Serum Free Light Chain Analysis: persisting limitations with new kids on the block

Lieve Van Hoovels[1,2*#], Martine Vercammen[3,4*], Louis Nevejan[1,5], Margot Cornette[6,7], Pieter-Jan Briers[3,5], Dries Deeren[8], Jan Van Droogenbroeck[9], Karel Fostier[10], Dieter De Smet[6]

*Shared first authorship

[1] Department of Laboratory Medicine, OLV Hospital Aalst, Aalst, Belgium

[2] Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

[3] Department of Laboratory Medicine, AZ Sint-Jan Hospital Brugge - Oostende AV, Bruges, Belgium

[4] Research group REIM, Vrije Universiteit Brussel (VUB), Brussels, Belgium

[5] Department of Laboratory Medicine, University Hospital Leuven, Leuven, Belgium

[6] Department of Laboratory Medicine, AZ Delta Hospital, Roeselare, Belgium

[7] Department of Laboratory Medicine, University Hospital Ghent, Ghent, Belgium

[8] Department of Hematology, AZ Delta Hospital, Roeselare, Belgium

[9] Department of Hematology, AZ Sint-Jan Hospital Brugge - Oostende AV, Bruges, Belgium

[10] Department of Hematology, OLV Hospital, Aalst, Belgium

#Corresponding author:
- Lieve Van Hoovels, OLV Hospital Aalst, Moorselbaan 164, 9300 Aalst, BELGIUM (e-mail: Lieve.Van.Hoovels@olvz-aalst.be)
- Martine Vercammen, AZ Sint-Jan Hospital Brugge-Oostende AV, Ruddershove 10, 8000 Brugge, BELGIUM (e-mail: martine.vercammen@azsintjan.be
- Dieter De Smet, AZ Delta Hospital, Deltalaan 1, 8800 Roeselare, BELGIUM (e-mail: dieter.desmet@azdelta.be)

Supplemental Tables

Supplemental data Table 1. Overview of assay characteristics of Diazyme FLC, Freelite FLC and Sebia FLC ELISA assays included in the study protocol.

	Diazyme FLC assay		Freelite FLC assay		Sebia FLC ELISA
Analytical platform	cobas c501	cobas c503	Optilite	cobas c501	AP22 ELITE
Measuring principle	Turbidimetry		Turbidimetry		ELISA sandwich
Antisera	κ: Polyclonal (rabbit, goat) λ: Polyclonal (rabbit)		κ: Polyclonal (sheep) λ: Polyclonal (sheep)		κ: Polyclonal (rabbit) λ: Polyclonal (rabbit)
Reference range*	κ: 2.4-20.7 mg/L λ: 4.2-27.7 mg/L κ/λ: 0.22-1.74		κ: 3.3-19.4 mg/L λ: 5.71-26.3 mg/L κ/λ: 0.3-1.7; κ/λ_{CKD}: 0.37-3.1		κ: 3.3-19.4 mg/L λ: 5.71-26.3 mg/L κ/λ: 0.3-1.7
LOQ (mg/L)	κ: 2.3 λ: 3.5	κ: 4.5 λ: 6.1	κ: 0.8 λ: 0.7	κ: 0.8 λ: 0.7	κ: 1.5 λ: 1.5
Measuring range (mg/L)	κ: 2.3-150 λ: 3.5-200	κ: 4.5-150 λ: 6.1-200	κ: 2.9-25.3 λ: 5.2-139	κ: 3.7-56.2 λ: 5.6-74.8	κ: 1.5-90 λ: 1.5-90
Serum dilution**	κ: 1/1-**1/20** λ: 1/1-**1/20**	κ: 1/1-**1/20** λ: 1/1-**1/20**	κ: 1/2-**1/10**-1/100000 λ: 1/1-**1/8**-1/80000	κ: 1/1-**1/5**- 1/500 λ: 1/1-**1/8**-1/800	κ:1/250- **1/1000**-1/10000-1/100000 λ: 1/250- **1/1000**-1/10000-1/100000

*Reference ranges for κ and λ sFLC are reported as 2.5th-97.5th percentile. Reference ranges for κ/λ ratio as min-max. κ/λ_{CKD} is adapted reference range for patients with chronic kidney disease (CKD)
**the standard routine dilution performed is highlighted in bold

Supplemental data Table 2. Analytical performance criteria of the different sFLC applications, evaluated using the criteria posed in (21).

		n	Mean (mg/L)	CV_{wr} (%)	CV_{br} (%)	CV_t (%)	Bias (%)	TE (%)
Freelite assay/ Optilite analyzer	**Serum κ FLC**							
	Commercial QC Low	20	14,5	3,9	4,4	**5,9**	3,8	**13,5**
	Commercial QC High	20	27,6	5,8	1,4	**5,9**	-0,8	10,6
	Serum Pool Low	20	9,3	2,6	7,0	**7,4**	NA	NA
	Serum Pool Medium	20	22,9	2,1	5,2	**5,6**	NA	NA
	Serum Pool High	20	103,8	4,5	5,9	**7,4**	NA	NA
	Serum λ FLC							
	Commercial QC Low	20	29,9	3,9	1,8	4,3	1,4	8,6
	Commercial QC High	20	53,8	4,4	1,7	4,7	4,7	12,6
	Serum Pool Low	20	7,5	6,2	4,0	**7,4**	NA	NA
	Serum Pool Medium	20	24,7	2,0	3,4	3,9	NA	NA
	Serum Pool High	20	113,3	6,5	3,7	**7,5**	NA	NA
Freelite asay/ cobas c501 analyzer	**Serum κ FLC**							
	Commercial QC Low	20	16,0	1,9	2,3	3,0	**13,7**	**18,7**
	Commercial QC High	20	32,7	2,6	0,1	2,6	**10,8**	**15,1**
	Serum Pool Low	20	8,9	6,3	2,5	**6,8**	-4,6	**15,8**
	Serum Pool Medium	20	22,1	3,6	1,6	**4,0**	-3,6	10,1
	Serum Pool High	20	90,8	3,2	3,4	**4,7**	-12,6	**20,3**
	Serum λ FLC							
	Commercial QC Low	20	24,3	2,1	0,3	2,1	-3,0	6,5
	Commercial QC High	20	50,7	2,3	1,3	2,6	-3,2	7,5
	Serum Pool Low	20	10,1	2,3	1,1	2,6	**33,8**	**38,1**
	Serum Pool Medium	20	24,5	1,7	2,1	2,7	-1,0	5,5
	Serum Pool High	20	113,1	5,1	2,2	**5,5**	-0,2	9,3
Diazyme FLC assay/ cobas c501 analyzer	**Serum κ FLC**							
	Commercial QC Low	20	16,6	5,8	3,7	**6,9**	-0,8	12,2
	Commercial QC High	20	31,6	1,7	1,7	2,4	-2,6	6,6
	Serum Pool Low	20	9,1	9,6	2,7	**9,9**	-1,9	**18,3**
	Serum Pool Medium	20	24,3	2,2	1,9	2,9	6,0	10,8
	Serum Pool High	20	89,4	1,5	2,7	3,1	**-13,9**	**19,0**
	Serum λ FLC							
	Commercial QC Low	20	17,8	5,2	1,9	**5,5**	-2,8	11,9
	Commercial QC High	20	37,8	4,1	1,2	4,2	-4,4	11,4
	Serum Pool Low	20	5,9	15,6	9,7	**18,3**	-21,4	51,7
	Serum Pool Medium	20	21,5	4,5	1,1	4,6	**-13,2**	**20,8**
	Serum Pool High	20	41,4	3,8	1,9	4,2	**-63,4**	**70,4**
Diazyme FLC assay/ cobas c503 analyzer	**Serum κ FLC**							
	Commercial QC Low	20	16,2	2,7	3,6	**4,5**	-2,7	10,1
	Commercial QC High	20	31,6	0,9	1,7	1,9	-2,7	5,8
	Serum Pool Low	20	8,6	3,6	9,6	**10,2**	-7,9	**24,7**
	Serum Pool Medium	20	24,5	1,7	4,7	**5,0**	7,2	**15,5**
	Serum Pool High	20	93,3	10,1	11,9	**15,6**	-10,2	**36,0**
	Serum λ FLC							
	Commercial QC Low	20	18,7	4,0	1,6	4,3	2,2	9,3
	Commercial QC High	20	40,9	1,2	2,4	2,7	3,5	8,0
	Serum Pool Low	20	5,5	10,7	9,2	**14,2**	-26,5	49,9
	Serum Pool Medium	20	23,0	3,0	1,1	3,2	-6,8	12,1
	Serum Pool High	20	47,8	2,1	2,7	3,4	**-57,8**	**63,4**
Sebia FLC ELISA assay/ AP22 analyzer	**Serum κ FLC**							
	Commercial QC Low	18	13,4	11,1	5,8	**12,5**	1,1	**21,8**
	Commercial QC High	20	51,1	10,6	3,2	**11,0**	11,7	**29,9**
	Serum Pool Low	20	8,0	9,5	2,3	**9,8**	-14,6	**30,7**
	Serum Pool Medium	20	17,2	8,5	3,7	**9,3**	-25,1	**40,3**
	Serum Pool High	20	44,1	6,6	4,8	**8,2**	-57,5	**71,0**
	Serum λ FLC							
	Commercial QC Low	20	21,0	13,0	11,5	**17,3**	-5,4	**34,0**
	Commercial QC High	20	40,6	12,4	11,8	**17,1**	-8,2	**36,4**
	Serum Pool Low	20	7,3	19,4	9,3	**21,6**	-3,6	**39,2**
	Serum Pool Medium	20	19,7	11,6	4,1	**12,3**	-20,3	**40,6**
	Serum Pool High	20	26,9	9,1	12,1	**15,2**	-76,2	**101,3**

$I_{optimum}$ κ/λ = 1,1%/1,8%; $I_{desirable}$ κ/λ = 2,2%/3,5%; $I_{minimum}$ κ/λ = 3,2%/5,3%
$Bias_{optimum}$ κ/λ = 2,7%/3,9%; $Bias_{desirable}$ κ/λ = 5,4%/7,7%; $Bias_{minimum}$ κ/λ = 8,0%/11,6%
$TEa_{optimum}$ κ/λ = 4,5% / 6,7%; $TEa_{desirable}$ κ/λ = 8,9% / 13,5%; $TEa_{minimum}$ κ/λ = 13,4% / 20,2%; $TEa_{intralab}$ = 25%; $TEa_{peer/insert}$ = 30%

Supplemental data Table 3. Overview of the coefficient of determination (r^2) obtained for the different sFLC applications by analysis of three serially dilution of three samples containing monoclonal κ or λ sFLC and one sample containing polyclonal κ and λ sFLC. Every sample of the linearity dilution series was analyzed in three different analysis runs. R^2 for every analysis run and global mean R^2 are presented.

Sample	Freelite assay/ Optilite analyzer				Freelite assay/ cobas c501 analyzer				Diazyme FLC assay cobas c501 analyzer				Diazyme FLC assay/ cobas c503 analyzer				Sebia FLC ELISA assay/ AP22 analyzer			
	Day 1	Day 2	Day 3	mean	Day 1	Day 2	Day 3	mean	Day 1	Day 2	Day 3	mean	Day 1	Day 2	Day 3	mean	Day 1	Day 2	Day 3	mean
KAPPA - 1	0,9988	0,9943	0,9863	0,9996	0,9736	0,9721	0,9753	0,9737	0,9225	0,8226	0,9502	0,9674	0,9989	0,9932	0,9872	0,9983	0,9743	0,9913	0,9979	0,9947
KAPPA - 2	0,9957	0,9957	0,9989	0,9974	0,9972	0,9980	0,9932	0,9979	0,9889	0,9391	0,9926	0,9938	0,9969	0,9846	0,9882	0,9970	0,9953	0,9977	0,9856	0,9951
KAPPA - 3	0,9983	0,9965	0,9985	0,9984	0,9988	0,9944	0,9983	0,9982	0,9991	0,9950	0,9969	0,9974	0,9943	0,9872	0,9406	0,9868	0,9811	0,9782	0,9868	0,9852
LAMBDA - 1	0,9969	0,9945	0,9952	0,9960	0,9978	0,9994	0,9983	0,9994	0,9798	0,9811	0,9797	0,9803	0,9969	0,9921	0,9684	0,9878	0,9815	0,9872	0,9932	0,9901
LAMBDA - 2	0,9924	0,9917	0,9960	0,9937	0,9930	0,9911	0,9980	0,9955	0,9933	0,9576	0,9893	0,9885	0,9936	0,9942	0,9889	0,9924	0,9716	0,9831	0,9615	0,9750
LAMBDA - 3	0,9991	0,9977	0,9984	0,9988	0,9861	0,9974	0,9982	0,9982	0,9950	0,9962	0,9973	0,9971	0,9954	0,9984	0,9826	0,9949	0,8903	0,9884	0,8907	0,9375
POLYCLONAL - KAPPA	0,9976	0,9877	0,9917	0,9967	0,9949	0,9811	0,9973	0,9954	0,9951	0,9988	0,9978	0,9976	0,9927	0,9972	0,9946	0,9966	0,9923	0,9956	0,9961	0,9956
POLYCLONAL - LAMBDA	0,9982	0,9987	0,9954	0,9983	0,9692	0,9897	0,9663	0,9860	0,9983	0,9992	0,9995	0,9996	0,9994	0,9995	0,9983	0,9997	0,9992	0,9904	0,9650	0,9978

Legend (r^2)				
<0,9	0,90-0,95	0,95-0,97	0,97-0,99	>0,99

Supplemental Table 4. Method Comparison (agreement) between different methods (Y) versus Freelite assay on Optilite analyzer (X). Method comparison is performed by Passing-Bablok regression analysis (statistically significant slopes and intercepts highlighted in bold) and diagnostic agreement analysis by Cohen's kappa coefficient for concordance. r_s, Spearman's coefficient of rank correlation; CI, confidence interval.

	n	r_s (95% CI)	Slope (95% CI)	Intercept (95% CI)	Cohen's kappa (95% CI)
Selected samples for method comparison					
Serum κ sFLC					
Freelite assay/ cobas c501 analyzer	120	0.998 (0.997 to 0.999)	**0.96 (0.92 to 0.98)**	0.01 (-0.27 to 0.58)	0.95 (0.89 to 1.00)
Diazyme FLC assay/ cobas c501 analyzer	120	0.991 (0.987 to 0.994)	1.00 (0.96 to 1.05)	**0.87 (0.14 to 1.68)**	0.98 (0.95 to 1.00)
Diazyme FLC assay/ cobas c503 analyzer	120	0.990 (0.986 to 0.993)	1.02 (0.98 to 1.07)	0.51 (-0.27 to 1.58)	0.98 (0.95 to 1.00)
Sebia FLC ELISA assay/ AP22 analyzer	120	0.984 (0.977 to 0.989)	**0.47 (0.45 to 0.50)**	**2.54 (1.82 to 3.20)**	0.76 (0.66 to 0.85)
Serum λ sFLC					
Freelite assay/ cobas c501 analyzer	120	0.996 (0.994 to 0.997)	**0.88 (0.86 to 0.91)**	**3.05 (2.61 to 3.52)**	0.80 (0.72 to 0.87)
Diazyme FLC assay/ cobas c501 analyzer	120	0.971 (0.958 to 0.979)	**0.90 (0.81 to 1.00)**	1.40 (-0.38 to 2.67)	0.78 (0.69 to 0.86)
Diazyme FLC assay/ cobas c503 analyzer	120	0.971 (0.958 to 0.980)	1.01 (0.90 to 1.12)	0.25 (-1.99 to 2.18)	0.80 (0.72 to 0.89)
Sebia FLC ELISA assay/ AP22 analyzer	120	0.940 (0.914 to 0.958)	**0.38 (0.31 to 0.45)**	**5.85 (4.66 to 7.93)**	0.83 (0.75 to 0.91)
Serum κ/λ ratio					
Freelite assay/ cobas c501 analyzer	120	0.976 (0.966 to 0.983)	**0.89 (0.86 to 0.94)**	**0.01 (0.00 to 0.02)**	0.91 (0.85 to 0.96)
Diazyme FLC assay/ cobas c501 analyzer	120	0.977 (0.968 to 0.984)	0.94 (0.87 to 1.02)	**0.08 (0.02 to 0.13)**	0.77 (0.69 to 0.86)
Diazyme FLC assay/ cobas c503 analyzer	120	0.978 (0.968 to 0.984)	0.96 (0.90 to 1.01)	**0.05 (0.00 to 0.09)**	0.82 (0.74 to 0.89)
Sebia FLC ELISA assay/ AP22 analyzer	120	0.960 (0.943 to 0.972)	**0.54 (0.50 to 0.62)**	**0.13 (0.09 to 0.18)**	0.84 (0.77 to 0.92)

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Supplemental data Table 5. Selection of results (measured with Diazyme FLC assays on cobas c501 analyzer and cobas c503 analyzer) with expected κ antigen excess and expected λ antigen excess with the conventional method of determining antigen excess revealing a relation between the high and standard dilution versus the extrapolated method (revealing a ratio between the extrapolated sFLC value and the measured sFLC result).

Sample number	Multiple myeloma	Cobas	Measured (mg/L) Standard dilution	High dilution	Ratio high/standard dilution	Extrapolated (mg/L)	Ratio extrapolated/measured
9	IgA λ	c501	146.3	1110	7.6	403.6	2.8
		c503	157	1310	8.3	452.2	2.9
55	LC λ	c501	97.5	970	9.9	558.6	5.7
		c503	97.9	1000	10.2	626.4	6.4
64	IgG λ	c501	178.3	760	4.3	786.8	4.4
		c503	196	764	3.9	882.8	4.5
25	κ LCMM	c501	5470	ND	ND	13000.5	2.4
		c503	2073	10700	5.2	13390	6.5

Abbreviation: ND, not determined

Supplemental Figures

Supplemental data Figure 1. sFLC linearity (5 dilutions/sample) for monoclonal κ (three different samples) and polyclonal κ (one sample) for the various sFLC assays included. Results of mean concentrations (determined at 3 different days) are included only.


Linearity – κ FLC
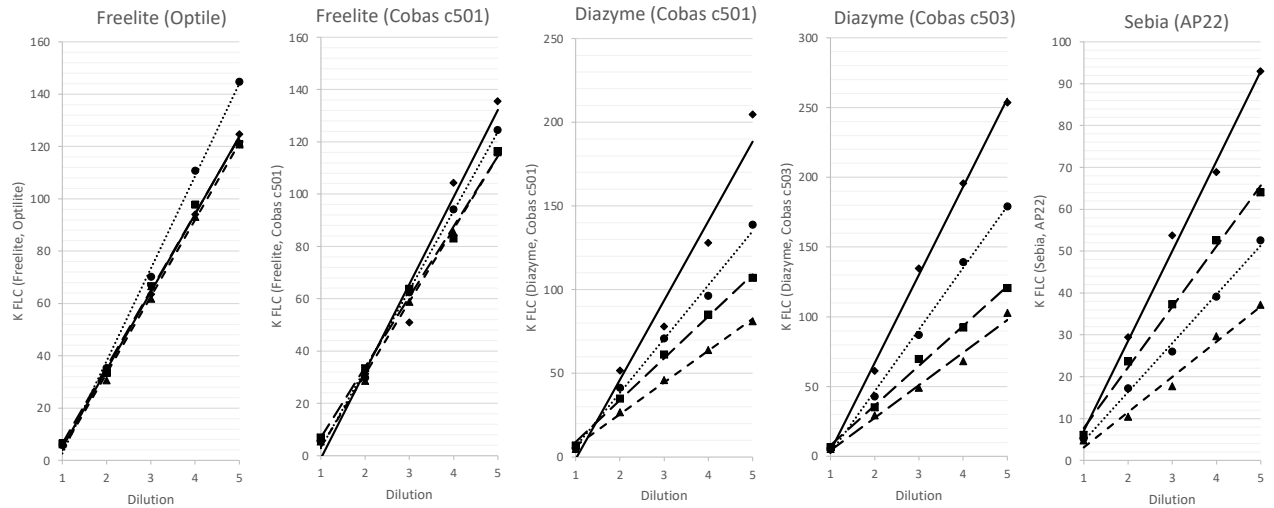
	Sample	Mean R²	Mean R²	Mean R²	Mean R²	Mean R²
━━◆	Kappa 1	0,9996	0,9737	0,9674	0,9983	0,9947
······●	Kappa 2	0,9974	0,9979	0,9938	0,9970	0,9951
---▲	Kappa 3	0,9984	0,9982	0,9974	0,9868	0,9852
— ■	Polyclonal Kappa	0,9967	0,9954	0,9976	0,9966	0,9956

Supplemental data Figure 2. sFLC linearity (5 dilutions/sample) for monoclonal λ (three different samples) and polyclonal λ (one sample) for the various sFLC assays included. Results of mean concentrations (determined at 3 different days) are included only.

**Linearity – λ FLC**
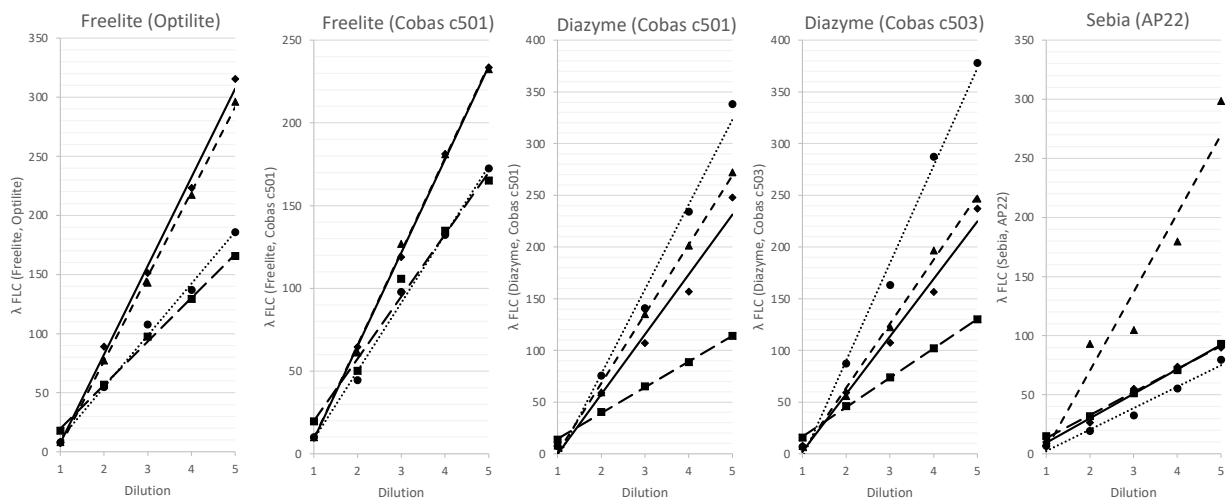
	Sample	Mean R²	Mean R²	Mean R²	Mean R²	Mean R²
━━◆	LAMBDA 1	0,9960	0,9994	0,9803	0,9878	0,9901
······●	LAMBDA 2	0,9937	0,9955	0,9885	0,9924	0,9750
---▲	LAMBDA 3	0,9988	0,9982	0,9971	0,9949	0,9375
— ■	POLYCLONAL LAMBDA	0,9983	0,9860	0,9996	0,9997	0,9978

Supplemental Figure 3. Relative Bland Altman analysis of lower range results (< 50 mg/L) of κ sFLC lower range for the Freelite assay on Optilite analyzer versus Freelite assay on cobas c501 analyzer (Figure 3.A), Diazyme FLC assay on cobas c501 analyzer (Figure 3.B), Diazyme FLC assay on cobas c503 analyzer (Figure 3.C) and Sebia κ FLC ELISA on AP22 analyzer (Figure 3.D). Dotted line represents line of identity. Mean differences (95% confidence interval) are presented.
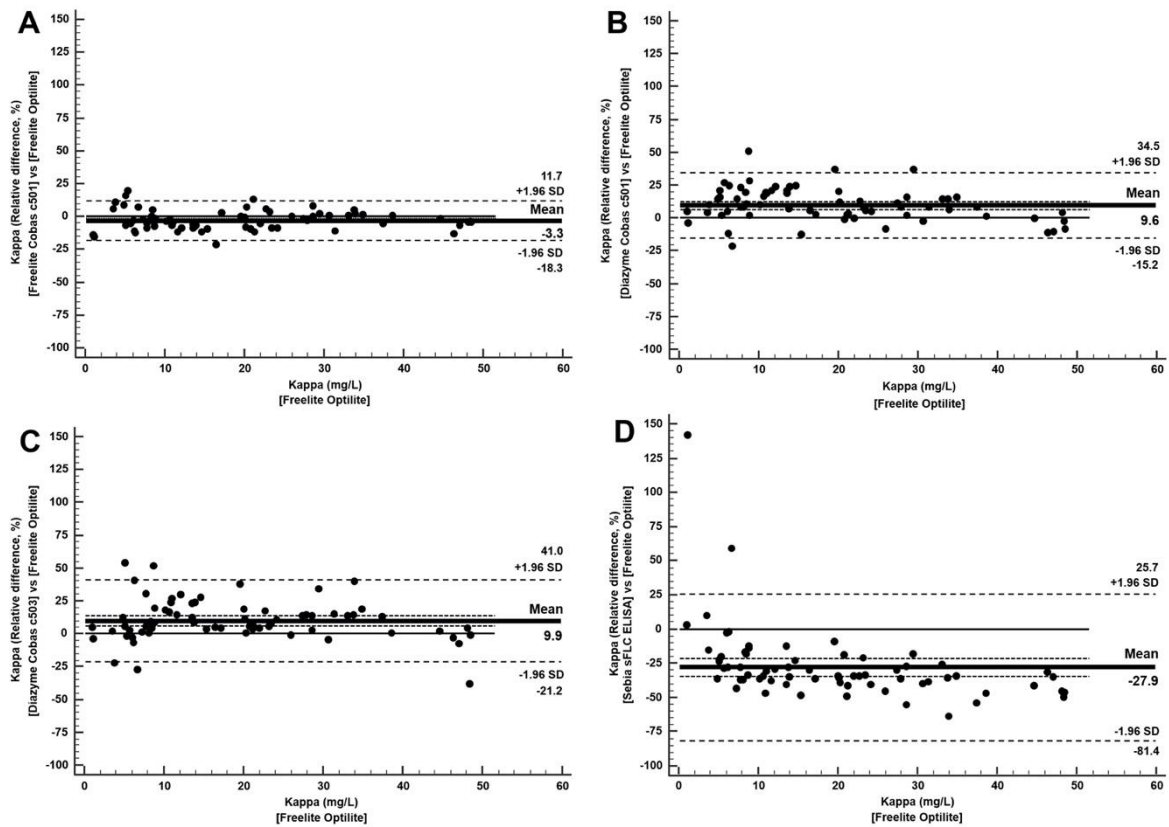
Supplemental Figure 4. Relative Bland Altman analysis of lower range results (< 50 mg/L) of λ sFLC lower range for the Freelite assay on Optilite analyzer versus versus Freelite assay on cobas c501 analyzer (Figure 4.A), Diazyme FLC assay on cobas c501 analyzer (Figure 4.B),), Diazyme FLC assay on cobas c503 analyzer (Figure 4.C) and Sebia λ FLC ELISA on AP22 analyzer (Figure 4.D). Dotted line represents line of identity. Mean differences (95% confidence interval) are presented.