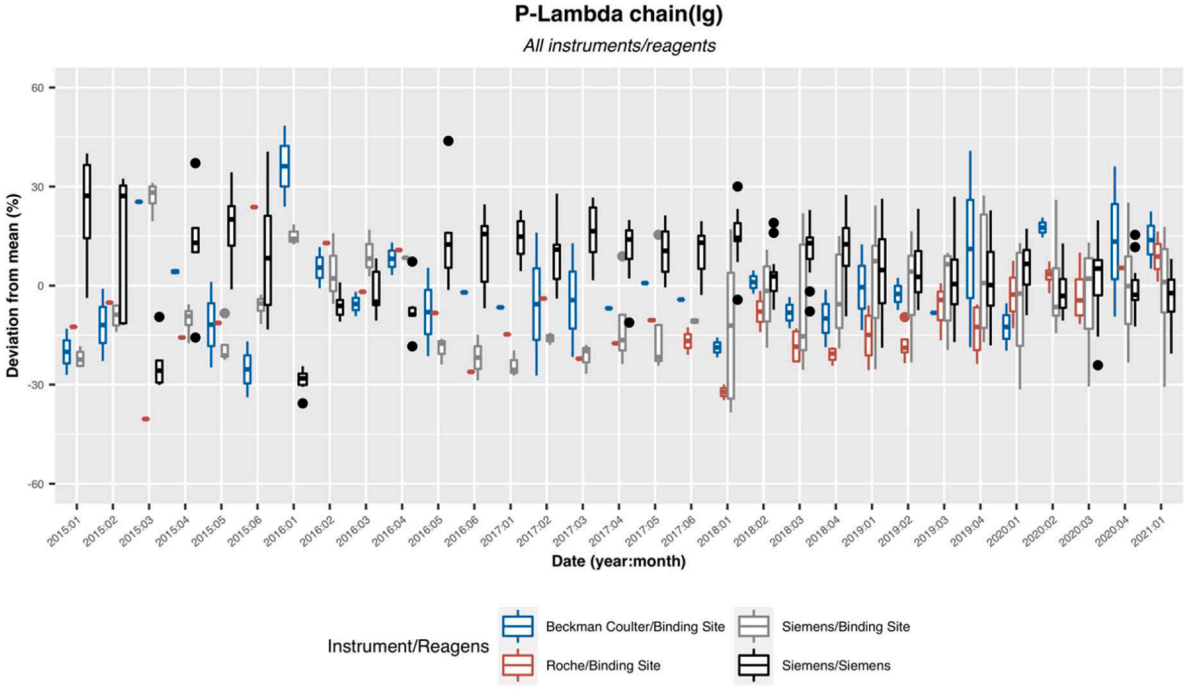

Fig. 2. Illustrates the deviation from the mean for the λFLC results of the EQA materials. In contrast to κFLC, there is no clear concordance between the combinations of instruments and reagents.

automatic instrument dilution. In contrast to κFLC, there is no clear concordance between the combinations of instruments and reagents (Fig. 2). Supplemental figure S2 shows results where only nephelometry has been used. In the time-period between 2016:05-2017:06 a statistically significant difference between the two reagents used was found ($p = 8.3e-16$). In contrast, from 2019:01 and onwards there was no significant difference between the reagents used.

Using a paired *t*-test, the CV was statistically significantly higher for SH/BS than SH/S ($p < 0.001$). There is a clear trend that the CV increases for SH/BS at concentrations > 25 mg/L and decreases for SH/S, and this is not dependent on the study period (not shown) (Fig. 4B). The CV mean for SH/BS was 13.9 % and SH/S was 9.9 %. The correlation coefficient was poorer between the combinations of reagents and instrument platforms used for λFLC, ranging between $r^2 = 0.59–89$ and the slope ranged between 0.48 and 1.5, compared to the κFLC results presented above (Supplemental Figure S5A-S5F). It is, again, the RD/BS combination resulting in the largest deviation of the correlation slope. As seen in the Bland Altman plots, the few measurements ($n = 5$) > 40 mg/L resulted in bias, seemingly independent of methods and reagents used (Supplemental Figure S8A-S8F).

3.3. Kappa chain / lambda Chain, free (κFLC/λFLC)

As expected, the ratio (κFLC/λFLC) also varies considerably between different methods and reagents used. Since the κFLC measurements had a better concordance than λFLC, it is logical that the κFLC/λFLC ratio resembles the λFLC observations (Fig. 3, Supplemental Figure S3 and S6A-S6F).

4. Discussion

Here we have investigated the agreement between different clinically used FLC methods using the Swedish external quality assurance (QA) for determination of κFLC and λFLC in serum and plasma. Our study has two major differences from other multicentre comparisons of the N latex and BS FLC assays, namely: 1. The plasma/serum samples analysed throughout our study are “normal” and do not include the higher FLC concentrations observed in MM etc. The levels of FLC expected should have meant that the automatic instrument dilution, and thus the non-linearity that has been described for FLC assays would not occur. 2. The variability of the two methods over time is the second observation, where the labs reported periods of time with concordance in the methods that are in agreement with the observations reported by others [15] and other periods of time that the methods significantly deviate. Although the results reported in this study deviated significantly over time for κFLC, a good correlation between all combinations of reagents and instrument platforms was found. In addition, a lower deviation from the mean value was found when only nephelometry was used. For λFLC measurements, concordance between the combinations of instruments and reagents was poorer than for the κFLC measurements. For some periods, there was also a statistically significant difference between the Freelite and N Latex FLC assays.

The imprecision of λFLC found in this study, directly affects the κFLC/λFLC ratio, which is important for the clinical assessment of individual patients. Method comparison of the clinically available assays for sFLC analysis has been reported, where good overall agreements for κFLC, λFLC, and FLC-ratio were observed [15], where the agreement became poorer as the analyte concentrations increased above 100 mg/ml. However, it is recognised that the sFLC assays used in clinical practice exhibit significant analytical limitations, including antigen excess, imprecision, lot-to-lot variations, and non-linear dose response curves [16–18]. In addition, the different assays report results that differ from each other and therefore the assays cannot be used interchangeably [17] and that patient follow-up should be performed using a single assay [19].

Some of the analytical differences reported have been ascribed to the lack of an international standard [20,21], thus making it difficult to calibrate the FLC assays correctly and consistently. To minimise this, the N Latex FLC assay has apparently been standardised to the Freelite reference ranges in healthy patient controls, [15,22]. Non-harmonised sFLC measurements have been previously suggested to be the cause of poor inter-laboratory agreement, with CVs ranging from 25 % to 70 % in EQA programs [20]. The results shown in this study for κFLC and the FLC ratio were typically higher for the Freelite than the N Latex FLC

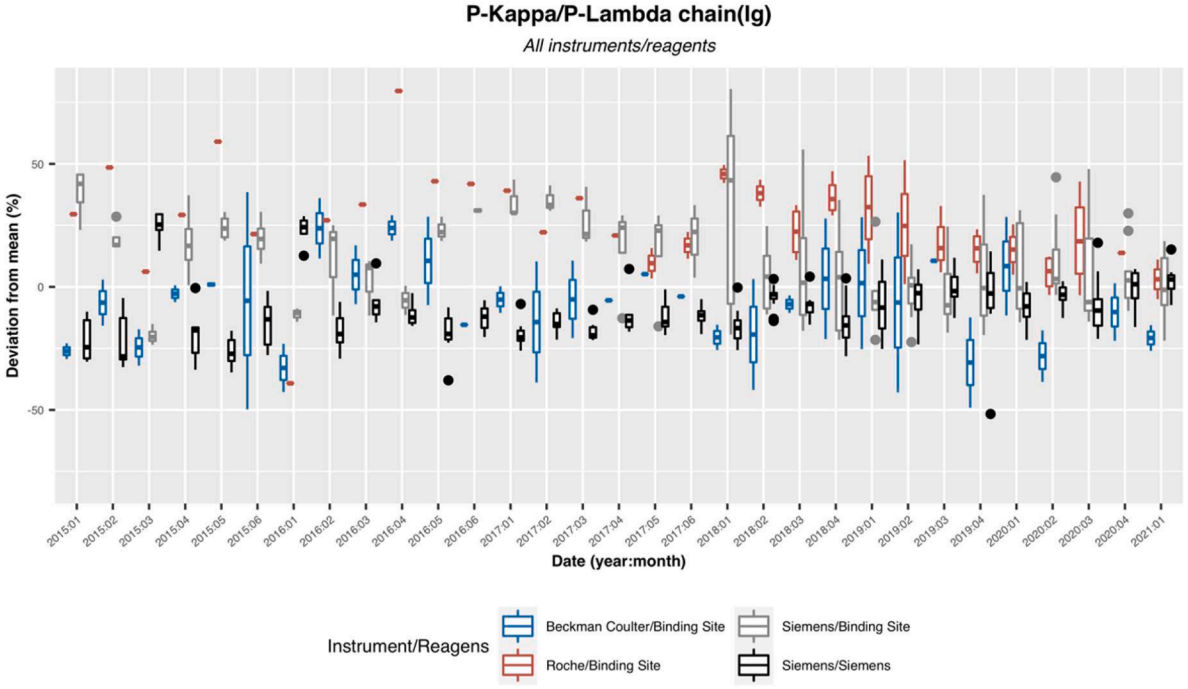

Fig. 3. Illustrates the ratio (κFLC/λFLC) of the EQA materials. The ratios vary considerably between different methods and reagents used. Since the κFLC measurements had a better concordance than λFLC, the κFLC/λFLC ratio resembles the λFLC observations.

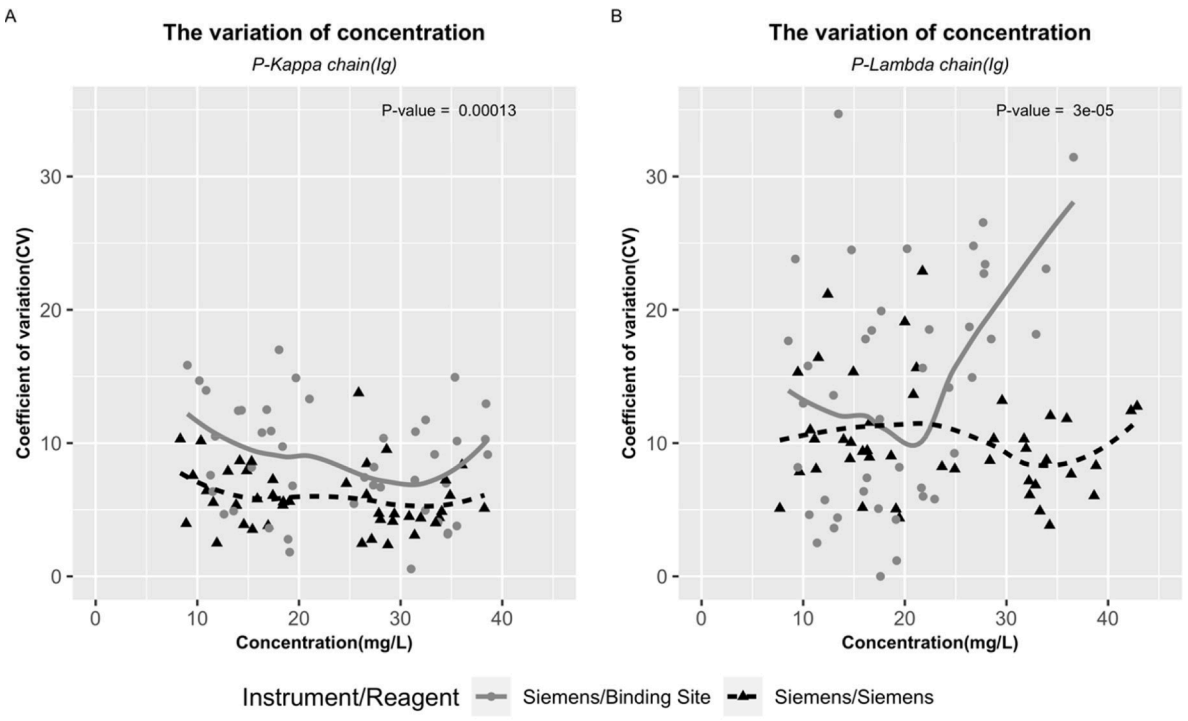

Fig. 4. A shows the CV results when only nephelometry had been used for the κFLC measurements. The CV was statistically significantly higher for SH/BS combination, in comparison to SH/S. Fig. 4B shows the CV results when only nephelometry had been used for the λFLC measurements. The CV was statistically significantly higher for SH/BS combination, in comparison to SH/S. There is a clear trend that the CV increases for SH/BS at concentrations > 25 mg/L and decreases for SH/S.

assay, with higher variability of the λFLC. These observations are in accordance with previously reported data [23,24].

There has been controversy in the literature regarding assays for serum FLC; however, there is consensus that measuring monoclonal proteins is not easy [25–27]. It is of note that CVs of over 100 % were observed in EQA trials using reagents calibrated with a common standard [27].

The results obtained in our study are similar to those obtained in previous multicentre studies in which the same analytical methods as we have used for measuring serum FLC were compared [23,28]. One study included both samples that were initially submitted for serum FLC analysis or were samples from healthy controls [28], whereas the other study included unselected routine samples submitted for serum FLC analysis [23]. In contrast with the study of Chae et al, our study did not include samples from patients with monoclonal gammopathies and therefore we should not have encountered the problem associated with auto-dilution [28].

The deviation from the mean oscillated for both κFLC and λFLC throughout the study period (2015–2020). However, when only nephelometry was used for κFLC, the deviation from the mean is small, from 2016:05 until the end of the study period. When only turbidimetry was used, this was not observed. Several different factors may explain these observations. For example, there were fewer turbidimetry measurements than nephelometry measurements; however, the precision of these two methods, in combination with how well the reagent antibody captures the epitope in any given sample, seems to have a greater role on the final result. FLC has many epitope variants, which means that some antibodies bind the antigen better in some patient samples and worse in other patient samples.

It is apparently more difficult to obtain concordant results for λFLC than for κFLC [29], as shown in this study. This could be explained by the fact that λFLC has more constant regions than κFLC [30], therefore, a broader spectrum of antibodies may be required for binding λFLC variants with variable immunoreactivity, dependent on the polymeric nature of the FLC [31]. Although we have no information on the polymeric nature of the FLCs used in this study, it is likely that the λFLC material used in the preparation of the EQA material exhibited a more diverse polymeric presentation than the κFLC material.

One problem reported for assays for FLCs is due to the FLCs being a heterogeneous group of molecules. The variable polymeric nature of FLC in serum has been documented since the 1970s [3,32]. Considerable variation has been shown, and polymeric forms often occur in blood, serum, plasma, and urine, for example as dimers, tetramers, or higher polymeric forms. For example, Sölling el al showed that a majority of the kappa chains in normal and anephric serum existed as monomers and non-covalently linked dimers, whereas the lambda chains mainly existed as stable, covalently linked dimers [3]. Abraham et al showed that monoclonal λ light chains may present as hexameric aggregates that cannot be cleared by renal excretion [33]. Kaplan et al showed that amyloid light-chain (AL) amyloidosis and multiple myeloma (MM) patients displayed an abnormally increased dimerization of monoclonal FLC, accompanied by higher clonality values of FLC dimers, as compared to that of monomers [34]. These abnormalities of FLC patterns were not observed in patients with monoclonal gammopathy of unknown significance (MGUS), smouldering myeloma (SMM), amyloid A (AA) amyloidosis, and healthy individuals.

However, unless anti-FLC antibodies recognise all molecular forms in an equimolar manner, sFLC assays will not provide equivalent results in all samples. Caponi et al highlighted the polymer associated discrepancy between the FLC assays [35]. Using gel chromatography and Western blotting, with and without disulfide bond reduction of the sample, the authors concluded that the Freelite assay may selectively recognize the dimeric λFLC in a patient with MM whereas the N Latex assay appeared to recognize the monomeric form. Caponi et al demonstrated that the Freelite assay recognised both kappa monomers and dimers, but preferably lambda dimers, and *N*-Latex preferably recognised kappa monomers and dimers, but only lambda monomers [31]. In our study, CV was higher for both κFLC and λFLC when the Freelite reagent was

used compared to N Latex. FLC assays using monoclonal Abs are probably more reproducible, but FLC assays using polyclonal Ab are known to detect a larger proportion of FLC hidden antigens [21].

For concentrations > 25 mg/L λFLC, the %CV increased using the Freelite assay, and this was not observed using N Latex. Higher variability of the Freelite compared to N Latex has been reported for λFLC by others [36,37]. Non-linearity of dilution is frequently observed when measuring FLC [16], however for this study, the analyte concentrations of 5–45 mg/L should not have been a source of variation, as extra dilution steps would not be required. It is interesting to note that the two most deviating results that were identified in this study, and excluded from the analysis (+98 % for kappa, and +109 % for lambda), were reported following an analyser automatic dilution step.

Lot-to-lot variation of the Freelite assay could be a factor in the observed variability of the λFLC results [38], and the analyser used could also be a contributing factor. In a study published by Pattenden et al [39] in which serum FLC values from 112 patients obtained with two analysers (Dade Behring BNII and the Olympus AU400) were compared, higher values were obtained with the BNII device than with the AU 400, with the difference being more marked for λ FLCs. The generation of local reference ranges are strongly recommended by the manufacturers of the assays.

In conclusion, EQA materials are valuable tools for monitoring method differences. There are clear method differences for FLC that are dependent on the reagents and instruments used. Considering that the decision limits for FLC do not take the methods into account, the choice of methods will influence patient treatments, and thus measurement methods have important clinical implications that medical practitioners should be made aware of. As the choice of reagents and assay platforms is expected to increase in the future, which could increase variations yet further, it is important to take this into account in the analytical laboratory, especially when moving to a new instrument or reagent. In addition, developers of platforms and reagents should attempt to minimise such variation. An important step forward would be developing an improved assay for the quantification of FLCs that has traceable calibration and for which the results exhibit less lot-to-lot reagent variation.

4.1. Limitations of the study

As this was a retrospective study, samples were not selected according to a predefined sampling plan, and as the participating labs were anonymised, difficulties were encountered in describing the changes in the methods over time. Also, samples from B-cell lymphoproliferative disorders (e.g. MM and MGUS patients) were not targeted. However, this retrospective sampling does provide information based on the type of samples routinely delivered for these analyses.

Another possible limitation of the study was the relatively low number of labs participating in this study. Nevertheless, this is more than included in some other multicentre studies [23], although fewer than others [28].

In addition, we had no information on the structure of the FLCs in the EQA material; this is relevant, as polymerisation factors may affect the assay results. Study bias may have been introduced due to differences in the number of samples analysed by the various labs, methodologies, and reagents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by Region Uppsala (ALF-grant and R&D funds) and Eurostars (grant number 114334).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2022.10.003.

References

[1] D.F. Keren, G. Bocsi, B.L. Billman, J. Etzell, J.D. Faix, S. Kumar, B. Lipe, C. McCudden, R. Montgomery, D.L. Murray, A.J. Rai, T.C. Redondo, L. Souter, C. B. Ventura, M.Q. Ansari, Laboratory detection and initial diagnosis of monoclonal gammopathies, Arch. Pathol. Lab. Med. 146 (2022) 575–590.

[2] S.V. Rajkumar, S. Vincent Rajkumar, M.A. Dimopoulos, A. Palumbo, J. Blade, G. Merlini, M.-V. Mateos, S. Kumar, J. Hillengass, E. Kastritis, P. Richardson, O. Landgren, B. Paiva, A. Dispenzieri, B. Weiss, X. LeLeu, S. Zweegman, S. Lonial, L. Rosinol, E. Zamagni, S. Jagannath, O. Sezer, S.Y. Kristinsson, J. Caers, S.Z. Usmani, J.J. Lahuerta, H.E. Johnsen, M. Beksac, M. Cavo, H. Goldschmidt, E. Terpos, R.A. Kyle, K.C. Anderson, B.G.M. Durie, J.F. San Miguel, International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma, The Lancet Oncology. 15 (2014) e538–e548. doi:10.1016/s1470-2045(14)70442-5.

[3] K. Sölling, Polymeric Forms of Free Light Chains in Serum from Normal Individuals and from Patients with Renal Diseases, Scand. J. Clin. Lab. Invest. 36 (1976) 447–452, https://doi.org/10.3109/00365517609054462.

[4] A. Solomon, Light chains of human immunoglobulins, Methods Enzymol. 116 (1985) 101–121.

[5] T.A. Waldmann, W. Strober, R.P. Mogielnicki, The renal handling of low molecular weight proteins. II. Disorders of serum protein catabolism in patients with tubular proteinuria, the nephrotic syndrome, or uremia, J. Clin. Invest. 51 (1972) 2162–2174.

[6] T.S. Jokiranta, A. Solomon, M.K. Pangburn, P.F. Zipfel, S. Meri, IMMUNOGLOBULIN LAMBDA LIGHT CHAIN DIMER LOI FROM A HUMAN PATIENT, (1999). Doi:10.2210/pdb2loi/pdb.

[7] F.A. Redegeld, M.W. van der Heijden, M. Kool, B.M. Heijdra, J. Garssen, A. D. Kraneveld, H. Van Loveren, P. Roholl, T. Saito, J.S. Verbeek, J. Claassens, A. S. Koster, F.P. Nijkamp, Immunoglobulin-free light chains elicit immediate hypersensitivity-like responses, Nat. Med. 8 (2002) 694–701.

[8] C. Napodano, K. Pocino, D. Rigante, A. Stefanile, F. Gulli, M. Marino, V. Basile, G. L. Rapaccini, U. Basile, Free light chains and autoimmunity, Autoimmun. Rev. 18 (2019) 484–492.

[9] S.K. Kumar, N.S. Callander, K. Adekola, L. Anderson, M. Baljevic, E. Campagnaro, J.J. Castillo, J.C. Chandler, C. Costello, Y. Efebera, M. Faiman, A. Garfall, K. Godby, J. Hillengass, L. Holmberg, M. Htut, C.A. Huff, Y. Kang, M. Hultcrantz, S. Larson, M. Liedtke, T. Martin, J. Omel, K. Shain, D. Sborov, K. Stockerl-Goldstein, D. Weber, J. Keller, R. Kumar, Multiple Myeloma, Version 3.2021, NCCN Clinical Practice Guidelines in Oncology, Journal of the National Comprehensive Cancer Network. 18 (2020) 1685–1717. doi:10.6004/jnccn.2020.0057.

[10] A.R. Bradwell, H.D. Carr-Smith, G.P. Mead, L.X. Tang, P.J. Showell, M.T. Drayson, R. Drew, Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine, Clin. Chem. 47 (2001) 673–680.

[11] Website, (n.d.). R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

[12] Ggplot2 : Elegant Graphics for Data Analysis - WorldCat, Https://www.worldcat.org ⟩ Isbnhttps://www.worldcat.org ⟩ Isbn. (n.d.). https://www.worldcat.org/isbn/978-3-319-24277-4.

[13] H. Wickham, M. Averick, J. Bryan, W. Chang, L.D. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T.L. Pedersen, E. Miller, S. M. Bache, K. Müller, J. Ooms, D. Robinson, D.P. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani, Welcome to the Tidyverse, J. Open Source Software. 4 (2019) 1686.

[14] Statistical methods for use in proficiency testing by interlaboratory comparison, (n.d.). doi:10.3403/30237515.

[15] C.K.A. Fleming, T. Swarttouw, C.M. de Kat Angelino, J.F.M. Jacobs, H. Russcher, Method comparison of four clinically available assays for serum free light chain analysis, Clin. Chem. Lab. Med. (CCLM) 58 (2019) 85–94, https://doi.org/10.1515/cclm-2019-0533.

[16] S.H.K. Murng, L. Follows, P. Whitfield, J.A. Snowden, K. Swallow, K. Green, R. Sargur, W. Egner, Defining the impact of individual sample variability on routine immunoassay of serum free light chains (sFLC) in multiple myeloma, Clin. Exp. Immunol. 171 (2013) 201–209.

[17] G. Cigliana, F. Gulli, C. Napodano, K. Pocino, E. De Santis, L. Colacicco, I. Cordone, L. Conti, U. Basile, Serum free light chain quantitative assays: Dilemma of a biomarker, J. Clin. Lab. Anal. 32 (2018) e22243.

[18] J.R. Tate, P. Mollee, Response to Letter to the Editor about immunochemical measurement of urine free light chains, Clin. Chem. Lab. Med. 57 (2019) e3–e4.

[19] G. Palladini, A. Jaccard, P. Milani, D. Lavergne, A. Foli, S. Bender, F. Lavatelli, T. Bosoni, V. Valentini, L. Pirolini, G. Ferraro, M. Basset, F. Russo, M. Nuvolone, R. Albertini, M. Cogne, G. Merlini, Circulating free light chain measurement in the diagnosis, prognostic assessment and evaluation of response of AL amyloidosis: comparison of Freelite and N latex FLC assays, Clin. Chem. Lab. Med. (CCLM). 55 (2017), https://doi.org/10.1515/cclm-2016-1024.

[20] J.F.M. Jacobs, J.R. Tate, G. Merlini, Is accuracy of serum free light chain measurement achievable? Clin. Chem. Lab. Med. 54 (2016) 1021–1030.

[21] M.S. Graziani, Measurement of free light chains - pros and cons of current methods, Clin. Chem. Lab. Med. 54 (2016) 1015–1020.

[22] H. Te Velthuis, M. Drayson, J.P. Campbell, Measurement of free light chains with assays based on monoclonal antibodies, Clin. Chem. Lab. Med. 54 (2016) 1005–1014.
[23] R.J. Lock, R. Saleem, E.G. Roberts, M.J. Wallage, T.J. Pesce, A. Rowbottom, S. J. Cooper, E.D. McEvoy, J.L. Taylor, S. Basu, A multicentre study comparing two methods for serum free light chain analysis, Ann. Clin. Biochem. 50 (2013) 255–261.
[24] X. Bossuyt, M. Delforge, M. Reynders, D. Dillaerts, B. Sprangers, K. Fostier, K. Poesen, M. Vercammen, Diagnostic thresholds for free light chains in multiple myeloma depend on the assay used, Leukemia (2017), https://doi.org/10.1038/leu.2017.335.
[25] J. Sheldon, Free light chains, Ann. Clin. Biochem. 44 (2007) 503–505, https://doi.org/10.1258/000456307782268183.
[26] J. Forsyth, P. Hill, Serum free light chains, Ann. Clin. Biochem. 45 (2008) 444–5; author reply 445–6.
[27] J. Sheldon, Free light chains: author's response, Ann. Clin. Biochem. 45 (2008) 445–446, https://doi.org/10.1258/acb.2008.072542.
[28] H. Chae, J. Yoo, J. Park, K. Cha, J.J. Lee, B. Cho, M. Kim, Y. Kim, Analysis of a 6-year pilot external quality assurance survey of free light chain using Sigma metrics, J. Lab. Med. 43 (2019) 235–242, https://doi.org/10.1515/labmed-2018-0175.
[29] Y. Yang, X. Han, G. Zheng, Z. Cai, Comparison of two serum free light chain assays for the diagnosis of primary plasma cell malignant proliferative disease, Health Sci. Rep. 2 (2019) e113.
[30] T.W. Mak, M.E. Saunders, The immune response, Shock. 27 (2007) 220–221, https://doi.org/10.1097/01.shk.0000258367.85587.7b.
[31] L. Caponi, E. Koni, N. Romiti, A. Paolicchi, M. Franzini, Different immunoreactivity of monomers and dimers makes automated free light chains assays not equivalent, Clin. Chem. Lab. Med. 57 (2018) 221–229.
[32] K. SøLling, Light chain polymerism in normal individuals, in patients with severe proteinuria and in normals with inhibited tubular protein reabsorption by lysine, Scand. J. Clin. Lab. Invest. 40 (1980) 129–134, https://doi.org/10.3109/00365518009093014.
[33] R.S. Abraham, M.C. Charlesworth, B.A.L. Owen, L.M. Benson, J.A. Katzmann, C. B. Reeder, R.A. Kyle, Trimolecular complexes of lambda light chain dimers in serum of a patient with multiple myeloma, Clin. Chem. 48 (2002) 1805–1811.
[34] B. Kaplan, S. Golderman, B. Aizenbud, K. Esev, O. Kukuy, M. Leiba, A. Livneh, I. Ben-Zvi, Immunoglobulin-free light chain monomer-dimer patterns help to distinguish malignant from premalignant monoclonal gammopathies: A pilot study, Am. J. Hematol. 89 (2014) 882–888, https://doi.org/10.1002/ajh.23773.
[35] L. Caponi, M. Franzini, E. Koni, S. Masotti, M. Petrini, A. Paolicchi, Discrepancy between FLC assays: only a problem of quantification? Clin. Chem. Lab. Med. (CCLM). 54 (2016) https://doi.org/10.1515/cclm-2015-1262.
[36] A. Schieferdecker, S. Hörber, M. Ums, B. Besemer, C. Bokemeyer, A. Peter, K. Weisel, Comparison of three different serum-free light-chain assays—implications on diagnostic and therapeutic monitoring of multiple myeloma, Blood Cancer J. 10 (2020), https://doi.org/10.1038/s41408-019-0267-8.
[37] C.J. Pretorius, S. Klingberg, J. Tate, U. Wilgen, J.P.J. Ungerer, Evaluation of the N Latex FLC free light chain assay on the Siemens BN analyser: precision, agreement, linearity and variation between reagent lots, Ann. Clin. Biochem. 49 (2012) 450–455, https://doi.org/10.1258/acb.2012.011264.
[38] J.R. Tate, P. Mollee, G. Dimeski, A.C. Carter, D. Gill, Analytical performance of serum free light-chain assay during monitoring of patients with monoclonal light-chain diseases, Clin. Chim. Acta. 376 (2007) 30–36.
[39] R.J. Pattenden, S.Y. Rogers, P.R. Wenham, Serum free light chains; the need to establish local reference intervals, Ann. Clin. Biochem. 44 (2007) 512–515.