510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172613

B. Purpose for Submission:

Modified IVD assay on previously cleared instrument

C. Measurand:

Immunoglobulin IgG Kappa (combined γ heavy and κ light chain) and Immunoglobulin IgG Lambda (combined γ heavy and λ light chain)

D. Type of Test:

Quantitative, Turbidimetry

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Optilite Hevylite IgG Kappa Kit
Optilite Hevylite IgG Lambda Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5510, Immunoglobulins A, G, M, D, and E Immunological Test System

2. Classification:

   Class II

3. Product codes:

   PCN - IgG Kappa (Heavy and Light chain Combined). Antigen, antiserum, control
   PCO - IgG Lambda (Heavy and Light chain Combined). Antigen, antiserum, control

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended uses:

   Optilite Hevylite IgG Kappa is a quantitative in vitro assay performed on the Optilite analyser for the measurement of IgG kappa (IgG heavy chain and kappa light chain intact immunoglobulin) in serum. Measurement of Hevylite Human IgG Kappa is used alongside Hevylite Human IgG Lambda to calculate the IgG Kappa / IgG Lambda ratio. The Hevylite Human IgG Kappa / IgG Lambda ratio can be used when monitoring previously diagnosed IgG multiple myeloma and is used in conjunction with other laboratory tests and clinical evaluations. The assignment of complete response is reliant upon other tests including immunofixation, bone marrow and urine assessments.

   Optilite Hevylite IgG Lambda is a quantitative in vitro assay performed on the Optilite analyser for the measurement of IgG lambda (IgG heavy chain and lambda light chain intact immunoglobulin) in serum. Measurement of Hevylite Human IgG Lambda is used alongside Hevylite Human IgG Kappa to calculate the IgG Kappa / IgG Lambda ratio. The Hevylite Human IgG Kappa / IgG Lambda ratio can be used when monitoring previously diagnosed IgG multiple myeloma and is used in conjunction with other laboratory tests and clinical evaluations. The assignment of complete response is reliant upon other tests including immunofixation, bone marrow and urine assessments.

2. Indications for use:

   Same as intended use.

3. Special conditions for use statements:

   For prescription use only.

   Warning: The result of Hevylite IgG Kappa in a given specimen determined with assays with different manufacturers or different instrument platforms can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Hevylite IgG Kappa assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of serially monitoring a patient, the assay method used for determining Hevylite IgG Kappa levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

   Warning: The result of Hevylite IgG Lambda in a given specimen determined with assays with different manufacturers or different instrument platforms can vary due to differences

in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Hevylite IgG Lambda assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of serially monitoring a patient, the assay method used for determining Hevylite IgG Lambda levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.

4. Special instrument requirements:

   The Binding Site Optilite analyzer

I. Device Description:

The Hevylite Human IgG Kappa and IgG Lambda Kits contain vials of ready-to-use polyclonal monospecific sheep anti-IgG antisera against combined γ heavy and κ light chain or combined γ heavy and λ light chain, calibrators (six levels), controls (low and high) and reaction buffer in liquid form. The reagents contain 0.099% sodium azide as preservative.

J. Substantial Equivalence Information:

1. Predicate device names:

   Hevylite® Human IgG Kappa Kit and Hevylite® Human IgG Lambda Kit for use on Siemens BN™ II Systems

2. Predicate 510(k) numbers:

   K132555

3. Comparison with predicate:

Similarities		
Item	Device Optilite® Hevylite® IgG Kappa (IgGκ) and IgG Lambda (IgGλ) Kits	Predicate Hevylite® IgG Kappa (IgGκ) and IgG Lambda (IgGλ) Kits
Intended Use	Quantitative in vitro assay for the measurement of IgG Kappa (IgG heavy chain and kappa light chain intact immunoglobulin) and IgG Lambda (IgG heavy chain and lambda light chain intact immunoglobulin) in serum.	Same

Similarities		
Item	Device Optilite® Hevylite® IgG Kappa (IgGκ) and IgG Lambda (IgGλ) Kits	Predicate Hevylite® IgG Kappa (IgGκ) and IgG Lambda (IgGλ) Kits
	Measurement of Hevylite Human IgG Kappa is used alongside Hevylite Human IgG Lambda to calculate the IgG Kappa/IgG Lambda ratio. The Hevylite Human IgG Kappa/IgG Lambda ratio can be used when monitoring previously diagnosed IgG multiple myeloma and is used in conjunction with other laboratory tests and clinical evaluations. The assignment of complete response is reliant upon other tests including immunofixation, bone marrow and urine assessments.	
Analyte	IgG Kappa and IgG Lambda	Same
Antibody	Polyclonal monospecific sheep anti-human combined γ heavy and κ light chain or combined γ heavy and λ light chain	Same
Control	Binding Site High and Low Controls	Same
Traceability	ERM-DA470k/IFCC	Same
Sample Matrix	Serum	Same
Capture Antibody	Sheep anti-human IgG combined	Same
Calibrator	Single level Binding Site Hevylite calibrator autodiluted by the analyser to six different concentrations	Same
Open Vial Stability	Three months	Same
Reference Interval	IgGκ: 4.03 – 9.78 g/L IgGλ: 1.97 – 5.71 g/L IgGκ/IgGλ Ratio: 0.98 – 2.75	Same

Differences		
Item	Device Optilite® Hevylite® IgG Kappa (IgGκ) and IgG Lambda (IgGλ) Kits	Predicate Hevylite® IgG Kappa (IgGκ) and IgG Lambda (IgGλ) Kits
Method	Turbidimetry	Nephelometry
Instrument	Binding Site Optilite	Siemens BN™ II Systems
On Board Stability	28 days	Not stated
Measuring Range	At standard 1/20 dilution: IgGκ: 2.3 – 30.0 g/L IgGλ: 1.5 – 17.5 g/L Extended Range for IgGκ: 1/1 dilution: 0.115 – 1.5 g/L 1/5 dilution: 0.575 – 7.5 g/L 1/80 dilution: 9.2 - 120 g/L Extended Range for IgGλ: 1/1 dilution: 0.075 – 0.875 g/L 1/5 dilution: 0.375 – 4.375 g/L 1/80 dilution: 6 - 70 g/L 1/120 dilution: 9 – 105 g/L	At standard 1/100 dilution: IgGκ: 1.72–27.5 g/L IgGλ: 0.88–14.0 g/L Extended Range for IgGκ: 1/5 dilution: 0.09–1.38 g/L 1/20 dilution: 0.34–5.50 g/L 1/400 dilution: 6.88–110.0 g/L 1/2000 dilution: 34.4–550 g/L Extended Range for IgGλ: 1/5 dilution: 0.04–0.70 g/L 1/20 dilution: 0.18–2.80 g/L 1/100 dilution:0.88–14.0 g/L 1/400 dilution: 3.50–56.0 g/L 1/2000 dilution: 17.5–280 g/L

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

CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.
CLSI EP07-A2 Interference Testing in Clinical Chemistry, Approved Guideline – Second Edition.
CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach.
CLSI EP05-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.
CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory.

L. Test Principle:

Evaluating the concentration of a soluble antigen (e.g. IgG Lambda) by turbidimetry involves the addition of the test sample to a solution containing the appropriate antibody (anti-IgG Lambda) in a reaction vessel or cuvette. A beam of light is passed through the cuvette and, as the antigen-antibody reaction proceeds, the light passing through the cuvette is scattered

increasingly as insoluble immune complexes are formed. 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 initially 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:

1. Analytical performance:

The results of all the studies met the Manufacturer's pre-specified acceptance criteria.

a. Precision/Reproducibility:

The within-run, between-run, between-day, between-lot and between-instrument imprecision were determined by testing six serum samples over 21 days with two runs per day on three different reagent lots and three different analyzers.
Results are summarised below.

IgGκ precision studies:

Sample	Mean (g/L)	Within Run		Between Run		Between Day		Between Lot		Between Instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	3.148*	0.048	1.5	0.104	3.3	0.124	3.9	0.098	3.1	0.036	1.1	0.169	5.4
2	4.260	0.184	4.3	0.209	4.9	0.162	3.8	0.14	3.3	0.033	0.8	0.323	7.6
3	5.601*	0.145	2.6	0.263	4.7	0.308	5.5	0.205	3.7	0.099	1.8	0.430	7.7
4	7.621	0.178	2.3	0.263	3.5	0.413	5.4	0.426	5.6	0.216	2.8	0.521	6.8
5	13.574	0.218	1.6	0.385	2.8	0.879	6.5	0.851	6.3	0.253	1.9	0.984	7.2
6	16.824	0.383	2.3	0.352	2.1	0.992	5.9	0.991	5.9	0.608	3.6	1.120	6.7

IgGλ precision studies:

Sample	Mean (g/L)	Within Run		Between Run		Between Day		Between Lot		Between Instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	1.313*	0.029	2.2	0.034	2.6	0.068	5.2	0.054	4.1	0.037	2.8	0.081	6.2
2	2.379*	0.059	2.5	0.049	2.1	0.064	2.7	0.061	2.6	0.027	1.1	0.100	4.2
3	2.894	0.118	4.1	0.120	4.2	0.198	6.8	0.119	4.1	0.142	4.9	0.260	9.0
4	4.262	0.096	2.2	0.144	3.4	0.267	6.3	0.182	4.3	0.190	4.4	0.318	7.5
5	6.657	0.138	2.1	0.112	1.7	0.301	4.5	0.256	3.8	0.200	3.0	0.350	5.3
6	13.941	0.297	2.1	0.237	1.7	0.414	3.0	0.541	1.7	0.096	0.7	0.561	4.0

*samples run at 1/5 dilution

b. Linearity/assay reportable range:

A linearity study was performed following CLSI guideline EP-6A. The linearity of the IgGκ and IgGλ assays have been confirmed using serially diluted serum samples to cover the standard measuring ranges of 2.3 – 30.0 g/L and 1.5 – 17.5 g/L, respectively. The results demonstrated that the IgGK and IgGL assays are linear over the ranges of 1.930 – 33.427 g/L and 1.380 – 19.300 g/L at 1+19 dilution.

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

Traceability:
An internal reference material was assigned by comparison with the Reference Material ERM-DA470k/IFCC.

Stability:
Open-vial stability was performed on three lots of Optilite Hevylite IgG Kappa and IgG Lambda kits with testing intervals at various time points up to 112 days. Data supports an open vial stability claim of 3 months at 2-8°C.

On-board stability was performed on three lots of Optilite Hevylite IgG Kappa Open-vial stability was performed on three lots of Optilite Hevylite IgG Kappa and IgG Lambda kits with testing intervals at various time points up to 112 days. Data supports an open vial stability claim of 3 months at 2–8°C.

On-board stability was performed on three lots of Optilite Hevylite IgG Kappa and IgG Lambda kits with testing intervals at various time points up to 37 days for IgGK and 49 days for IgGL. Data supports an on-board stability claim of 28 days, provided that the power is left switched on as stated in the product insert.

d. Detection limit:

The analytical sensitivity was determined in accordance with CLSI EP17-A. The Limit of Blank was based on 60 determinations of analyte depleted sample and was calculated as the 95th percentile of the distribution. The Limit of Detection was calculated from the LoB and the combined SD of the five LoQ samples. The LoQ was calculated from five independent samples (serum samples diluted with analyte depleted serum to achieve a concentration close to the bottom of the measuring range) tested 12 times over five days. The tabulated summary of results is shown below:

	LoB	LoD	LoQ
IgG Kappa	0.000 g/L	0.009 g/L	0.115 g/L
IgG Lambda	0.000 g/L	0.005 g/L	0.075 g/L

e. Analytical specificity:

Interference:

Interferences were assessed according to CLSI guideline EP07-A2 by testing three serum samples with different IgG Kappa and IgG Lambda concentration ranges, including a sample close to the lower limit of the reference interval, a sample within the reference interval and an elevated serum sample. Each sample was spiked with interfering substances and tested in multiple replicates. No significant assay interference effects were observed when the samples were tested with bilirubin at 200mg/L, hemoglobin at 5g/L, triglyceride at 1000mg/dL, intralipid at 125mg/dL or the 16 commonly used drugs at the concentrations given below.

Substance	Concentration
Acetaminophen	1324µmol/L
Acetylsalicylic Acid	3.63mmol/L
Ascorbic Acid	342µmol/L
Bortezomib	6mg/mL
Caffeine	308µmol/L
Cimetidine	79.2µmol/L
Cyclophosphamide Monohydrate	60µg/mL
Digoxin	3.9nmol/L (IgGK) 7.8nmol/L (IgGL)
Furosemide	181µmol/L
Ibuprofen	2425µmol/L
Methotrexate	2mmol/L
Penicillin	75mg/L
Phenytoin	198µmol/L
Pomalidomide	100µg/mL
Prednisolone	100µg/mL
Theophylline	222µmol/L

Cross-reactivity:

No significant cross reaction was observed during testing for the predicate device. The specificity of the antisera is unchanged.

Antigen Excess:

The possibility of antigen excess occurring when using the device on the Binding Site Optilite was evaluated with eight monoclonal IgG Kappa and six monoclonal IgG Lambda samples with concentrations above the respective standard measuring ranges. No antigen excess was observed up to 100.5g/L and 102.5g/L for IgG Kappa and IgG Lambda respectively.

f. Assay cut-off:

Refer to Expected values

2. Comparison studies:

a. Method comparison with predicate device:

IgG Kappa:
A comparison study was performed by analysing 284 serum samples (including 135 IgG Kappa paraprotein and 60 IgG Lambda paraprotein samples, 60 donor samples and 29 other samples, covering the range 0.2 – 47.69 g/L) using the Optilite Hevylite IgG Kappa kit and an alternative commercially available assay. Passing-Bablok regression analysis generated the results below.

IgG Lambda:
A comparison study was performed by analysing 172 serum samples (including 42 IgG Kappa paraprotein and 59 IgG Lambda paraprotein samples, 59 donor samples and one AL Amyloidosis sample, covering the range 0.09 – 40.65 g/L) using the Optilite Hevylite IgG Lambda kit and an alternative commercially available assay. Passing-Bablok regression analysis generated the results below.

IgG Kappa/Lambda Ratio:
A comparison study was performed by analysing 143 serum samples (including 39 IgG Kappa paraprotein and 44 IgG Lambda paraprotein samples, 59 donor samples and one AL Amyloidosis sample, covering the range 0.01 – 277.50 g/L) using the Optilite Hevylite IgG Kappa and IgG Lambda kits and alternative commercially available assays. Passing-Bablok regression analysis generated the results below.

	N	Sample Range	Passing & Bablok	Slope 95% CI	Intercept 95% CI
IgG Kappa	284	0.200-47.69 g/L	y = 1.100x – 0.68	1.05 to 1.15	-1.05 to -0.43
IgG Lambda	172	0.090-40.65 g/L	y = 0.950x + 0.00	0.88 to 1.01	-0.18 to 0.14
IgGκ/IgGλ ratio	143	0.014-277.5	y = 1.124x – 0.17	1.07 to 1.21	-0.30 to -0.10

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and clinical specificity:

Not applicable

b. Other clinical supportive data:

Transformation of the BNII Study onto the Optilite

The purpose of this study was to compare the clinical Heavy+Light Chain (HLC) Response categorization of the predicate and the Optilite Hevylite IgG Kappa and Lambda Kits obtained from samples taken at multiple time points from patients with IgG Kappa and IgG Lambda multiple myeloma during the course of their disease. Passing-Bablok regression equations were generated for the comparison study of the Optilite kits against the predicate kits. The regression equation was then modeled with the existing 437 monitoring sample results from the original BNII submission to evaluate the clinical validity of the new device.

HLC Monitoring Response Categories	
Complete Response (CR)	HLC ratio within the normal range and negative urine immunofixation.
Very Good Partial Response (VGPR)	>91% reduction of HLC ratio from baseline and reduction in 24 hour urinary M-protein to ≤100mg per 24 hours
Partial Response (PR)	Reduction of HLC ratio from baseline between 47 - 91% and reduction in 24 hours urinary M-protein by ≥90% or to ≤200mg/24 hours.
Stable Disease (SD)	A change in HLC ratio from baseline < 32% increase but < 47%
Progressive Disease (PD)	> 32% increase in HLC ratio from baseline (the absolute increase in involved IgG must be ≥5g/L) or a ≥25% increase in urine M-protein from baseline (the absolute increase must be ≥200mg/24 hours)
Relapse from CR	> 32% increase in HLC ratio from baseline (the absolute increase in involved IgG must be ≥5g/L)

Optilite HLC Response Category Study

Assignment of classification was based on the HLC Monitoring Response Category detailed in the above table, using all assay data available. Responses were categorized in accordance with NCCN Guidelines v1.2011 by using the percentage change from baseline. Responses were characterised as progressive disease (PD), stable disease (SD), partial response (PR), very good partial response (VGPR), and complete response (CR). Kappa statistics were used to evaluate agreement between the test and predicate devices.

Optilite Monitoring Study Design

A comparison of 69 monitoring samples from 10 IgG Kappa patients and 12 IgG Lambda patients was performed to compare the BNII HLC response category assigned to those observed with the Optilite Hevylite IgG Kappa and Lambda kits (Note: The Optilite monitoring response category is not to be used interchangeably with other manufacturer's assays or with any other instrument platform monitoring

response category). The results of the comparison study using 69 monitoring samples yielding 43 response classifications are shown in the table below:

Observed		Predicate HLC response					
		CR	VGPR	PR	SD	PD	Total
Optilite HLC Response	CR	0	0	0	0	0	**0**
	VGPR	0	1	0	0	0	**1**
	PR	0	0	11	1	0	**12**
	SD	0	0	1	23	1	**25**
	PD	0	0	0	2	3	**5**
	Total	**0**	**1**	**12**	**26**	**4**	**43**
Kappa (95% CIs)		0.79 (0.62 – 0.96)					
Weighted Kappa (95% CIs)		0.87 (0.74 – 0.98)					

These monitoring data were also supported by additional statistical regression equation modelling data.

Data Modelling

A data modelling procedure was carried out on monitoring sample results from the original BNII submission. The Passing-Bablok regression equations derived from the previously cleared 437 BNII data set were mathematically transformed using the Optilite regression equation. The statistical regression equation modeling (Cohen's Kappa) results are summarized in the tables below.

H/L*		Predicate Assigned Response					
		CR	VGPR	PR	SD	PD	Total
Transformed Data Response	CR	29	5	2	0	0	**36**
	VGPR	24	72	27	0	0	**123**
	PR	7	4	165	4	0	**180**
	SD	0	1	4	92	0	**97**
	PD	0	0	0	0	1	**1**
	Total	**60**	**82**	**198**	**96**	**1**	**437**
Kappa (95% CIs)		0.75 (0.70 – 0.80)					
Weighted Kappa (95% CIs)		0.86 (0.82 – 0.89)					

* H/L: H = highest kappa, L = lowest lambda; the imprecision values were applied to generate the highest possible kappa and the lowest possible lambda results.

L/H**		Predicate Assigned Response					
		CR	VGPR	PR	SD	PD	Total
Transformed Data Response	CR	55	16	6	1	0	78
	VGPR	5	63	23	0	0	91
	PR	0	2	166	3	0	171
	SD	0	1	3	92	0	96
	PD	0	0	0	0	1	1
	Total	60	82	198	96	1	437
Kappa (95% CIs)		0.81 (0.76 – 0.85)					
Weighted Kappa (95% CIs)		0.90 (0.86 – 0.93)					

** L/H: L = lowest kappa, H = highest lambda; the imprecision values were applied to generate the lowest possible kappa and the highest possible lambda results.

The Otpilite HLC Monitoring Response Category in the two Tables above were modified from the NCCN v1.2011 *Guidelines on Treatment Response Classification* as shown in the Table below.

Response	NCCN v1.2011	Disease Monitoring Using HLC Optilite IgG
Complete Response (CR)	**Negative IFE** on the serum and urine and disappearance of any soft tissue plasmacytomas and ≤ 5% plasma cells in bone marrow	**HLC ratio** within the **norma**l range (SPAPLUS® IgGκ/IgGλ Ratio 0.98–2.75) and negative urine immunofixation
Very Good Partial Response (VGPR)	Serum and urine **M protein detectable by IFE but not SPEP** or ≥ 90% reduction in serum M protein level plus urine M protein level < 100 mg per 24 hours	> 91% reduction of HLC ratio from baseline and urine M protein level < 100 mg per 24 hours
Partial Response (PR)	**≥ 50% reduction of serum M protein** and reduction in 24 hour urinary M protein by ≥ 90% or to < 200 mg per 24 hours.	**Reduction of HLC ratio from baseline between 47–91%** and reduction in 24 hour urinary M protein by ≥ 90% or to < 200 mg per 24 hours.
Stable Disease (SD)	Not meeting criteria for CR, VGPR, PR or progressive disease	A change in HLC ratio from baseline **< 32% increase but < 47% reduction.**

Response	NCCN v1.2011	Disease Monitoring Using HLC Optilite IgG
Progressive Disease (PD)	**Increase of ≥ 25%** from baseline in 1 or more: • Serum M-component and/or (the absolute increase must be ≥ 0.5 g/dL) • Urine M component and/or (the absolute increase must be ≥ 200 mg per 24 hours) • Bone marrow plasma cell percentage: the absolute percentage must be ≥ 10% • Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas • Development of hypercalcemia	**≥ 32% increase in HLC ratio** from baseline (the absolute increase in involved IgG must be ≥ 5 g/L) or a ≥ 25% increase in urine M-component from baseline (the absolute increase must be ≥ 200 mg per 24 hours)
Relapse from CR		**> 32% increase in HLC ratio** from baseline (the absolute increase in involved IgG must be ≥5g/L)

4. Clinical cut-off**:**

   Refer to discussion above.

5. Expected values/Reference range:

   The reference intervals were transferred from the predicate devices and were verified by testing 50 normal adult donor samples.

	95 Percentile Range
IgG kappa (g/L)	4.03 – 9.78 g/L
IgG lambda (g/L)	1.97 – 5.71 g/L
IgG kappa/ IgG lambda ratio	0.98 – 2.75

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 substantial equivalence decision.