510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k062372

B. Purpose for Submission:
New analyzer

C. Analyte:
Kappa (κ) free light chains and Lambda (λ) free light chains

Type of Test:
Quantitative nephelometry

Applicant:
The Binding Site, Ltd

Proprietary and Established Names:
FREELITE™ Human Kappa Free Kit for use on the SPA_{plus}™
FREELITE™ Human Lambda Free Kit for use on the SPA_{plus}™

D. Regulatory Information:

1. Regulation section:
   21CFR §866.5550 Immunoglobulin (light chain specific) immunological test system
2. Classification:
   Class II
3. Product Code:
   DFH - Kappa antigen, antiserum, control
   DEH - Lambda antigen, antiserum, control
4. Panel:
   Immunology (82)

Intended Use:

5. Intended use(s):
   The intended use for each kit is the same as the predicate with the exception of the matrices and analyzer.

   FREELITE™ Human Kappa Free Kit for use on the SPA_{plus}™: The kit is intended for quantitation of kappa free light chains in serum on The Binding Site SpaPlus. Measurement of the different types of light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.

   FREELITE™ Human Lambda Free Kit for use on the SPA_{plus}™: The kit is intended for quantitation of lambda free light chains in serum on The Binding Site SpaPlus. Measurement of the different types of light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, amyloidosis, light chain deposition disease and connective

tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.

6. Indication(s) for use:

Same as above

7. Special condition for use statement(s):

Prescription use only.

8. Special instrument Requirements:

The Binding Site SPA_{plus}™

Device Description:

Latex reagent consists of polyclonal monospecific sheep antibody coated onto polystyrene latex in the presence of preservatives. The calibrator and controls consists of human sera that contain free polyclonal kappa or lambda free light chains, stabilized in a liquid form with preservatives.

E. Substantial Equivalence Information:

1. Predicate device name(s):
   FREELITE™ Human Kappa Free Kit for use on the Dade Behring Nephelometer™ II
   FREELITE™ Human Lambda Free Kit for use on the Dade Behring Nephelometer™ II
2. Predicate K number(s):
   k040009
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for Use	Measurement of the different types of light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.	Same
Detection Method	Nephelometry	Same
Differences		
Stability	**Device**	**Predicate**
Instrument	The Binding Site SPA_{plus}™	Dade Behring Nephelometer™ BN II
Matrix	Serum only	Serum or urine

Standard/Guidance Document Referenced (if applicable):

CLSI document EP5-A: Evaluation of Precision Performance of Clinical Chemistry; CLSI document EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures - A Statistical Approach.

Test Principle:

A 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 as the antibody-antigen reaction proceeds. The antibody in the cuvette is in excess so that the amount of immune complex formed is proportional to the antigen concentration. A series of calibrators of known antigen concentration are assayed to generate a calibration curve which is then used to determine the unknown concentration of the sample. The polyclonal, sheep antibody that is specific for the free light chain of interest, is coated onto polystyrene latex to increase the relative light-scattering signal of the immune complex formation, and thereby increase the sensitivity of the assay.

F. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed according to CLSI document EP5-A: Evaluation of Precision Performance of Clinical Chemistry Approved Guideline. Three different serum samples representing the low, medium and high levels of the measuring range were tested over 21 days, 2 runs per day, in duplicate. Acceptance criteria ±20% were met.

Kappa Precision	Within run		Between-run		Between-day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low (mean 7.21 mg/L)	0.24	3.3	0.30	4.2	0.81	11.2	0.90	12.5
Medium (mean 35.72 mg/L)	0.56	1.6	0.69	1.9	3.17	9.0	3.30	9.3
High (mean 123.77 mg/L)	2.22	1.8	2.90	2.3	8.22	6.6	8.99	7.3
Lambda Precision	Within run		Between-run		Between-day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low (mean 10.35 mg/L)	0.35	3.4	0.23	2.2	0.74	7.2	0.85	8.2
Medium (mean 35.09 mg/L)	0.85	2.4	0.0	0.0	1.55	4.4	1.76	5.0
High (mean 142.10 mg/L)	2.81	2.0	3.40	2.4	9.62	6.8	10.58	7.4

κ/λ ratio Precision: A precision study was also performed testing two levels of a control serum sample for both kappa and lambda for 10 separate runs in one day, in replicates of three, on 3 different SPA_{plus}™ analyzers to determine the precision of the contrived κ/λ ratios: SD< 0.08, %CV <6.65%.

b. Linearity/assay reportable range:

Linearity studies were performed according to CLSI document EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures - A Statistical Approach. Both monoclonal (myeloma) and polyclonal (SLE) samples previously identified as containing high levels of free kappa or

lambda light chains were diluted in a normal serum sample. Polyclonal samples were serially diluted 1:10 and linearity measured across the measuring range (kappa 4.0-180 mg/L; lambda 6.0-220mg/L).

Light Chain	**Type**	**Slope (95% CI)**	**Y-intercept (95% CI)**	**R^2**	**% recovery range**
Kappa (224161 kit lot 7699)	Polyclonal	1.0283 (0.9814 to 1.075)	-0.8611 (-4.513 to 2.7907)	0.9969	83.19 to 109.20
Lambda (224161 kit lot 7695)	Polyclonal	1.0035 (0.9925 to 1.015)	0.1024 (-0.731 to 0.936)	0.9998	95.56 to 104.98

Myeloma (monoclonal antibody) samples were diluted to give final percentages of 100%, 80%, 60%, 40%, 20%, 10% and 0% which covers the measuring ranges Three samples for each kappa and lambda were evaluated and % recovery ranged 85.68 to 102.92% for kappa and 84.65 to 115.39% for lambda. The linear regression data below represents data from one myeloma sample. Because linearity for monoclonal samples varies, a limitation was added to the package insert with regard to linearity of free light chains.A limitation was added to the package insert with regard to linearity of free light chains.

Sample	Slope (95% CI)	Y-intercept (95% CI)	R^2
Kappa (monoclonal sample)	1.0036 (0.963 to 1.044)	0.543 (-4.1595 to 5.2455)	0.9975
Lambda (monoclonal sample)	0.9942 (0.9766 to 1.0118)	-2.4962 (-8.6025 to 3.61)	0.9994

c. *Traceability (controls, calibrators, or method):*
There are no reference standards for this method. A calibrator and two controls are provided with each kit. No changes were made to the calibrator and controls from the previous cleared submission.

d. *Detection limit:*
The limit of detection was evaluated by assaying a blank 20 times for both kappa and lambda free light chain assays. The mean plus 3SD was calculated. The lower limit of detection equates to -0018 delta absorbance units for kappa free and 0.0006 delta absorbance units for lambda free. The limit of detection was demonstrated to be below the measuring range of the assay.

e. *Analytical specificity:*
   i. *Interference*- Susceptibility to interference was assessed by adding high concentrations of triglycerides (Intralipid), hemoglobin and bilirubin to a test serum sample containing known concentrations of polyclonal free kappa and free lambda light chains and compared to matched samples without interferent. Samples tested in triplicate.

Analyte (mg/L.)	% Interference							
	κ (~ 1.4 mg/L)	κ (~10.0 mg/L)	λ (~2.5 mg/L)	λ (~ 6.8 mg/L)	κ high control (~31.2 mg/L)	κ low control (~16.5 mg/L)	λ high control (~59.4 mg/L)	λ low control (~28.5 mg/L)
Bilirubin 200 mg/L		-5.0%		2.7%				
Hemoglobin 3 g/L	2.10%		-1.55%		-2.68%	-2.5%	-9.01%	-5.28%
Triglycerides (Intralipid)	-9.1% (0.1% Intralipid)			-3.0% (0.3% Intralipid)				

Interference by whole immunoglobulins IgG, IgA and IgM serum samples and rheumatoid arthritis was evaluated by spiking into human sera containing low and high levels of kappa or lambda, and compared to saline spiked matched samples.

	% Difference			
Sample (conc. in mg/L)	IgG to 10 g/L	IgA to 2 g/L	IgM to 1 g/L	RF to 320 IU/mL
Kappa high (~148.43)	2.77%	2.21%	0.96%	15.52%
Kappa low (~21.31)	14.9%	19.8%	-1.85%	-2.68%
Lambda high (~148.29)	-0.80%	-3.76%	0.00%	-3.63%
Lambda low (~19.35)	4.67%	8.07%	-0.75%	1.33%

ii. *Cross-Reactivity*-cross-reactivity to other disease conditions was not evaluated.

iii. *Antigen excess*- The SPA_{plus}™ analyzer is set up to flag the user to prozone effects based on the kinetics of the reaction. 147 myelomas (monoclonal) were used to establish prozone parameters. A limitation warns that due to the unpredictable nature of monoclonals, falsely low values may be reported. Antigen excess for polyclonal samples does not occur up to 330 mg/L for both free kappa and lambda light chains.

f. Assay cut-off:

See Expected values

2. Comparison studies:

a. Method comparison with predicate device:

The Binding Site FREELITE™ assays for kappa and lambda were evaluated on the new analyzer SPA_{plus}™ and compared to the results obtained with the kits on the predicate analyzer Dade Behring BNII. A total of 176 samples were assayed spanning the measuring ranges (kappa sample range 5.38 to 1978 mg/L; lambda sample range 2.1 to 6620 mg/L) of which 119 were normal serum samples and 56 were known/suspected monoclonal gammopathy patients (including multiple myeloma) or systemic lupus erythematosus (SLE) patients. Samples were obtained from a university clinical lab and a blood transfusion service located in the UK (patient age 18-65). All samples were stored at -20°C prior to use. Passing-Bablock linear regression analysis was performed and yielded the following data:

	Sample	n	Slope (95% CI)	Y-intercept (95% CI)	r
Kappa	Normal	119	1.000 (0.945 to 1.063)	-1.700 (-2.525 to -1.088)	0.9564
	Clinical	56	1.162 (1.092 to 1.212)	1.800 (0.278 to 2.911)	0.9912
	Overall	175	1.091 (1.043 to 1.162)	-2.426 (-3.281 to -1.902)	0.9564
Lambda	Normal	120	0.912 (0.842 to 0.984)	1.192 (0.157 to 2.222)	0.9612
	Clinical	56	1.026 (0.983 to 1.067)	0.483 (-0.042 to 1.833)	0.9975
	Overall	176	1.003 (0.982 to 1.032)	0.095 (0.982 to 1.032)	0.9977
κ/λ Ratio	All	176	0.892 (0.841 to 0.934)	0.003 (-00.23 to 0.049)	0.9954

b. Matrix comparison:
Serum is the only sample specimen.

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:
   Refer to expected values
5. Expected values/Reference range:
   Adult serum ranges were obtained by measuring the light chain concentrations of 282 normal adult sera (age 20-90 years old) using the assays on the Dade Behring BNII.

Normal Adult serum	Mean conc.	Median conc.	95th percentile range
Free Kappa	8.36 mg/L	7.30 mg/L	3.30 to 19.40 mg/L
Free Lambda	13.43 mg/L	12.40 mg/L	5.71 to 26.30
	Mean	Median	Total Range
κ/λ ratio	0.63	0.60	0.26 to 1.65

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.