

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k083601

B. Purpose for Submission:

New Device

C. Measurand:

Immunoglobulin Lambda light chains (bound and free)

D. Type of Test:

Quantitative determination of the turbidity of the immune complex

E. Applicant:

Sentinel CH. SpA

F. Proprietary and Established Names:

Lambda light chains assay

G. Regulatory Information:

1. Regulation section:

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

2. Classification:

Class II

3. Product code:

DEH-Lambda antigen, antiserum, control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

The Lambda light chains assay is *in vitro* diagnostic test used for the quantitative determination of Immunoglobulin bound and free Lambda light chains (LAMBDA) in serum and Li-heparin plasma by immunoturbidimetry. It is intended to measure Immunoglobulin Lambda light chains (bound and free) using Synchron LX20 System. Measurement of various amounts of the different types of light chains aids in the diagnosis of multiple myeloma, 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:

Synchron LX20 System

I. Device Description:

The Lambda 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 System Kappa (KAP) and Lambda (LAM) Light Chain Reagents
2. Predicate 510(k) number(s):
k964260
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indication for Use:	Measurements of immunoglobulin bound and free Lambda 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 Lambda light chains	Same
Reagent format	Utilize reagents in R1 and R2 format	Same
Analysis Medium	Aqueous solution	Same
Use of Calibrators	Yes	Yes
Calibration model	Nonlinear-multi point calibration	Same
Reference	Traceable to ERM-DA 470 (European Reference Material) from BCG, 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 light chains	Measure the molecular weight of whole IgG
Assay Range	20-400 mg/dL	180 to 1400 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

L. Test Principle:

When a sample is added to the cuvette, the antiserum against human Immunoglobulin Lambda 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 Lambda 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:

Within run precision:

Within run precision was determined by running four serum controls at different detection levels and one human sera pool once a day for 20 days. Level 1 represented the low to mid detection range (25% -30%), Level 2 represented the low detection range (<20%), Level 4 represented the mid level of detection range (~50%), and level 3 represented the high detection range (>80%). The %CV from all 5 serum controls tested was less than 5%.

Within-run Precision

Serum Controls	N	Mean (mg/dL)	SD (mg/dL)	CV%
Level 1	20	125.9	2.78	2.2
Level 2	20	96.1	3.48	3.6
Level 3	20	413.5	5.30	1.3
Level 4	20	209.0	3.42	1.6
Human sera pool	20	301.1	6.13	2.0

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

Total precision was determined by running two levels of serum control materials twice a day for 20 days with each sample ran in duplicate (20 X 2 X 2). Level 1 represented the low detection range, level 2 represented the mid detection range. Three additional human sera pools that span the region of 150 mg/dL to 450 mg/dL were added later. The result was summarized in the

following table. The CV% of each test level was $\leq 5\%$.

Total Precision study

	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	67.6	3.19	4.7	1.78	2.6	2.35	3.5
Quality Control Material Level 2	80	243.2	7.05	2.9	6.04	2.5	3.64	1.5
Human sera pool Level 1	80	168.7	5.77	3.4	4.62	2.7	2.68	1.6
Human sera pool Level 2 (*)	80	387.0	16.56	4.3	11.94	3.1	4.27	1.1
Human sera pool Level 3 (*)	80	415.9	17.89	4.3	14.08	3.4	6.19	1.5

(*) Data obtained on ORDAC mode on Synchron LX20

b. *Linearity/assay reportable range:*

Linearity:

Linearity was assessed using two different pools of human sera to cover all measuring range. Pool 1 was used to determine the linearity of the assay over “Whole Analytical Measurement Range (Whole AMR),” and Pool 2 was used to determine the linearity of the assay over “Low Analytical Measurement Range (AMR).” Pool 1 was created by spiking concentrated Lambda light chains into a human pool serum, followed by serially diluting the spiked sample with saline to obtain 10 samples with concentrations of Lambda light chains from 423 mg/dL – 0 mg/dL. Pool 2 was created by serially diluting a human pooled serum sample to create 10 samples with concentrations ranging from 54 mg/dL – 0 mg/dL. Each sample was run in triplet in both AMR test and Whole AMR tests. 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 ²	Relative Bias range	% CV range
Low AMR	1.8 – 54.3	1.05 (0.97 to 1.12)	-3.82 (-6.15 to -1.49)	0.9916	-67.5% to 0%	0.3% to 2.4%
Whole AMR	37.8 – 424.1	1.01 (0.98 to 1.04)	-5.74 (-12.57 to 1.08)	0.9987	-11.2% to 2%	1.0% to 5.8%

ORDAC range (Over Range Detection and Correction):

For a sample that is out of the linear range but between 400 to 800 mg/dL, the machine will follow the automated protocol ORDAC to achieve an acceptable degree of accuracy. To verify the accuracy of the ORDAC protocol that is performed automatically by the machine, a set of 25 samples, equally distributed within the range of 300 to 800 mg/dL were run in duplicate on Synchron Lx20 according to the ORDAC parameters setting. The results were compared with manually pre-diluted saline. Data was evaluated by plotting the result from automatically run with ORDAC protocol against the result from manually pre-diluted protocol. The data was summarized in the following table:

Test Range (mg/dL)	Slope (95% CI)	Y-intercept (95% CI)	R ²	Average Bias (95% CI)
285 to 1055	1.017 (0.987 to 1.047)	-12.88 (-28.69 to 2.92)	0.998	-4.72 (-10.74 to 1.30)

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
 Calibrator: 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 test:

Three levels of serum controls containing Lambda light chains (62.93 mg/dL, 145.93 mg/dL, and 226.10 mg/dL) were tested on Synchron LX 20 System with opened on-board reagents up to 29 days. Percent recovery for each control level was tested within 90% – 110% throughout the 29 days period.

Unopened Reagent Stability test:

Three different lots of unopened Reagent 1 and three different lots of unopened Reagent 2 were tested at different time frame up to 25 months. The calibrator is used as sample to be tested for the same time frame as the reagent. Percent recovery ranged from 85% – 115% for Reagent 1 and 2, 95% – 105% for calibrator. The shelf life for both reagents were claimed to be stable up to 24 months when stored at 2°C – 4°C.

- d. *Detection limit:*

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 was calculated to be 0.55 mg/dL based on formula of Mean + 1.65 x SD.

Limit of Detection:

To determine the Limit of Detection (LoD), a human sera pool was diluted with saline to obtain a concentration higher than the LoB (0.55 mg/dL). The diluted pool was measured in three different runs with 20 replicates each on Synchron LX20 System. If the result of Mean – 3 SD from all three runs is below the measuring range yet greater than LoB, then the concentration of the diluted pool is considered to be the LoD.

	Limit of Detection Mean (mg/dL)	SD (mg/dL)	Mean - 3 x SD (mg/dL)	Assessment Vs LOB	LOB (mean +1.645*SD)
Run 1 (n=20)	6.05	0.259	5.27	Pass	0.55 mg/dL
Run 2 (n=20)	5.77	0.593	3.99	Pass	
Run 3 (n=20)	5.32	0.494	3.83	Pass	
N= 60	<u>5.71</u>	0.554	4.05	Pass	

The LoD of this assay is 5.71 mg/dL.

Limit of Quantification:

A pool of human sera with a concentration around 20 mg/dL was serially diluted with saline to obtain a set of samples with decreased concentrations of human Lambda light chains. Each sample was tested on Synchron LX20 system with 10 replicates. The LoQ is defined by %CV being $\leq 10\%$ and the % relative bias, which is defined as the absolute bias (theoretical value – observed value) divided by theoretical value, being $\leq 20\%$. The Limit of Quantification is 20 mg/dL.

e. *Analytical specificity:*

Interference on Endogenous compounds in serum:

For the interference study on bilirubin, hemoglobin and triglycerides, human serum samples were spiked with various concentrations of interferents. A minimum of three replicates of each interference level were run. Absolute bias at each concentration level was determined against concentration of non-spiked pool. For the determination of potential interference by Rheumatoid Factor (RF), a human serum with a RF concentration of about 1012 U/mL was diluted with a human serum without RF. Absolute bias for RF interference was determined against theoretical values which were calculated based on the dilution ratio.

Interfering Substance	Max. concentration tested	No interference found up to
Bilirubin, conjugated	60 mg/dL	60 mg/dL
unconjugated	66 mg/dL	66 mg/dL
Hemoglobin	1000 mg/dL	1000 mg/dL
Triglycerides	1000 mg/dL	500 mg/dL
Rheumatoid Factor	1012 U/mL	303 mg/dL

Interferences were found in samples containing triglycerides higher than 500 mg/dL and Rheumatoid Factor higher than 300 mg/dL.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

To determine the degree of correlation between the Lambda light chains assay on the Synchron LX20 System vs. the IMMAGE Immunochemistry System Lambda light chain (k964260) on IMMAGE nephelometer Analyzer, 79 serum samples ranged from 21.2 mg/dL – 1120 mg/dL [corresponding to 78.9 mg/dL – 3643.1 mg/dL based on Whole IgG (MW 150,000)] were tested in duplicate with both devices. The result from the current device (calibration based on Lambda light chains as Whole IgG content), was plotted against the result from the predicate Nephelometer.

Regression Parameter	Acceptance Criteria	Equivalent to Whole IgG content MW 150,000	
		First replicates	Second replicates
Slope	0.90 – 1.10	0.950 (0.916 – 0.984)	1.017 (0.992 – 1.062)
Y-Intercept	NA	35.35 (8.77 – 61.93)	0.41 (-33.55 – 34.37)
Correlation Coefficient	>0.975	0.988	0.981
Range of samples tested (mg/dL)	NA	75 - 3840	75.4 - 3450
Std. Error of estimate (Sy/X)	NA	81.578	99.943
Average Bias (mg/dL)	NA	6.715 (-12.44 – 25.87)	9.943 (-12.38 – 32.26)

Results from additional 309 samples from patients aged 19 – 93y with Lambda light chain concentrations within the detectable range including ORDAC range were added later.

The results were summarized in the following table:

Regression Parameter	Acceptance Criteria	Equivalent to Whole IgG content MW 150000	
		First replicates	Second replicates
Slope	0.90 – 1.10	0.9850 (0.964 – 1.007)	0.928 (0.908 – 0.949)
Y-Intercept	NA	28.61 (14.45 – 42.77)	58.59 (44.53 – 72.65)
Correlation Coefficient	>0.975	0.982	0.981
Range of samples tested (mg/dL)	NA	42 - 3550	90 - 3730
Std. Error of estimate (Sy/X)	NA	65.65	66.73
Average Bias (mg/dL)	NA	20.30 (12.92 – 27.68)	17.54 (9.50 – 25.58)

b. Matrix comparison:

To demonstrate that the Lambda light chains assay can be used in both human serum and Li-heparin plasma, blood from 45 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 $R^2 \geq 0.98$.

Summary of results

Range (mg/dL)	80.50 – 1066	
Linear regression analysis	Specs	Found (95% CI)
Slope	0.95 to 1.05	0.991 (0.974 to 1.008)
Intercept	NA	-3.785 (-8.155 to 0.585)
R	≥ 0.98	0.998
Sy/x	NA	9.647
Bland/Altman analysis	Specs	Found (95% CI)
Bias	+/- 8.5mg/dL	-5.547 mg/dL (-8.451 to -2.642)

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 donors and 102 male donors, 20 – 66 years old. The Lambda light chains were found to be ranged from 62 mg/dL – 231 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.