510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k140396

B. Purpose for Submission:
Modification of the device (addition of urine matrix to SPA_{PLUS} instrument)

C. Measurand:
Lambda (λ) free light chains

D. Type of Test:
Turbidimetry, Quantitative

E. Applicant:
The Binding Site Group, Ltd.

F. Proprietary and Established Names:
Freelite® Human Lambda 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:
   DEH, Lambda, antigen, antiserum, control

4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):

   Freelite® Human Lambda Free Kit for use on the SPA_{PLUS}: The kit is intended for the quantitation of lambda 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):

   Prescription use only

4. Special instrument requirements:

   The Binding Site SPA_{PLUS} analyzer (k040958)

I. Device Description:

The 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 lambda 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) and 510(K) number(s):

   Freelite® Human Lambda Free Kit for use on Beckman Coulter IMMAGE™ (k003671)

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	Quantitation of lambda free light chains	Same
Indication 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 free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström'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	Lambda free light chains	Same
Measurement	Quantitative	Same
Detection Method	Turbidimetry	Same
Sample type	Serum and Urine	Same
Antibody	Polyclonal sheep antibody	Same

Differences		
Item	Device	Predicate
Instrument	Binding Site SPA_{PLUS}	Beckman Coulter IMMAGE/IMMAGE 800
Measuring Range (mg/L, Urine)	At 1/10 standard diluton: 4.5 – 165 Extended range: Neat: 0.45 – 16.5 1/100 dilution: 45 – 1650 1/1000 dilution: 450 – 16500 1/10000 dilution: 4500 – 165000	At 1/10 standard dilution: 4.8 – 162 Extended range: Neat: 0.48 – 16.2 1/5 dilution: 2.4 – 81 1/250 dilution: 120 – 4050 1/5000 dilution: 2400 – 81000
Reference Range (Urine)	<0.45 – 4.994 mg/L	0.81 – 10.1 mg/L

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

CLSI EP05-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition".

CLSI EP06-A "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline".

CLSI EP17-A "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline"

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

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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

The analytical performance of serum samples on the Freelite Human Lambda Free Kit for use on SPA_{PLUS} was established in k062372.

a. Precision/Reproducibility:

The precision was evaluated based on CLSI EP05-A2 by testing three urine samples with low, medium and high concentrations of the analyte. Each sample was assayed in duplicate with 2 runs per day for 21 days (n=84) using one lot of reagents and three instruments. The sponsor's specified acceptance criterion for total precision is CV% ≤15%. The results are summarized in the following table.

Mean Conc. (mg/L)	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Instrument**		**Total**	
	SD (mg/L)	CV %	SD (mg/L)	CV %	SD (mg/L)	CV %	SD (mg/L)	CV %	SD (mg/L)	CV %
7.54	0.28	3.70	0.29	3.90	0.43	5.70	0.32	4.16	0.59	7.80
48.95	0.87	1.80	1.21	2.50	2.75	5.60	1.68	3.48	3.13	6.40
149.55	4.15	2.80	8.59	5.70	10.25	6.90	4.05	2.71	14.00	9.40

To evaluate the lot-to-lot variability of the assay, two urine samples with low and medium concentrations of the measuring range were tested. Each sample was

assayed in duplicate with 2 runs per day for 21 days using three lots of reagents and three analyzers. The results are summarized in the following table:

Mean Conc. (mg/L)	**Within-Run**		**Between-Run**		**Between-Instrument**		**Between-Lot**		**Total**	
	SD (mg/L)	CV %	SD (mg/L)	CV %	SD (mg/L)	CV %	SD (mg/L)	CV %	SD (mg/L)	CV %
6.49	0.18	2.80	0.20	3.00	0.36	5.54	0.43	6.65	0.62	9.50
62.91	1.86	3.00	2.13	3.40	4.23	6.63	5.03	8.00	6.34	10.10

b. Linearity/assay reportable range:

Linearity: The linearity across the assay range was conducted based on CLSI EP06-A. Two dilution series were prepared by diluting high and medium level clinical samples with a normal urine pool: one dilution series of 10 samples was prepared with a concentration range of 2.10 to 405.28 mg/L, another dilution series of 11 samples was prepared with a concentration range of 3.51 to 81.29 mg/L. Each dilution was tested in triplicate. The observed values were graphed against the expected values, and the regression analyses are presented below:

	Range (mg/L)	**Slope (95% CI)**	**Intercept (95% CI)**	**R**
1	2.21 – 405.28	0.97 (0.94 – 1.00)	-2.06 (-10.31 – 6.19)	0.99
2	3.61 – 81.29	0.96 (0.92 – 1.00)	-1.81 (-3.41 – -0.21)	0.99
Pooled	2.21 – 405.28	0.97 (0.96 – 0.99)	-2.22 (-4.78 – 0.34)	1.00

The assay was shown to give a linear response over the claimed measuring range, 4.5 – 165 mg/L (at standard 1/10 sample dilution).

Antigen excess (hook effect): A study evaluating the effect of antigen excess in urine on the Freelite® Lambda Free assay was performed using the same protocol as the antigen excess study in serum samples performed in k062372. The suitability of the previously determined prozone limits was assessed by analyzing four normal and four monoclonal antigen excess urine samples at standard dilution (1/10). The optical density (OD) values from the calibration curve were obtained. The results showed that the samples with analyte levels above the 1/10 measuring range were correctly flagged as antigen excess samples by the SPA_{PLUS} analyzer. The antigen excess parameters have been demonstrated to give antigen excess protection in urine samples with concentrations up to 98 times the top point of the calibration curve.

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

Traceability: 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 previously cleared submission (k062372).

Stability:

Kit stability: The stability of the kit is supported by the studies presented in the previously cleared submission k062371. No change was made for the stability claims for the device.

Sample stability: The stability of lambda free light chains in urine was tested by evaluating two (2) samples with low and high analyte concentration level at different time points: 0, 14, 23, and 28 days. Each sample was divided into two batches: one was stored at 4°C and the other at -20°C. The sponsor's acceptance criterion for sample stability was recovery (%) at each time point of within ±15% of the initial reading taken at Day 0. The results support that stability of the analyte in urine sample stored at 2-8°C is up to 21 days.

d. Detection limit:

The study was performed in accordance with CLSI EP17-A. The limit of blank (LoB) for this assay was determined by testing instrument diluent with sixty (60) replicates. The LoB value was estimated to be 0.1 mg/L. The limit of detection (LoD) was determined by testing five (5) urine samples with low analyte concentration. Each sample was tested with 12 replicates. The LoD value was calculated as the LoB + 1.645 x SD of the replicates for the low level samples and was found to be 0.127 mg/L. The LoD lies below the lower end of measuring range 0.45 mg/L.

e. Analytical specificity:

Endogenous interference: Interference by endogenous substances was evaluated by using a base pool of urine containing 5 mg/L lambda free light chain (close to the top of the reference range). Hemoglobin, bilirubin, ascorbic acid and albumin were added to aliquots of the urine base pool to generate spiked samples. The interferent free samples were similarly prepared by spiking the same volume of interferent diluent into aliquots of the urine base pool. The resulting samples were tested in triplicate and the mean values were used to calculate % interference. The results are summarized in the following table:

Interferent	Concentration	% Interference
Hemoglobin	240 mg/L	0.5
Bilirubin	200 mg/L	-5.4
Ascorbic Acid	200 mg/ L	-1.7
Albumin	1.25 g/L	8.9

f. Assay cut-off:

See Expected values/reference range.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare the Freelite® Human Lambda Free Kit on the SPA_{PLUS} (y) and the predicate device on Beckman Coulter IMMAGE™ analyzer (x). A total 168 samples including 129 retrospective clinical urine samples from a hospital in the United Kingdom and 39 normal urine samples from healthy donor were tested. Out of 168 samples, only 124 samples were within the measuring range for both the proposed and the predicate device and the results were used for the regression analysis (Passing/Bablok). The following regression equation was obtained:

N=	Sample range (mg/L)	Regression analysis
124	0.52 – 45,450	y =1.03x + 0.14 Slope (95% CI): 0.95 – 1.09 Intercept (95% CI): -0.01 – 0.27

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**:**

Not applicable

5. Expected values/Reference range:

The reference range was established in accordance with CLSI C28-A3. Free light chain concentrations of 120 healthy individuals were measured. The results are summarized below:

	Mean Conc (mg/L)	**Median Conc (mg/L)**	**95% Reference Range (mg/L)**
Lambda Free Light Chains	0.934	0.545	<0.45 – 4.994

In the product insert, the sponsor recommends that each user of the kit should generate their own ranges.

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.