510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K210623

B Applicant

Sebia, Inc.

C Proprietary and Established Names

FLC Kappa
FLC Lambda

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DFH DEH	Class II	21 CFR 866.5550 - Immunoglobulin (Light Chain Specific) Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Kappa (κ) Free Light Chain (FLC)
Lambda (λ) Free Light Chain (FLC)

C Type of Test:

Manual enzyme-linked immunosorbent assay (ELISA), quantitative

III Intended Use/Indications for Use:

A Intended Use(s):

The FLC Kappa kit is intended for the quantification of Kappa free light chains in human serum from adults with an Enzyme-Linked Immunosorbent Assay (ELISA) procedure. Measurement of free light chains aids in the diagnosis of multiple myeloma and AL amyloidosis. It must be used in conjunction with other laboratory and clinical findings. For In Vitro Diagnostic Use only.

The FLC Lambda kit is intended for the quantification of Lambda free light chains in human serum from adults with an Enzyme-Linked Immunosorbent Assay (ELISA) procedure. Measurement of free light chains aids in the diagnosis of multiple myeloma and AL amyloidosis. It must be used in conjunction with other laboratory and clinical findings. For In Vitro Diagnostic Use only.

B Indication(s) for Use:

Same as Intended Use

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Warning: The result of the FLC Kappa, FLC Lambda in a given specimen determined with assays and/or instrument platforms from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different assay methods cannot be used interchangeably.

D Special Instrument Requirements:

No special instrument requirements.

IV Device/System Characteristics:

A Device Description:

The FLC Kappa is comprised of the following reagents:

- Kappa microplate: coated with polyclonal rabbit anti-human kappa FLC antibody
- Dilution Buffer
- Wash Solution
- Anti-Kappa Antiserum
- Stop Solution
- Calibrators: A five level set in liquid form with target concentrations: 2.7 mg/L, 5.3 mg/L 16 mg/L, 48 mg/L and 96 mg/L, ready to use.

The FLC Lambda is comprised of the following reagents:

- Lambda Microplate: coated with polyclonal rabbit anti-human lambda FLC antibody
- Dilution Buffer
- Wash Solution
- Anti-Lambda Antiserum
- Stop Solution
- Calibrators: A five level set in liquid form with target concentrations: 2.9 mg/L, 5.9 mg/L, 17.7 mg/L, 53.0 mg/L and 106.0 mg/L, ready to use.

The following materials are required but not provided in the kits:

- Densitometer for microplate reading by absorbance spectrophotometry at 450 nm.
- FLC Control Level 1 with target concentrations for Kappa: 9–25 mg/L and Lambda: 15–30 mg/L
- FLC Control Level 2 with target concentrations for Kappa and Lambda > 35 mg/L

FLC Control Level 1 and FLC Control Level 2 are intended for the quality control of SEBIA immunoenzymatic procedures and are obtained from a pool of human sera. The controls are packaged in a stabilized lyophilized form. It must be noted that the high limit of the FLC Control Level 2 must not exceed the values of the Calibrator 5. Additionally, the exact concentrations for FLC Control Level 1 and FLC control Level 2 are lot dependent.

B Principle of Operation:

The FLC Kappa and FLC Lambda assays are intended for the quantification of FLCs in human serum from adults with an Enzyme-Linked Immunosorbent Assay (ELISA) procedure utilizing specific anti-Kappa and anti-Lambda FLC antibodies. Kappa FLC or Lambda FLC in the sample binds to specific anti-Kappa FLC antibody or anti-Lambda FLC antibody coated on the wells of microplates. Following the washing step, the samples in wells are incubated with an anti-FLC antiserum (Kit specific) conjugated to horseradish peroxidase followed by another washing of wells to remove the excess of the conjugated antiserum. Reading of the optical density is performed by absorbance spectrophotometry at 450 nm of the colored product. Finally, the calculation of the FLC concentration of the sample is performed using a calibration curve obtained with calibrators that have been analyzed on the same microplate.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Freelite Human Kappa And Lambda Free Kits for use on the Siemens BN II Nephelometer

B Predicate 510(k) Number(s):

K031016

C Comparison with Predicate(s):

Device & Predicate Device(s):	K210623	K031016
Device Trade Name	FLC Kappa FLC Lambda	Freelite Human Kappa Free and Freelite Human Lambda Free kits for use on the Siemens BN II
General Device Characteristic Similarities		
Intended Use/ Indications for Use	The FLC Kappa kit is intended for the quantification of Kappa free light chains in human serum from adults with an Enzyme-Linked Immunosorbent Assay (ELISA) procedure. Measurement of free light chains aids in the diagnosis of multiple myeloma and AL amyloidosis. It must be used in conjunction with other laboratory and clinical findings. For *In Vitro* Diagnostic Use only. The FLC Lambda kit is intended for the quantification of Lambda free light chains in human serum from adults with an Enzyme-Linked Immunosorbent Assay (ELISA) procedure. Measurement of free light chains aids in the diagnosis of multiple myeloma and AL amyloidosis. It must be used in conjunction with other laboratory and clinical findings. For *In Vitro* Diagnostic Use only.	Kappa: This kit is intended for the quantitation of kappa free light chains in serum and urine on the Siemens BN II. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings. Lambda: This kit is intended for the quantitation of lambda free light chains in serum and urine on the Siemens BN II. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.
Analyte	Kappa and Lambda	Same
Measurement	Quantitative	Same
General Device Characteristic Differences		
Specimen Type	Serum	Serum, Urine
Detection Method	ELISA	Nephelometric
Assay format	Manual	Automatic, on BN II instrument

Capture Antibody	Polyclonal rabbit anti-human kappa/lambda FLC coated on the well of the microplate	Polyclonal sheep anti-human kappa/lambda antibody coated onto latex particles
Detection Antibody	Horseradish peroxidase (HRP) conjugated polyclonal rabbit anti-human kappa/lambda FLC antibody	Not Applicable
Analytical Measuring Interval	Kappa: 4.5–76.2 mg/L Lambda: 3.8–66.8 mg/L	Kappa: 0.3 –190 mg/L Lambda: 0.25 –160 mg/L
Reference Interval	Kappa: 6.4–17.4 mg/L Lambda: 8.4–21.8 mg/L Ratio: 0.46–1.51	Kappa: 3.30–19.40 mg/L Lambda: 5.71–26.30 mg/L Ratio: 0.26–1.65
Calibrators (number of levels)	5-level	1-level

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP6-A2, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within-Lab Precision:

Six samples including four serum samples (samples 1 to 4) and two controls (samples C1 and C2) were tested using the FLC Kappa and FLC Lambda kit. Each day, during the 20 days, one operator analyzed the samples (three replicates per sample) on one microplate (two runs with minimum two hours between runs) using one reagent lot (same microplate design each day), yielding a total of 120 results per sample. The standard deviation (SD) and %CV were

evaluated for repeatability (within-run), between-run, within-day, between-day, and within-laboratory precision. The results are summarized in the following table:

FLC Kappa

Sample	Mean (mg/L)	Within-Run (Repetability)		Between-Run		Within-Day		Between-Day		Within-Lab Precision	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	6.3	0.41	6.6	0.74	11.9	0.85	13.6	0.36	5.8	0.92	14.8
2	14.4	0.54	3.7	1.30	9.0	1.40	9.7	0.81	5.6	1.62	11.2
3	39.2	1.47	3.8	4.02	10.2	4.28	10.9	1.29	3.3	4.47	11.4
4	62.0	2.89	4.7	6.03	9.7	6.69	10.8	1.77	2.9	6.92	11.2
C1	11.5	0.67	5.8	1.09	9.5	1.28	11.2	0.00	0.0	1.28	11.2
C2	42.2	1.64	3.9	4.00	9.5	4.32	10.2	0.66	1.6	4.37	10.4

FLC Lambda

Sample	Mean (mg/L)	Within-Run (Repetability)		Between-Run		Within-Day		Between-Day		Within-Lab Precision	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	13.2	0.55	4.2	0.96	7.3	1.11	8.4	0.63	4.8	1.28	9.7
2	20.0	0.83	4.1	1.25	6.2	1.5	7.5	0.74	3.7	1.67	8.3
3	33.7	1.55	4.6	2.67	7.9	3.09	9.2	1.61	4.8	3.48	10.3
4	79.5	5.13	6.5	5.15	6.5	7.27	9.1	6.91	8.7	10.03	12.6
C1	19.7	1.33	6.8	1.4	7.1	1.93	9.8	0.00	0.0	1.93	9.8
C2	37.1	1.66	4.5	2.76	7.5	3.22	8.7	1.55	4.2	3.57	9.6

Operator-to-Operator Variability

Six samples including four serum samples (samples 1 to 4) and two controls (samples C1 and C2) were tested using the FLC Kappa and FLC Lambda kit. Each day, during five days, three different operators analyzed the same samples (five replicates / sample) on a microplate using one reagent lot (same microplate design each day), yielding a total of 75 results per sample. The precision including operator-to-operator variance precision is summarized in the following table.

FLC Kappa

Sample	Mean (mg/L)	Within-Day		Between-Days		Within-Operator		Between-Operators		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	6.8	0.46	6.8	0.63	9.2	0.78	11.4	0.0	0.0	0.78	11.4
2	15.7	0.72	4.6	1.30	8.3	1.49	9.5	0.0	0.0	1.49	9.5
3	44.1	2.49	5.7	2.05	4.7	3.23	7.3	0.5	1.1	3.27	7.4
4	67.9	3.52	5.2	5.58	8.2	6.60	9.7	0.0	0.0	6.60	9.7
C1	12.7	1.25	9.9	0.93	7.3	1.56	12.3	0.5	3.9	1.64	12.9
C2	44.8	1.95	4.3	2.94	6.6	3.53	7.9	0.0	0.0	3.53	7.9

FLC Lambda

Sample	Mean (mg/L)	Within-Day		Between-Days		Within-Operator		Between-Operators		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	13.4	0.87	6.5	0.83	6.2	1.20	9.0	0.0	0.0	1.20	9.0
2	21.1	0.98	4.6	1.49	7.1	1.79	8.5	0.0	0.0	1.79	8.5
3	35.4	2.7	7.6	2.87	8.1	3.94	11.10	0.0	0.0	3.94	11.1
4	81.5	5.88	7.2	5.58	6.8	8.11	10.0	3.99	4.9	9.03	11.1
C1	20.7	1.59	7.7	1.41	6.8	2.13	10.3	0.00	0.0	2.13	10.3
C2	37.1	2.09	5.6	2.81	7.6	3.51	9.4	1.93	5.2	4.0	10.8

Lot-to-Lot Variability

Six samples including four serum samples (samples 1 to 4) and two controls (samples C1 and C2) were tested using the FLC Kappa and FLC Lambda kit. Each day, during five days, one operator analyzed the same samples (five replicates / sample) on a microplate using three reagent lots (one microplate/lot, same design each day), yielding a total of 75 results per sample. The results including lot-to-lot imprecision is summarized in the following table.

FLC Kappa

Sample	Mean (mg/L)	Within-Day		Between-Days		Within-Lot		Between-Lots		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	6.8	0.36	5.3	0.50	7.4	0.62	9.1	0.17	2.6	0.64	9.4
2	15.3	0.50	3.3	0.55	3.6	0.74	4.9	0.40	2.6	0.84	5.5
3	38.7	2.00	5.2	1.72	4.4	2.64	6.8	0.76	2.0	2.75	7.1
4	62.2	3.59	5.8	1.69	2.7	3.96	6.4	1.65	2.6	4.29	6.9
C1	12.1	0.86	7.1	0.54	4.4	1.01	8.3	0.0	0.0	1.01	8.3
C2	38.7	2.00	5.2	1.72	4.4	2.64	6.8	0.76	2.0	2.75	7.1

FLC Lambda

Sample	Mean (mg/L)	Within-Day		Between-Days		Within-Lot		Between-Lots		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	13.8	1.14	8.3	1.20	8.7	1.66	12.0	1.40	10.1	2.17	15.7
2	20.8	0.99	4.8	1.98	9.5	2.21	10.7	2.20	10.6	3.12	15.0
3	33.7	2.91	8.6	3.12	9.3	4.27	12.7	0.70	2.1	4.33	12.9
4	71.4	5.71	8.0	7.63	10.7	9.53	13.4	0.00	0.0	9.53	13.4
C1	20.9	1.70	8.2	1.51	7.2	2.27	10.9	2.32	11.1	3.25	15.6
C2	38.5	1.84	4.8	3.29	8.5	3.77	9.8	1.66	4.3	4.12	10.7

Site-to-Site Reproducibility:

Six samples including four serum samples (samples S1 to S4) and two controls (samples C1 and C2) were tested using the FLC Kappa and FLC Lambda kit. Each day, for five days, an operator analyzed the same samples (five replicates per sample) on a microplate using one reagent lot (same microplate design each day), yielding a total of 25 results per sample. The same protocol was followed by two other operators in two other laboratories with the same reagent lot. The SD and %CV were evaluated for within-day, between-day, within-site, between-sites, and total reproducibility. The results are summarized in the table below:

FLC Kappa

Sample ID	Mean (mg/L)	Within-Day		Between-Days		Within-Site		Between-Sites		Reproducibility	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	6.2	0.45	7.2	0.39	6.3	0.60	9.6	0.00	0.0	0.60	9.6
2	15.0	0.87	5.8	1.48	9.9	1.71	11.4	0.00	0.0	1.71	11.4
3	32.5	1.29	4.0	2.76	8.5	3.05	9.4	1.48	4.5	3.38	10.4
4	71.9	4.23	5.9	5.78	8.0	7.16	9.9	2.86	4.0	7.71	10.7
C1	13.8	1.08	7.9	0.70	5.0	1.29	9.3	1.69	12.2	2.12	15.4
C2	57.2	2.67	4.7	2.68	4.7	3.78	6.6	3.79	6.6	5.36	9.4

FLC Lambda

Sample ID	Mean (mg/L)	Within-Day		Between-Days		Within-Site		Between-Sites		Reproducibility	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	11.4	0.76	6.7	0.91	8.0	1.19	10.5	0.51	4.5	1.30	11.4
2	20.7	1.51	7.3	1.62	7.8	2.22	10.7	1.12	5.4	2.48	12.0
3	31.3	2.12	6.8	3.39	10.8	4.00	12.8	1.87	6.0	4.42	14.1
4	68.8	4.44	6.5	6.13	8.9	7.57	11.0	4.81	7.0	8.97	13.0
C1	24.2	1.62	6.7	2.27	9.4	2.78	11.5	2.40	9.9	3.68	15.2
C2	50.1	3.87	7.7	5.81	11.6	6.98	13.9	2.83	5.7	7.53	15.1

2. Linearity:

The linearity of the FLC Kappa and FLC Lambda assays was evaluated based on three panels using native patient samples. Each panel was prepared by proportional mixing of one low sample with concentration close to the concentration of the Calibrator 1 and one high sample with concentration close to the concentration of the Calibrator 5 in order to reach 12 concentration levels. Each concentration level sample was tested in seven replicates using one reagent lot of each FLC Kappa and FLC Lambda. The summary data for linearity study of FLC Kappa and FLC Lambda are summarized in the table below.

	Range Tested (mg/L)	Slope (95% CI)	Y-intercept (95% CI)	Recovery % (min-max)
FLC Kappa				
Panel 1	(4.4; 81.7)	1.01 (0.95; 1.07)	-0.30 (-0.88; 0.29)	94.2–100.6
Panel 2	**(4.5**; 79.9)	1.02 (0.97; 1.07)	-0.20 (-0.72; 0.32)	97.1–101.3
Panel 3	(3.5; **76.2)**	0.93 (0.86; 1.00)	0.50 (-0.11; 1.10)	94.0–107.5
FLC Lambda				
Panel 1	**(3.8**; 74.1)	1.05 (0.94; 1.15)	-1.54 (-2.52; -0.55)	64.1*–102.5
Panel 2	(3.3; 77.7)	0.96 (0.93; 0.98)	-0.62 (-0.84; -0.40)	76.9*–94.8
Panel 3	(3.5; **66.8)**	0.96 (0.89; 1.03)	-0.31 (-0.87; 0.25)	87.3–95.6

* Low-level sample in Panel 1 (3.8 mg/L) and Panel 2 (3.3 mg/L).

The results support the linearity of the following claimed AMR:

FLC Kappa: 4.5 – 76.2 mg/L
FLC Lambda: 3.8 – 66.8 mg/L

3. Analytical Specificity/Interference:

Endogenous Interference Study

Three serum samples containing normal, medium and high analyte concentrations were tested for the presence of endogenous interferants using FLC Kappa and FLC Lambda kits based on the CLSI EP7-A2 and the CLSI EP07, 3rd Edition. The test samples were prepared by supplementing interferants in the solvent (either saline or NaOH) to reach the desired concentration of the interferant to be tested. Additionally, neat serum samples were supplemented with the same volume of solvent, and these were considered blanks samples. The test samples and blank samples were analyzed by one operator through one run and 10 replicates each for test and blank samples. Mean relative deviation was calculated for blank and test sample and no significant interference (deviation < ±10%) was observed for each tested interferant up to the following concentration levels:

FLC Kappa

Interferants	Concentration Level
Conjugated Bilirubin	66.6 mg/dL
Unconjugated Bilirubin	40 mg/dL
Total Protein*	150 g/L
Hemoglobin	10 g/L
Triglycerides	20 g/L
Rheumatoid Factor*	2000 IU/mL

* Maximum deviation for total protein and Rheumatoid factor is 11.7% and 12.2%, respectively.

FLC Lambda

Interferants	Concentration Level
Conjugated Bilirubin	66.6 mg/dL
Unconjugated Bilirubin*	40 mg/dL
Total Protein*	150 g/L
Hemoglobin*	10 g/L
Triglycerides*	20 g/L
Rheumatoid Factor	250 IU/mL

* Maximum deviation for unconjugated bilirubin, total protein, hemoglobin and triglycerides and rheumatoid factor is 11.7%, 11.6%, 11.7% and 12.2%, respectively.

Exogenous Interference Study

Three serum samples containing normal, medium and high analyte concentrations were tested for the presence of exogenous interferants using FLC Kappa and FLC Lambda kits based on the CLSI EP7-A2 and the CLSI EP07, 3rd Edition. The test samples were prepared by supplementing interferants in the solvent (either saline or NaOH) to reach the desired concentration of the interferant to be tested. Additionally, neat serum samples were supplemented with the same volume of solvent, and these were considered blanks samples. The test samples and blank samples were analyzed by one operator through one run and 10 replicates each for test and blank samples. Mean relative deviation was calculated for blank and test sample and no significant interference (deviation < ±10%) was observed for each tested interferant up to the following concentration levels:

Interferants	Concentration Level
Melphalan	4 mg/L
Dexamethasone*	12 mg/L
Daratumumab*	1 g/L
Bortezomib	2 mg/L
Lenalidomide	4 mg/L
Pomalidomide	1 mg/L
Carfilzomib*	1 mg/L
Isatuximab*	1 g/L

* Maximum deviation for FLC Kappa assay for Carfilzomib is 11.2%. Maximum deviation for FLC Lambda assay for Dexamethasone, Daratumumab and Isatuximab is 11.3%, 10.8% and 10.9% respectively.

4. Assay Reportable Range:

The assay reportable range for the FLC Kappa and the FLC Lambda is the same as the analytical measuring range for these two assays:

FLC Kappa: 4.5 – 76.2 mg/L
FLC Lambda: 3.8 – 66.8 mg/L

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability

In the absence of an international reference standard, the calibration of the FLC Kappa and the FLC Lambda assays is traceable to an internally assigned master material.

Stability

Reagent Kit

a. Shelf-life stability: The real-time shelf-life stability of the FLC Kappa and FLC Lambda reagent kits was determined over a 10-month period using three reagent lots of each assay. Each reagent lot stored at 2–8°C was tested at 0 (T_0), 1, 2, 3 6, 9 and 10 months. At each testing point, three serum samples (low, medium and high concentration) as well as two controls (FLC Control Level 1 and FLC Control Level 2) were tested in triplicate using all three reagent lots of FLC Kappa and FLC Lambda, respectively. At each time point, the mean concentrations for Kappa and Lambda were compared to the initial target range concentration obtained at T_0. The results are acceptable at all tested time points and hence support the shelf-life of FLC Kappa and FLC Lambda for 9 months at 2–8°C.

b. Open-vial stability: The open-vial (in-use) kit stability of the FLC Kappa and FLC Lambda reagent kits was determined over a 5-week period using two reagent lots. Each reagent lot after first opening was stored at room temperature (22°C ± 3°C) and was tested each week for five weeks (weeks 1–5). At each testing point, three serum samples (low, medium, and high concentration) were tested in triplicate on two reagent lots of FLC Kappa and FLC Lambda, respectively. The results are acceptable at all tested time points and hence support the claimed in-use stability of the FLC Kappa and FLC Lambda up to 4 weeks at room temperature (22°C ± 3°C) after the first opening.

FLC Controls

a. Shelf-life stability: The real-time stability of the FLC Control Level 1 and FLC Control Level 2 was determined over a 24-month period using three reagent lots. Each freeze-dried control stored at 2–8°C was tested at 0 (T_0), 4, 8, 12, and 24 months. At each testing point, FLC Control Level 1 and FLC Control Level 2 were tested in triplicate using three reagent lots of FLC Kappa and FLC Lambda, respectively. At each time point, the mean concentrations for Kappa and Lambda were compared to the initial target range concentration obtained at the zero-month (T_0) time point. The results were acceptable at all tested time points and hence support the claimed shelf-life stability of the FLC Control Level 1 and FLC Control Level 2 up to 12 months at 2–8°C.

b. In-use (reconstituted) stability: The stability of reconstituted FLC Control Level 1 and FLC Control Level 2 were tested for the following storage conditions: -18°C to -30°C, 2–8°C, and room temperature (22°C ± 3°C). The stability of freeze-thaw cycles for the Controls (freezing at -18°C to -30°C, thawing at room temperature) was also tested. For each test condition, three lots of the Controls were tested. At each time point post first reconstitution, the mean concentrations for the FLC Controls were compared to the initial target range concentration obtained when the open vials of the Controls were reconstituted for the first time. The results support the stability claim for the Controls: 3

months at -18ºC to -30ºC; 4 days at 2–8ºC; 24 hours (one day) at room temperature (22ºC ± 3ºC), and up to 40 freeze-thaw cycles.

6. Detection Limit:

The limit of blank (LoB) for the FLC Kappa and FLC Lambda was determined by testing the blank samples. All measurements yielded no detectable value. Hence the claimed LoB for the FLC Kappa and FLC Lambda is 0 mg/L.

The limit of detection (LoD) for the FLC Kappa and FLC Lambda assays was determined by assaying five samples with low Kappa free light chain concentration and five samples with low Lambda free light chain concentration. The samples were tested in two runs of four replicates over the course of 7 days using two reagent lots yielding a total of 56 replicates per sample for each lot. The precision profile analysis was used to calculate the LoD. Since LoD values were lower than the calibrator 1 value, the LoD was considered as equal to the LoQ value.

The limit of quantitation (LoQ) for the FLC Kappa and FLC lambda assays was determined by assaying five samples with low Kappa free light chain concentration and five samples with low Lambda free light chain concentration. The samples were tested in two runs of four replicates over the course of 7 days using two reagent lots yielding a total of 56 replicates per sample for each lot. The LoQ was defined to be the lowest concentration level that meets the within-laboratory imprecision of < 20% for each lot. The calculated LoQ for Kappa was determined to be 0.8 mg/L and 1.1 mg/L for Lambda. The data for linearity supported the lower level of measuring interval for Kappa and Lambda to be 4.5 mg/L and 3.8 mg/L, respectively. Hence the claimed LoQ for the FLC Kappa is 4.5 mg/L and the claimed LoQ for the FLC Lambda is 3.8 mg/L.

7. Assay Cut-Off:

See expected values/reference range.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A total of 222 samples were tested with the FLC Kappa and FLC Lambda kits and the predicate devices: Freelite Human Kappa Free and Freelite Human Lambda Free kits for use on the Siemens BN II. The samples included 96 Multiple Myeloma (MM) and 83 Amyloid Light chain (AL) serum samples. The regression analysis was performed based on the results of 216 samples for the FLC Kappa assay and its predicate, and the results of 221 samples for the FLC Lambda assay and its predicate.

	N	Sample Range mg/L	Slope (95% CI)	Y-Intercept (95% CI)	R^2
FLC Kappa	216	1.0 – 1947.0	0.56 (0.51–0.60)	0.91 (0.75–1.07)	0.92
FLC Lambda	221	1.6 – 860.4	0.61 (0.52–0.69)	2.243 (1.15–3.34)	0.75

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity:

A total of 510 samples were tested in the clinical validation study for the FLC Kappa and Lambda assay. This validation set included 177 samples from MM patients, 144 samples from AL amyloidosis patients and 189 samples from non-myeloma patients with various clinical conditions: 35 samples from polyclonal immunoglobulin stimulation patients; 19 samples from chronic kidney disorders (CKD) patients and 126 samples from patients with other clinical conditions.

For MM, a total of 366 samples were included in the clinical validation study for the FLC Kappa and Lambda assays. This validation set included 177 samples from multiple myeloma patients, and 189 samples from non-myeloma patients with various clinical conditions.

Clinical sensitivity and specificity summary of the FLC Kappa and Lambda Ratio for MM are shown in the table below using the FLC Kappa and Lambda ratio reference interval as cut-off (i.e., ratio within the reference interval is considered as positive; Ratio outside the reference interval is considered as negative):

		Clinical Diagnosis of MM		
		Positive	Negative	Total
FLC Kappa and Lambda Ratio	Positive	171	28	199
	Negative	6	161	167
	Total	177	189	366

Clinical Sensitivity: 96.6% (95% CI: 94.0 – 99.3)
Clinical Specificity: 85.1% (95% CI: 79.4 – 89.5)

For AL amyloidosis, a total of 333 samples were included in the clinical validation study for the FLC Kappa and Lambda assay. This validation set included 144 samples from AL amyloidosis patients, and 189 non-AL amyloidosis samples from patients with various clinical conditions.

Clinical sensitivity and specificity summary of the FLC Kappa and Lambda Ratio for AL are shown in the table below using the FLC Kappa and Lambda ratio reference interval as cut-off (i.e., ratio within the reference interval is considered as positive; ratio outside the reference interval is considered as negative):

		Clinical Diagnosis of AL		
		Positive	Negative	Total
FLC Kappa and Lambda Ratio	Positive	131	28	159
	Negative	13	161	174
	Total	144	189	333

Clinical Sensitivity: 91.0% (95% CI: 86.3 – 95.7)
Clinical Specificity: 85.1% (95% CI: 79.4 – 89.5)

2. Other Supportive Data:

In the clinical study described above, all samples were also tested with the predicate device. The clinical performance of the FLC Kappa and FLC Lambda as an aid in diagnosis of MM and as an aid in diagnosis of AL was compared to the predicate device. The results are summarized in the following table.

Sebia FLC Kappa/FLC Lambda		**Freelite Human Kappa Free/ Freelite Human Lambda Free kits for use on the Siemens BN II**	
Diagnosis of MM			
Sensitivity (n/N) (95% CI)	**Specificity (n/N) (95% CI)**	**Sensitivity (n/N) (95% CI)**	**Specificity (n/N) (95% CI)**
96.6% (171/177) (94.0%; 99.3%)	85.1% (151/189) (79.4%; 89.5%)	96.6% (173/179) (94.0%; 99.3%)	68.3% (129/189) (61.3%; 74.4%)
Diagnosis of AL			
Sensitivity (n/N) (95% CI)	**Specificity (n/N) (95% CI)**	**Sensitivity (n/N) (95% CI)**	**Specificity (n/N) (95% CI)**
91.0% (131/144) (86.3%; 95.7%)	85.1% (151/189) (79.4%; 89.5%)	89.6% (129/144) (84.6%; 94.6%)	68.3% (129/189) (61.3%; 74.4%)

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

The reference interval for the FLC Kappa and FLC Lambda was determined by testing the values for Kappa FLC concentration, Lambda FLC concentration, and [Kappa FLC] / [Lambda FLC] ratio on 238 apparently healthy adults using one reagent lot of the FLC Kappa and FLC Lambda, respectively. The reference interval for FLC Kappa & FLC Lambda assays were defined as 95% of the dataset. The results are summarized as follows:

	Mean	**95% Reference Range**
FLC Kappa	10.9 mg/L	6.4 – 17.4 mg/L
FLC Lambda	13.4 mg/L	8.4 – 21.8 mg/L
Kappa/Lambda Ratio	0.83	0.46 – 1.51

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.