510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k120056

B. Purpose for Submission:

Addition of urine matrix

C. Measurand:

Kappa (κ) free light chains

D. Type of Test:

Quantitative turbidimetry

E. Applicant:

The Binding Site, Ltd.

F. Proprietary and Established Names:

Freelite® Human Kappa Free Kit for use on the SPA_{PLUS}

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):

   Freelite® Human Kappa Free Kit for use on the SPA_{PLUS}: This kit is intended for the quantitation of kappa free light chains in serum and urine on Binding Site SPA_{PLUS}. Measurement of free light chains in serum aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. Measurement of free light chains in urine aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis and light chain deposition disease in conjunction with other laboratory and clinical 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:

   Binding Site SPA_{PLUS} (This is the same analyzer that was previously cleared under k040958. A minor software modification to the standard sample dilution was covered in k062372.)

I. Device Description:

Each Freelite® kit contains latex reagent consisting of polyclonal monospecific sheep antibody coated onto polystyrene latex in the presence of preservatives. The calibrator and controls consist of human sera that contain polyclonal kappa free light chains, stabilized in a liquid form with preservatives. A supplementary reagent containing preservatives is also included.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Freelite® Human Kappa and Lambda Free Kits for use on Hitachi Modular P

2. Predicate 510(k) number(s):

   k023009

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	Measurement of free light chains in serum aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. Measurement of free light chains in urine aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis and light chain deposition disease in conjunction with other laboratory and clinical findings.	Measurement of the various amounts of the different types of light chains aid in the diagnosis of and monitoring of multiple myelomas, lymphocytic neoplasms, Waldenstrom's macroglobulinemia and connective tissue diseases, such as systemic lupus erythematosus.
Measurement	Quantitative	Same
Detection Method	Turbidimetry	Same
Matrix	Serum and Urine	Same
Standards and Controls	Human sera containing human free light chains	Same

Differences		
Item	Device	Predicate
Instrument	Binding Site SPA_{PLUS}	Roche Hitachi 911, Hitachi 912, Hitachi 917 and Modular P
Measuring Range (mg/L)	Kappa: 4.0-180 (at standard 1/10 dilution) Extended Range for Kappa:	Kappa: 3.7-56.2 (at standard 1/5 dilution) Lambda: 5.6-74.8 (at standard 1/8 dilution)

Differences		
Item	Device	Predicate
	Neat: 0.4-18 1/100 dilution: 40-1800 1/1000 dilution: 400-18000 1/10000 dilution: 4000-180000	 Extended Range for Kappa: Neat: 0.8-11.2 1/50 dilution: 37-562 1/500 dilution: 370-5620 1/5000 dilution: 3700-56200 Extended Range for Lambda: Neat: 0.7-9.35 1/80 dilution: 56-748 1/800 dilution: 560-7480 1/8000 dilution: 5600-74800
Urine Reference Interval (mg/L)	Kappa: 0.012-32.71	Kappa: < 13.48 Lambda: < 5.9

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.
CLSI EP6-A: Evaluation of the Linearity of the Quantitative Measurement Procedure: A Statistical Approach; Approved Guideline.
CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition.
CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation.
CLSI C28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition.

L. Test Principle:

The concentration of the soluble antigen is assessed by turbidimetry. The test sample is added to a solution containing the appropriate antibody in a reaction cuvette. A beam of light is passed through the cuvette and is increasingly scattered by the formation of insoluble immune complexes. Light scatter is monitored by measuring the decrease in intensity of the incident beam of light. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. A series of calibrators of known antigen concentration are assayed to produce a calibration curve of measured light scatter versus antigen concentration. Samples of unknown antigen concentration can then be assayed and the results read from the calibration curve. The polyclonal sheep anti-free light chain antibody is coated onto polystyrene latex in order to increase the relative light-scattering signal of the antigen-antibody complexes, and thereby increase the sensitivity of the assay.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed according to CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition. Three urine samples with low, medium and high concentrations of the measuring range were tested over 21 days with 2 runs per day and each sample in duplicate per run. One lot of reagents and three analyzers were used. The acceptance criterion of total precision CV $\leq$ 15% was met.

	Within-Run		Between-Run		Between-Day		Between-Instrument		Total Precision	
Mean (mg/L)	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Low 5.821	0.133	2.30	0.398	6.80	0.475	8.20	0.307	5.23	0.634	10.90
Med 44.630	0.748	1.70	1.498	3.40	3.039	6.80	0.822	1.84	3.469	7.80
High 143.647	2.806	2.00	4.347	3.00	11.685	8.10	6.336	4.41	12.779	8.90

A lot-to-lot variability study was also performed. Two urine samples with low and medium concentrations of the measuring range were tested over 21 days with 2 runs per day and each sample in duplicate per run. Three lots of reagents and three analyzers were used. The acceptance criterion of total precision $\leq$ 15% was met.

		Kappa	
		Low	**Med**
Mean		**6.350**	**45.362**
Within-Run	**SD**	0.163	0.687
	%CV	2.6	1.5
Between-Run	**SD**	0.333	2.991
	%CV	5.3	6.6
Between-Day	**SD**	0.631	4.937
	%CV	9.9	10.9
Between-Batch	**SD**	0.638	0.566
	%CV	10.04	1.25
Between-Instrument	**SD**	0.291	2.936
	%CV	4.55	6.40
Total Precision	**SD**	0.732	5.814
	%CV	11.5	12.80

b. Linearity/assay reportable range:

A linearity study was conducted based on CLSI EP6-A: Evaluation of the Linearity of the Quantitative Measurement Procedure: A Statistical Approach;

Approved Guideline. Linear ranges were established using a dilution series of 9-11 concentrations (kappa 1.10-324.493 mg/L). An unprocessed urine sample with high analyte concentration was diluted with instrument diluent. Clinical samples were monoclonal samples from myeloma patients. The acceptance criteria for each concentration were met (i.e., mean recovery ±20% and % CV < 10%).

	Kappa	
Range (mg/L)	1.10-324.49	1.44-14.36
Slope (95% CI)	0.99 (0.9, 1.01)	0.96 (0.93, 1.00)
Y-intercept (95% CI)	0.75 (-2.82, 4.33)	-0.36 (-0.68, -0.04)
R^2	0.9977	0.9914
% Recovery	90.6-117.4	87.1-100

The measuring ranges when using the standard 1:10 dilution are 4.0-180 mg/L for kappa free light chains. This is consistent with previously cleared submission for serum (k062372).

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

There are no reference analytes or standards for this method. A calibrator set and two controls are provided in each kit. No changes were made from the previous cleared submissions (k062372 and k040009).

Kit Stability: No change from previous cleared submission.

Urine Stability: The stability of free light chains in urine was evaluated. A high and a low sample were evaluated at three time points: day 0, two weeks, and one month. Each sample was divided into two lots; one was stored at 4°C and the other at -20°C. The difference in results compared to the initial time point (day 0) was determined, and a difference ≤ 20% was considered acceptable. Kit controls were processed in each run as a quality control measure. From the results below, it was concluded that free light chains are stable in urine at 4°C for at least 28 days.

Kappa Free Light Chains

Days in storage	High sample		Low sample	
	% difference (from day 0)		% difference (from day 0)	
	4°C	-20°C	4°C	-20°C
Day 0	--	--	--	--
Day 13	3.3	7.2	-1.2	1.9
Day 31	-8.5	-6.4	-18.7	-13.6

d. Detection limit:

A study was performed in accordance with CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation. Instrument diluent (saline) was used as the blank sample, and diluted urine samples of

known low analyte concentrations were used to determine the limit of detection. The limit of detection was below the measuring range.

Free Light Chains	LoB (mg/L)	LoD (mg/L)
Kappa	0.030	0.102

e. Analytical specificity:

i. *Interference:* Testing was conducted according to CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition. High concentrations of hemoglobin, bilirubin, ascorbic acid and albumin were spiked into urine samples (containing approximately 32 mg/L kappa) and compared to matched samples without interferent. For albumin, a concentration series up to 5 g/L was evaluated, and the maximum level of acceptable interference (≤ 15%) was observed at 1.25 g/L.

Interferent	Concentration	% Interference
		Kappa
Hemoglobin	240 mg/L	8.2
Bilirubin	40 mg/L	-4.9
Ascorbic Acid	200 mg/ L	-10.3
Albumin	1.25 g/L	14.4

ii. *Antigen Excess:* Antigen excess was studied previously for serum samples (under k062372). The suitability of the previously determined prozone limits was assessed for urine samples. A series of four high concentration monoclonal urine samples with analyte levels from 3904 mg/L to 16249 mg/L were tested at 1/10 dilution. All samples were correctly flagged as antigen excess by the SPA_{PLUS} analyzer. The product insert warns that the amino acid composition of monoclonal free light chains may influence the level at which a sample may show antigen excess. Antigen excess for polyclonal serum samples remains unchanged.

iii. *Cross Reactivity:* Cross reactivity to whole immunoglobulins was previously carried out with serum samples.

f. Assay cut-off:

See Expected values.

2. Comparison studies:

a. Method comparison with predicate device:

Results from the Freelite® kits evaluated on the SPA_{PLUS} analyzer were compared to results obtained with the kits on the Hitachi Modular P analyzer (predicate). Clinical samples (myeloma) were obtained from a medical school in the UK and normal samples were obtained from adult donors in the UK. All samples were tested within 14 days of collection and stored at $4^{o}C$. 121 samples were evaluated (concentrations ranging from 0.88 mg/L to 87110 mg/L); one sample was a within method outlier with insufficient volume for

reanalysis. Passing-Bablok linear regression analysis was performed with the following results:

Regression equation y=1.04x + 0.92
The 95% confidence intervals for the slope are 0.99 – 1.07.
The upper limit of the reference range is 32.71 mg/L.
The bias is 2.12 mg/L at the upper limit of the reference range (6.5% bias).
The bias is 148 mg/L at 4000 mg/L (3.7% bias).

b. Matrix comparison:

Not applicable.

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:

There are no clinical guidelines for the medical decision points.

5. Expected values/Reference range:

Reference ranges were established in accordance with CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. Free light chain concentrations of 120 healthy individuals were measured. Users of the kits should generate their own ranges, as stated in the product insert.

Free light chains	Mean Conc (mg/L)	Median Conc (mg/L)	95th Percentile Range (mg/L)
Kappa	8.147	4.93	0.012-32.71

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.