

## 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

**A. 510(k) Number:**

k083602

**B. Purpose for Submission:**

New device

**C. Measurand:**

Immunoglobulins, Kappa light chains (bound and free)

**D. Type of Test:**

Quantitative, immunoturbidimetry

**E. Applicant:**

Sentinel CH. SpA

**F. Proprietary and Established Names:**

Kappa light chains assay

**G. Regulatory Information:**

1. Regulation section:

21 CFR § 866.5550, Immunoglobulin (light chain specific) immunological test system

2. Classification:

Class II

3. Product code:

DFH – Kappa, antigen, antiserum, control

4. Panel:

Immunology (82)

**H. Intended Use:**

1. Intended use(s):

The Kappa light chains assay is an *in vitro* diagnostic test used for the quantitative determination of immunoglobulin bound and free kappa light chains (KAPPA) in serum and in Li-heparin plasma by immunoturbidimetry on Synchron LX20 System. Measurement of type of light chains aids in the diagnosis of multiple myeloma (cancer of antibody-forming cells), lymphocytic neoplasms (cancer of lymphoid tissue), Waldenstrom's macroglobulinemia (increased production of large immunoglobulins), and connective tissue diseases such as rheumatoid arthritis or systemic lupus erythematosus, in conjunction with other clinical and laboratory findings.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For Prescription use only

4. Special instrument requirements:

Beckman Coulter Synchron LX20 System (k965240)

**I. Device Description:**

The Kappa light chains assay consists of 2 bottles of 50-mL Reagent 1 containing 20 mmol/L of phosphate buffer (pH 7.5),  $\geq 7\%$  of PEG, 150 mmol/L sodium chloride,  $<0.1\%$  sodium azide, and 2 bottles of 10 mL of Reagent 2 containing goat anti-

lambda polyclonal antiserum, 50 mmol/L of good's buffer (pH 7.5), 150 mmol/L of sodium chloride and < 0.1% sodium azide.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Beckman IMMAGE® Immunochemistry Systems Kappa light chain
2. Predicate 510(k) number(s):  
k964260
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indication for Use:	Measurement of type of light chains aids in the diagnosis of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, and connective tissue diseases such as rheumatoid arthritis or systemic lupus erythematosus, in conjunction with other clinical and laboratory findings.	Same
Measurement	Quantitative	Same
Analyte Measured	Bound and free Kappa light chains	Same
Reagent format	Utilize reagents in R1 and R2 format	Same
Analysis Medium	Aqueous solution	Same
Use of Calibrators	Yes	Same
Calibration model	Nonlinear-multi point calibration	Same
Reference	Traceable to ERM-DA 470 (European Reference Material) from BCR (EG Community Bureau of Reference), corresponding to RPPHS (Reference Preparation for Protein in Human Serum).	Same

Differences		
Item	Device	Predicate
Matrix	Human serum and Li-heparin plasma	Human serum and urine
Method	Immunoturbidimetry	Nephelometry
Measurement	Only measure the molecular weight of kappa light chains	Measure the molecular weight of whole IgG
Assay Range	35-750 mg/dL Extended range – 600-1250 mg/dL	133 to 1467 mg/dL Extended range – 11.1-26,400 mg/dL
Analyzer/Instrument	Synchron LX20 System	Beckman IMMAGE nephelometer Analyzer

**K. Standard/Guidance Document Referenced (if applicable):**

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation.

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP9-A2 – Method Comparison and Bias Estimation Using Patient Samples

CLSI EP07-A2 – Interference Testing in Clinical Chemistry

CLSI C28-A3 – Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

**L. Test Principle:**

When a sample is added to the cuvette, the antiserum against human Immunoglobulin Kappa light chains will form a complex with its corresponding antigen in the sample under optimal pH conditions and in the presence of PEG. These complexes scatter a beam of light passing through the sample. The intensity of the light that passed through the complex can be captured and measured by the instrument. The intensity of the light passed through the complex is proportional to the concentration of the respective antigen (free and bound Kappa light chains) in the sample. A series of calibrators of known antigen concentration are assayed to generate a calibration curve which is used to determine the unknown concentration of the sample. The memory can be stored in the machine and used for the calculation of the unknown samples in the future runs.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. Precision/Reproducibility:

Testing was performed according to CLSI EP5-A

Within run precision:

Within run precision was determined on four levels of serum control materials and on one human serum pool in 20 replicates one run per day.

**Within run Precision**

		N	Mean (mg/dL)	SD (mg/dL)	CV%
Serum control	Level 1	20	125.9	2.78	2.2
	Level 2	20	164.7	4.08	2.5
	Level 3	20	413.5	5.30	1.3
	Level 4	20	392.5	7.27	1.9
Human sera pool		20	558.7	10.78	1.9

Total Precision (Run- to-Run and day-today)

Total precision was determined on Levels 1 and 2 of serum control materials in duplicate two runs per day for 20 days. Four additional human sera pools that span the assay range of 93.5 mg/dL to 695.0 mg/dL were added later. The total imprecision results on 6 levels of human sera met the acceptance criteria of  $CV\% \leq 7\%$ . The results are summarized in the following table:

	N	Mean (mg/dL)	Total Imprecision		Between days		Within run (Repeatability)	
			SD (mg/dL)	CV%	SD (mg/dL)	CV%	SD (mg/dL)	CV%
Quality Control Material Level 1	80	125.7	8.55	6.8	6.88	5.5	5.08	4.0
Quality Control Material Level 2	80	476.9	14.54	3.0	12.34	2.6	7.7	1.6
Human sera pool Level 1	80	93.5	5.63	6.0	3.33	3.6	3.44	3.7
Human sera pool Level 2	80	104.4	4.46	4.3	3.74	3.6	1.55	1.5
Human sera pool Level 3 (*)	80	651.9	25.27	3.9	22.40	3.4	11.69	1.8
Human sera pool Level 4 (*)	80	695.0	16.76	2.4	11.59	1.7	12.11	1.7

(\*) Data obtained on ORDAC mode on Synchron LX20

*b. Linearity/assay reportable range:*

Linearity was assessed using two different pools of human sera: Pool 1 was used to determine the linearity of the assay over the “Whole Analytical Measurement Range (Whole AMR)” and Pool 2 was used to determine the linearity over the “Low Analytical Measurement Range (Low AMR)”. Pool 1 was created by spiking concentrated Kappa light chains into a human pool serum, followed by serially diluting the spiked sample with saline to obtain at least 10 samples with concentrations of Kappa light chains of 0 mg/dL - 750 mg/dL. Pool 2 was created by serially diluting a human pooled serum sample to create at least 10 samples with concentrations ranging from 0 mg/dL - 77 mg/dL. Each diluted sample was run in triplicate in both Whole AMR and Low AMR tests. Data were analyzed in accordance with CLSI EP6-A. The measured value from each sample was plotted against the theoretical value. The data is summarized in the following table:

Range	Test Range (mg/dL)	Slope (95% CI)	Y-intercept (95% CI)	R	Relative Bias Range	%CV range
Low AMR	0.0 to 77.9	1.05 (0.98 to 1.11)	-5.34 (-8.32 to -2.36)	0.997	-70.0% to 0.0%	0.2% to 5.1%
Whole AMR	0.0 to 750.1	1.01 (0.99 to 1.03)	-6.62 (-15.49 to 2.26)	1.000	-7.1% to 1.8%	0.1% to 2.4%

**ORDAC (Over Range Detection and Correction):**

For samples that are out of the linear range but between 600 to 1250 mg/dL, the instrument will automatically dilute the sample following the automated protocol ORDAC. Study was done to verify that serum samples diluted by the automated protocol ORDAC, achieve an acceptable degree of accuracy as determined by comparison with manually diluted samples. The ORDAC function was verified by using a panel of 25 samples, equally distributed within the range of 591 to 1825 mg/dL. Samples were run in duplicate on Synchron LX20 according to the ORDAC parameters setting. The results were compared with paired aliquots of each sample that was manually pre-diluted. Data generated met the Preapproved Acceptance Criteria of slope 0.95 to 1.05;  $R \geq 0.975$  and demonstrated the accuracy of the assay within the range of 600 mg/dL to 1250 mg/dL. The following table summarizes the results:

Test Range (mg/dL)	Slope (95% CI)	Y-intercept (mg/dL) (95% CI)	r	Average Bias (95% CI)
591 to 1825	0.963 (0.925 to 1.002)	48.29 (10.39 to 86.19)	0.996	14.36 (1.24 to 27.48)

**Assay reportable range** - 35 to 750 mg/dL. For the extended range, it is 600 to 1250 mg/dL.

**Prozone effect** – samples from 1250 up to 10000 mg/dL will be reported as >750 mg/dL.

- c. **Traceability, Stability, Expected values (controls, calibrators, or methods):**  
SENTINEL Plasmaproteins Cal 3X, previously cleared in k081533 is traceable to ERM-DA 470 (European Reference Material) from BCR (EG Community Bureau of Reference), corresponding to RPPHS (Reference Preparation for Protein in Human Serum).

**On board stability**

Three levels of serum controls containing Kappa light chains (117.9 mg/dL, 266.6 mg/dL, and 435.6 mg/dL) were tested on Synchron LX 20 System with opened on-board reagents over 29 days. Percent recovery for each control level against measurement at time zero was 98.5% to 104.9%. The claimed On Board Stability will be 28 days.

**On board calibration stability**

The study for on-board stability described above was used to determine calibration stability of the Kappa light chains assay reagents on the Synchron LX20 System. For the study, a calibration with fresh reagents was performed at Day 0 and was used throughout. The acceptance criteria of percent recovery against measurement at Day 0 (90% to 110%) were met through the Study from Day 0 to Day 29. The claimed on Board Calibration Stability will be 14 days.

*Unopened Reagent Stability Test:*

A Real Time Study was carried out on three different lots of Kappa light chains assay for up to 38 months. Reagents were stored at +2 to +8°C throughout the duration of the study. Accuracy was tested on two levels of serum control material. During the analysis, a fresh material was used to calibrate the tests. Three replicates were tested for each level. Percent recovery ranged from 98.4% – 104.0% for calibrator and 98.2% – 110.6% for controls. The shelf life of the Kappa Light chains assay was claimed to be stable up to 24 months when stored at 2°C - 8°C.

d. *Detection limit:*

Analytical sensitivity studies were conducted following the preapproved internal protocol and acceptance criteria according to CLSI document EP 17-A.

*Limit of Blank:*

The limit of Blank (LOB) was determined by 20 blank measurements of saline in three different runs (total 20 X 3). The LOB is based on formula of (Mean+1.645SD) and was calculated to be 0.25 mg/dL.

*Limit of Detection:*

To determine the Limit of Detection (LOD), a human serum pool was diluted with saline to obtain a concentration higher than the LOB (0.25 mg/dL). The diluted pool was measured on three different runs with 20 replicates each, on Synchron LX20 System. LOD was defined as the concentration at which (Mean–3SD) is below the measuring range yet greater than LOB.

	Mean (mg/dL)	SD (mg/dL)	Limit of Blank Mean - 3SD (mg/dL)
Run 1 (n=20)	7.91	0.717	5.76
Run 2 (n=20)	9.14	0.332	8.15
Run 3 (n=20)	8.72	0.747	6.48
N= 60	8.59	0.804	6.18

The LOD of this assay is 6.18 mg/dL

*Limit of Quantitation (LOQ):*

A pool of human sera with a concentration around 40 mg/dL was serially diluted with saline to obtain a set of samples with decreased concentrations of human Kappa light chains. Each sample was tested on Synchron LX20

System with 10 replicates. LOQ is defined by the %CV being  $\leq 10\%$  and the percent bias must be  $\leq 20\%$ .

% Bias was defined as: 
$$\frac{(\text{Theoretical value} - \text{Found value})}{(\text{Theoretical value})} \times 100.$$

The LOQ of this assay is 30 mg/dL.

e. *Analytical specificity:*

Endogenous compounds in serum:

Interference study was done to evaluate interference caused by bilirubin (conjugated and unconjugated), hemoglobin, lipids (triglycerides), and Rheumatoid Factor (RF) in the Kappa light chains assay on the Synchron LX20 System. Human serum samples were spiked with various concentrations of the interferents. A minimum of three replicates of each interferent level were run. Absolute bias at each concentration level was determined against concentration of unspiked pool. For the determination of potential interference by RF, a human serum with a concentration of RF of about 1012 U/mL was diluted with a human serum without RF. Absolute bias at each concentration level was determined against theoretical values. The table below shows that the data generated met the acceptance criteria:

<b>Interfering Substance</b>	<b>Maximum Interfering Substance Concentration tested</b>	<b>No Interference found up to</b>
<b>Bilirubin (conjugated)</b>	60 mg/dL	60 mg/dL
<b>Bilirubin (unconjugated)</b>	66 mg/dL	60 mg/dL
<b>Hemoglobin</b>	1000 mg/dL	1000 mg/dL
<b>Lipids (Triglycerides)</b>	1000 mg/dL	1000 mg/dL
<b>Rheumatoid Factor</b>	1012 U/mL	1000 U/mL

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

To determine the degree of correlation between the Kappa light chains assay on the Synchron LX20 System vs. the IMMAGE Immunochemistry System Kappa light chain (k964260) on IMMAGE nephelometer Analyzer, 79 samples ranges from 56.8 mg/dL to 842.1 mg/dL (corresponding to 229.2 mg/dL to 3572.50 mg/dL based on whole IgG 150,000) were tested in duplicate with both devices. The data, including regression analysis results, are summarized in the table below:

<b>Regression Parameter</b>	<b>Synchron LX20 System vs. Immage Nephelometer</b>	<b>Acceptance criteria</b>
Slope	0.919 (0.873 to 0.965)	0.90 – 1.10
Y – Intercept	63.35 (4.92 to 121.77)	NA
Correlation Coefficient	0.977	> 0.975
Range of samples tested (mg/dL)	189.00 to 4285.00	NA
Std. Error of estimate (Sy/X)	123.93	NA
Average Bias (mg/dL)	-27.42 (-57.36 to 2.52)	NA
N	78	> 60
Units definition of Kappa light chains on Synchron LX20 System	Equivalent to Whole IgG content MW 150000	

Additional study was performed in accordance with CLSI Document EP9-A2. 308 samples were collected from patients ages 4–94y within the detectable range, including ORDAC range. Kappa light chains assay on Synchron LX20 System was calibrated with a calibrator material with assigned Kappa Light chains concentration based on definition of Kappa light chains as whole IgG content (MW 150000). The following table summarizes the results for all samples:

<b>Regression Parameter</b>	<b>Mean of two replicates</b>	<b>First replicate only</b>	<b>Second replicate only</b>	<b>Acceptance criteria</b>
<b>N</b>	306	306	306	≥ 300
<b>Range of samples tested (mg/dL) Predicate device</b>	353.5 to 7545.0	352.0 to 7430.0	306.0 to 7660.0	NA
<b>Range of samples tested (mg/dL) Synchron LX20</b>	228.5 to 7172.0	230.0 to 7002.0	227.0 to 7342.0	NA
<b>Average Bias (mg/dL) (95% CI)</b>	12.54 (-3.85 to 28.92)	9.57 (-8.11 to 27.25)	15.50 (-0.22 to 31.22)	NA



<b>Regression Parameter</b>	<b>Mean of two replicates</b>	<b>First replicate only</b>	<b>Second replicate only</b>	<b>Acceptance criteria</b>
<b>Passing&amp;Bablok Slope (95% CI)</b>	0.983 (0.955 to 1.009)	0.983 (0.955 to 1.009)	0.979 (0.954 to 1.006)	0.90 – 1.10
<b>Passing&amp; Bablok Y – Intercept (95% CI)</b>	40.57 (10.88 to 70.49)	36.50 (9.50 to 65.43)	47.06 (17.13 to 72.84)	NA

The table below summarizes results for samples with a Kappa light chains concentration < 2000 mg/dL:

<b>Regression Parameter</b>	<b>Mean of two replicates</b>	<b>First replicate only</b>	<b>Second replicate only</b>	<b>Acceptance criteria</b>
<b>N</b>	283	283	283	$\geq 300$
<b>Range of samples tested (mg/dL) Predicate Device</b>	353.5 to 1950.0	352.0 to 1940.0	306.0 to 1960.0	NA
<b>Range of samples tested (mg/dL) Synchron LX20</b>	228.5 to 2117.5	230.0 to 2127.0	227.0 to 2108.0	NA
<b>Average Bias (mg/dL) (95% CI)</b>	25.83 (16.47 to 35.18)	24.29 (13.87 to 34.71)	27.36 (18.04 to 36.68)	NA
<b>Passing&amp;Bablok Slope (95% CI)</b>	0.994 (0.961 to 1.027)	0.992 (0.960 to 1.028)	0.988 (0.957 to 1.022)	0.90 – 1.10
<b>Passing&amp; Bablok Y – Intercept (95% CI)</b>	29.50 (-4.37 to 64.61)	27.10 (-8.45 to 60.45)	35.82 (5.00 to 70.56)	NA

*b. Matrix comparison:*

To demonstrate that the Kappa light chains assay can be used in both human serum and Li-heparin plasma, blood from 77 volunteers was collected in Li-heparin plasma and serum tubes. The result from Li-heparin specimens were plotted against the results from serum specimens. The slope of the linear regression fell into the acceptance range of 0.95 – 1.05, and the  $R \geq 0.98$ .

Summary of results:

X	data on Serum		
Y	data on Li-Heparin		
N	77		
Range (mg/dL)	33.00 to 804.00		
<b>Linear regression analysis</b>	<b>Specs</b>	<b>Found (95% CI)</b>	<b>Assessment (Pass/Fail)</b>
Slope	0.95 to 1.05	0.950 (0.925 to 0.975)	Pass
Intercept	NA	8.974 (2.191 to 15.756)	NA
R	$\geq 0.98$	0.994	Pass
Sy/x	NA	14.777	NA
<b>Bland/Altman analysis</b>	<b>Specs</b>	<b>Found (95% CI)</b>	<b>Assessment (Pass/Fail)</b>
Bias	$\pm 13$ mg/dL	-2.958 (-6.634 to 0.717)	Pass

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable

4. Clinical cut-off:

See expected values

5. Expected values/Reference range:

Normal reference range was established by testing 100 female and 102 male voluntary healthy donors, 20 – 69 years old. Reference range was calculated at 95% of the samples distribution. The Kappa light chains were found to range from 122 mg/dL – 437 mg/dL among the tested healthy donors.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

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