510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k040009

B. Analyte:
Kappa free light chains and lambda free light chains

C. Type of Test:
Nephelometric or turbidimetric, quantitative

D. Applicant:
The Binding Site, Ltd.

E. Proprietary and Established Names:
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
FREELITE™ Human Kappa Free Kit for use on the Beckman Coulter IMMAGE™
FREELITE™ Human Lambda Free Kit for use on the Beckman Coulter IMMAGE™
FREELITE™ Human Kappa Free Kit for use on the HITACHI 911/912/917/Modular P
FREELITE™ Human Lambda Free Kit for use on the HITACHI 911/912/917/Modular P

F. 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
   DEH, Lambda, antigen, antiserum, control
4. Panel:
   IM (82)

G. Intended Use:
The amyloidosis claim has been added to the intended use of the FREELITE™ Human Kappa Free kit and the FREELITE™ Human Lambda Free kit for use on the Beckman Coulter IMMAGE™ and the Hitachi 911/912/917/Modular P. No change was made to the FREELITE™ Human Kappa Free kit and the FREELITE™ Human Lambda Free kit for use on the Dade Behring Nephelometer™ II.
The intended use for the FREELITE™ Human Kappa or Lambda Free Kit is as follows:
The Binding Site FREELITE™ Human Kappa or Lambda Free Kit is intended for the quantitation of kappa free light chains or lambda free light chains in serum and urine on the Dade Behring Nephelometer II (BN™ II) or Beckman Coulter IMMAGE or Roche HITACHI 911, HITACHI 912, HITACHI 917 and Modular P. Measurement of the various amounts 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.

1. Indication(s) for use:

Same as intended use.

2. Special condition for use statement(s):
   For prescription use only.
3. Special instrument Requirements:
   Dade Behring Nephelometer II (BNII), Beckman Coulter IMMAGE, Roche HITACHI 911, 912, 917 and Modular P analyzers

H. Device Description:

The FREELITE™ Human Kappa or Lambda Free Kit consists of the following: latex reagent (polystyrene beds coated with monospecific antibody), a standard, a high control, a low control and supplementary reagent.

I. 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
   FREELITE Human Kappa Free Kit for use on the Beckman Coulter IMMAGE™
   FREELITE Human Lambda Free Kit for use on the Beckman Coulter IMMAGE ™
   FREELITE Human Kappa Free Kit for use on the HITACHI 911/912/917/Modular P
   .
2. Predicate K number(s):
   k003669, k003671, k023009, k023131, k031016
3. Comparison with predicate:
   The purpose for this submission was to provide data to support changing the format of the latex reagent from freeze dried to liquid for the Dade Behring BNII and Beckman IMMAGE FREELITE™ Human Kappa Free kits and FREELITE™ Human Lambda Free Kits and the addition of the amyloidosis claims to the Beckman IMMAGE and Roche HITACHI FREELITE™ kits.

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

None referenced.

K. Test Principle:

The FREELITE™ Human Kappa Free kits and FREELITE™ Human Lambda Free kits are nephelometric or turbidimetric assays. The test sample is added to a solution containing the appropriate antibody in a reaction vessel. A beam of light is passed through the vessel and as the antigen-antibody reaction proceeds, the light passing through is increasingly scattered as insoluble immune complexes are formed. The amount of immune complex formed is proportional to the antigen concentration in the test sample. In nephelometry, the light scatter is monitored by measuring the light intensity at an angle away from incident light whereas in turbidimetry, light scatter is by measuring the increase in intensity of the incident light beam. A series of calibrators of known antigen concentration are assayed to construct a calibration curve which will be used for determining the antigen concentration of test samples.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
   The precision studies were performed at the Binding Site with the liquid latex reagents. Studies for each analyzer were performed at different times using different reagent lots. The kappa light chain assays used three concentrations of Level 1 whereas the lambda light

chain assays used Level 1 and two other positive samples. The samples were assayed in replicates of 10 for the within-run precision and in singlicate on 10 separate runs for the between-run precision. Results were summarized below:

BN II

Within-run precision – Kappa				Within-run precision - Lambda			
Sample	Level 1	Level 2	Level 3	Sample	Level 1	Level 2	Level 3
Mean (mg/L)	51.06	32.76	13.56	Mean (mg/L)	71.84	21.89	15.12
SD (mg/L)	2.16	1.58	0.42	SD (mg/L)	3.28	1.08	1.21
%CV	4.23	4.84	3.10	%CV	4.8	5.2	8.4

Between-run precision – Kappa				Between-run precision - Lambda			
Sample	Level 1	Level 2	Level 3	Sample	Level 1	Level 2	Level 3
Mean (mg/L)	54.66	32.17	12.02	Mean (mg/L)	71.71	24.20	18.43*
SD (mg/L)	3.84	2.56	0.71	SD	5.06	1.08	1.40
%CV	7.4	8.38	6.27	%CV	7.45	4.72	8.08

*One outlier sample (13.1 mg/L) was excluded from the calculation. If included, the mean±SD and % CD are 17.9±2.08 mg/L and 11.6% respectively.

IMMAGE

Within-run precision – Kappa				Within-run precision - Lambda			
Sample	Level 1	Level 2	Level 3	Sample	Level 1	Level 2	Level 3
Mean (mg/L)	57.1	27.11	18.3	Mean (mg/L)	98.78	46.79	31.83
SD (mg/L)	1.09	0.88	0.43	SD (mg/L)	2.25	1.81	1.93
%CV	2.00	3.41	2.48	%CV	2.4	4.08	6.4

Between-run precision – Kappa				Between-run precision - Lambda			
Sample	Level 1	Level 2	Level 3	Sample	Level 1	Level 2	Level 3
Mean (mg/L)	57.59	27.5	20.09	Mean (mg/L)	101.62	64.52	33.85
SD (mg/L)	3.51	1.89	2.38	SD (mg/L)	2.76	5.75	2.16
%CV	6.42	7.23	12.47	%CV	2.86	9.39	6.74

.

b. Linearity/assay reportable range:

A serum sample containing high concentrations of free kappa and lambda was serially diluted and each dilution was assayed at the normal sample dilution of 1:100 for BNII and 1:10 for IMMAGE. The samples were also run at 1:20 for BNII, 1:5 for IMMAGE Lambda and 1:1 for both IMMAGE Kappa and Lambda to confirm linearity at the low and high regions of the measuring range. All studies were performed at the Binding Site. No 95% CI for the slope or intercept was provided but was calculated by the reviewer.

<u>BNII</u>

Kappa – Linearity at 1:100 gave y = 1.0052x – 0.1154 (r^2 = 0.9955). The 95% CI for slope and the intercept were (0.9532 to 1.0542) and (-1.3597 to 1.1767) respectively (9 samples)

Linearity at 1:20 gave y = 1.0423x + 3.6932 (r^2 = 0.9834). The 95% CI for the slope and the intercept were (0.9215 to 1.1631) and (-6.0518 to 13.4383) respectively (9 samples)
The combined data gave y = 1.0774x – 0.2537 (r^2 = 0.9882) with 95% CI of slope (1.0151 to 1.1397) and the intercept (-3.9761 to 3.4687).
The assay measuring range is 6-190 mg/L

Lambda – Linearity at 1:100 gave y = 0.9839x + 0.0893 (r^2 = 0.9769). The 95% CI for the slope and the intercept were (0.8487 to 1.1192) and (-6.8080 to 6.9866) respectively (9 samples)
Linearity at 1:20 gave y = 1.0353x -7.3410 (r^2 = 0.9985). The 95% CI for the slope and the intercept were (0.9959 to 1.0747) and (-14.3463 to -0.3357) respectively (8 samples)
The combined data gave y = 1.0151x – 2.7463 (r^2 = 0.9979) with 95% CI of the slope and the intercept (0.9894 to 1.0408) and (-6.0199 to 0.5726) respectively.
The assay measuring range is 8-260 mg/L

IMMAGE

Kappa - Linearity at 1:10 gave y = 0.9826x – 1.0617 (r^2 = 0.9938). The 95% CI for slope and the intercept were (0.9134 to 1.0518) and (-3.4615 to 1.3381) respectively (9 samples)
Linearity at 1:1 gave y = 1.0402x - 9.1018 (r^2 = 0.9977). The 95% CI for the slope and the intercept were (0.9491 to 1.1314) and (-19.9493 to 1.7456) respectively. (5 samples)
The combined data gave y = 1.0065x – 3.2801 (R^2 = 0.9962) with 95% CI of slope (0.9672 to 1.0458) and the intercept (-6.2814 to -0.2789).
The assay measuring range 6-180 mg/L

Lambda – Linearity at 1:10 gave y = 1.0678x – 4.0516 (r^2 = 0.9951). The 95% CI for the slope and the intercept were (1.0006 to 1.1350) and (-7.6061 to -0.4981) respectively (9 samples)
Linearity at 1:5 gave y = 1.0348x + 0.8347 (r^2 = 0.9962). The 95% CI for the slope and the intercept were (0.9709 to 1.0986) and (-5.2347 to 6.9040) respectively (10 samples)
Linearity at 1:1 gave y = 1.0308x – 15.4130 (r^2 = 0.9782). The 95% CI for the slope and the intercept were (0.9491 to 1.1314) and (-80.2294 to 49.4033) respectively (4 samples).

The combined data gave y = 1.0077x – 1.3177 (r^2 = 0.9764) with 95% CI of the slope and the intercept (0.9365 to 1.0788) and (-7.8189 to 5.1835) respectively. The assay measuring range is 8-270 mg/L

The combined linearity data for both analytes on the BNII and IMMAGE were also reanalyzed using NCCLS EP6 and found to be acceptable.

c. Traceability (controls, calibrators, or method):
No reference standards or method available. For each assay kit, a calibrator, a low and a high positive control are included.

d. Detection limit (functional sensitivity):
Not performed for the purpose of this submission.

e. Analytical specificity:
Interference was assessed by spiking high concentrations of triglycerides (1930 formazine turbidity units), hemoglobin (5g/L) and bilirubin (200 mg/L) into aliquots of a test serum sample containing known concentrations of free kappa and free lambda light chains. In addition, saline was used as control for hemoglobin and bilirubin and chyle blank for triglycerides. All assays were run in triplicate. Results of spiked in samples were compared to control samples to determine % interference. No significant interference was observed with the BNII or the IMMAGE assays.

f. Assay cut-off:
Not performed for the purpose of this submission

2. Comparison studies:

a. Method comparison with predicate device:
The modified kappa and lambda kits were compared with the previously cleared kits. All normal and patient samples were from Birmingham. The normal samples were collected from a blood transfusion service and the patient samples were from a university hospital clinical immunology laboratory. All samples were stored frozen at -20ºC prior to use.

BNII

Kappa - 95 serum samples consisted of 21 normal, 8 SLE and 66 myeloma samples were analyzed. The concentration of kappa free light chains ranged from 0.12 mg/L to 2615 mg/L with a mean of 165.57 mg/L and a median of 7.66 mg/L. Linear regression analysis yielded y = 0.9818x + 4.5206 and the correlation coefficient (r) was 0.9956,. No 95% CI for the slope or the y-intercept was provided. As calculated by the reviewer, the 95% CI for the slope is 0.9627 to 1.0009 and for the intercept, -4.6485 to 13.6072.

Lambda - 54 samples consisted of 5 normal, 6 SLE and 43 myeloma samples were assayed. The concentration of lambda free light chains ranged from 2.15 mg/L to 3455 mg/L with a mean of 442.65 mg/L and a median of 17.03 mg/L. Linear regression yielded y = 0.9266x + 9.5556 and the correlation coefficient (r) was 0.9752. Similar to kappa, no 95% CI for the slope and intercept was provided. As calculated by the reviewer, the 95% CI for the slope is 0.8681 to 0.9851 and for the intercept, -47.5224 to 66.5807.

IMMAGE

Kappa - 63 serum samples consisted of 21 normal, 2 SLE and 40 myeloma samples were analyzed. The concentration of kappa free light chains ranged from 1.6 mg/L to 2198 mg/L with a mean of 155.49 mg/L and a median of 6.83 mg/L. Linear regression analysis yielded y = 1.0314x + 2.6792 and the correlation coefficient (r) was 0.9805. No 95% CI for the slope or the y-intercept was provided. As calculated by the reviewer, the 95% CI for the slope is 0.9785 to 1.0843 and for the intercept, -19.962 to 25.3215.

Lambda - 65 samples consisted of 21 normal, 2 SLE and 42 myeloma samples were assayed. The concentration of lambda free light chains ranged from 2.4 mg/L to 3738 mg/L with a mean of 425.13 mg/L and a median of 11.89 mg/L. Linear regression yielded y = 0.8503x -11.42 and the correlation coefficient (r) was 0.9651. Similar to kappa, no 95% CI for the slope and intercept was provided. As calculated by the reviewer, the 95% CI for the slope is 0.7922 to 0.9083 and for the intercept, -62.82 to 39.95.

To demonstrate equivalent performance between the IMMAGE and BNII FREELITE Kappa and Lambda Free kits, the following comparison studies were performed:

Kappa - Samples for this study consisted of 21 normal samples, 2 SLE samples and 34 myeloma samples. The normal samples were from a blood transfusion service and the clinical samples from a clinical laboratory. Using the BNII kit, the mean kappa free light chain concentration was 126 mg/L (ranged from 0.08 mg/L to 2330 mg/L) as compared to a mean concentration of 140.5 mg/L (ranged from 0.69

mg/L to 2460 mg/L) with the IMMAGE kit. The difference between the sample means was not statistically significant. Linear regression analysis yielded y = 1.07x + 5.89 and the correlation coefficient (r) was 0.99. There was no statistical analysis of the slope or y-intercept. As calculated by the reviewer, the 95% confidence interval for the y-intercept was -12.66 to 24.45 and for the slope, 1.02 to 1.11. The standard error with y (IMMAGE) for a given x (BNII) was 66 mg/L.

Lambda - Samples for this study consisted of 21 normal samples, 2 SLE samples and 29 myeloma samples. The normal samples were from a blood transfusion service and the clinical samples from a clinical laboratory. Using the BNII kit, the mean lambda free light chain concentration was 296.5 mg/L (ranged from 0.78 mg/L to 1980 mg/L) as compared to a mean concentration of 296.6 mg/L (ranged from 0.69 mg/L to 2420 mg/L) with the IMMAGE kit. The difference between the sample means was not statistically significant. Linear regression analysis yielded y = 0.97x + 10.08 and the correlation coefficient (r) was 0.97. There was no statistical analysis of the slope or y-intercept. As calculated by the reviewer, the 95% confidence interval for the y-intercept was -34.64 to 54.79 and for the slope, 0.90 to 1.05. The standard error with y (IMMAGE) for a given x (BNII) was 140 mg/L.

No new comparison study was performed for the HITACHI kits since these kits have demonstrated equivalent performance to the BNII kits in previous submissions.

b. Matrix comparison:
Since the sample matrices are the same, .no comparison study is needed.

3. Clinical studies:
   a. Clinical sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.
   c. Other clinical information
   Not applicable
4. Clinical cut-off:
   Not applicable.
5. Expected values/Reference range:
   Not applicable for the purpose of this submission.

M. Conclusion:

Based on the review of the information provided in this 510 (k) submission, the modified devices are Substantially Equivalent to the predicate devices regulated under 21 CFR §866.5555, Immunoglobulin (light chain specific) immunological test system (Class II, product code DFH, Kappa, antigen, antiserum, control and DEH, Lambda, antigen, antiserum, control, Immunology Device Panel 82).