

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

A. 510(k) Number:

k140105

B. Purpose for Submission:

New monitoring claim

C. Measurand:

Immunoglobulin IgA Kappa (combined α heavy and κ light chain) and
Immunoglobulin IgA Lambda (combined α heavy and λ light chain)

D. Type of Test:

Quantitative, Nephelometry

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Hevylite™ Human IgA Kappa Kit for use on Siemens BN™ II Systems
Hevylite™ Human IgA Lambda Kit for use on Siemens BN™ II Systems

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 code:

OPX - IgA kappa (Heavy and Light chain Combined). Antigen, antiserum, control
OPY - IgA lambda (Heavy and Light chain Combined). Antigen, antiserum,
control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

Hevylite Human IgA Kappa is a quantitative in vitro assay performed on the Siemens BN II nephelometer for the measurement of IgA kappa (IgA heavy chain and lambda light chain intact immunoglobulin) in serum. Measurement of Hevylite Human IgA Kappa is used alongside Hevylite Human IgA Lambda to

calculate the IgA kappa/IgA lambda ratio. The Hevylite Human IgA kappa/IgA lambda ratio can be used when monitoring previously diagnosed IgA 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.

Hevylite Human IgA Lambda is a quantitative in vitro assay performed on the Siemens BN II nephelometer for the measurement of IgA lambda (IgA heavy chain and lambda light chain intact immunoglobulin) in serum. Measurement of Hevylite Human IgA Lambda is used alongside Hevylite Human IgA Kappa to calculate the IgA kappa/IgA lambda ratio. The Hevylite Human IgA kappa/IgA lambda ratio can be used when monitoring previously diagnosed IgA 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. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only.

Warning: The result of Hevylite Human IgA Kappa in a given specimen determined with assays with different manufacturers 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 Human IgA Kappa assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining Hevylite IgA Kappa levels serially 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 Human IgA Lambda in a given specimen determined with assays with different manufacturers 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 Human IgA Lambda assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining Hevylite Human IgA Lambda levels serially 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:

Siemens Dade Behring Nephelometer™ II (BN™ II) (k943997)

I. Device Description:

The Hevylite™ Human IgA Kappa and IgA Lambda Kits contain vials of ready-to-use polyclonal monospecific sheep anti-IgA antisera against combined α heavy and κ

light chain or combined α heavy and λ light chain, a single level calibrator, controls (low and high) and supplementary reagent in liquid form. The reagents contain 0.099% sodium azide as preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s) and predicate 510(k) number(s):

Hevylite IgA Kappa and IgA Lambda kit (k082823)

2. Comparison with predicate:

The following devices were also used to establish substantial equivalence in the method comparison evaluation of this device: Sebia Hydragel 30 B1-B2 SPE Kit (k960029), Sebia Hydragel 4 IF Kit (k960669), and Siemens Dade Behring Total IgA (k042735).

Similarities		
Item	Device Hevylite™ IgA Kappa and IgA Lambda Kit	Predicate Hevylite IgA Kappa and IgA Lambda kit
Method	Nephelometric	Same
Instrument	Siemens BN™ II	Same
Analyte	IgA Kappa and Lambda	Same
Antibody	Sheep anti-human combined α heavy and κ light chain or combined α heavy and λ light chain	Same
Control	Binding Site High and Low Control	Same
Sample Matrix	Serum	Same
Measuring Range	At standard 1/100 dilution: IgA Kappa: 0.35 - 11.2 g/L IgA Lambda: 0.33 - 10.4 g/L Extended Range for IgA Kappa: 1/5 dilution: 0.018 – 0.56 g/L 1/20 dilution: 0.07 – 2.24 g/L 1/400 dilution: 1.40 – 44.8 g/L 1/2000 dilution: 7.0 – 224 g/L Extended Range for IgA Lambda: 1/5 dilution: 0.016 – 0.520 g/L 1/20 dilution: 0.065 – 2.08 g/L 1/400 dilution: 1.40 – 41.6 g/L 1/2000 dilution: 6.5 – 208 g/L	Same
Calibrator	Single level Binding Site	Same

Similarities		
Item	Device	Predicate
	Hevylite™ IgA Kappa and IgA Lambda Kit	Hevylite IgA Kappa and IgA Lambda kit
	Hevylite Calibrator autodiluted by BN II to six different concentrations	
Reference Interval	IgA Kappa: 0.48 - 2.82 g/L IgA Lambda: 0.36 - 1.98 g/L IgA Kappa/IgA Lambda ratio: 0.80 - 2.04	Same
Capture antibody	Sheep anti-human IgA combined	Same

Differences		
Item	Device	Predicate
Intended Use	Quantitative in vitro assay for the measurement of IgA kappa (IgA heavy chain and lambda light chain intact immunoglobulin) and IgA lambda (IgA heavy chain and lambda light chain intact immunoglobulin) in serum. Measurement of Hevylite Human IgA Kappa is used alongside Hevylite Human IgA Lambda to calculate the IgA kappa/IgA lambda ratio. The Hevylite Human IgA kappa/IgA lambda ratio can be used when monitoring previously diagnosed IgA 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.	In vitro quantification of IgA Kappa (combined α heavy and κ light chain) concentration and IgA Lambda (combined α heavy and λ light chain) concentration in human serum. The test result is to be used with previously diagnosed IgA multiple myeloma, in conjunction with other clinical and laboratory findings.
Sample Stability	21 days at 2 - 8°C	48 hours at 2 - 8°C

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

None provided?

L. Test Principle:

Hevylite antibodies bind specifically to junctional epitopes formed where the immunoglobulin heavy chain is in contact with the light chain.

Evaluating the concentration of a soluble antigen by nephelometry involves the addition of the test sample (with either IgA kappa (IgA κ) or IgA lambda (IgA λ)) to a solution containing the appropriate antibody (anti-IgA κ or anti-IgA λ) 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. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. In nephelometry, the light scatter is monitored by measuring the light intensity at an angle away from incident light. The single calibrator included with the kits is automatically diluted on the BNII to produce a five point 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:

a. *Precision/Reproducibility:*

See k082823 for original precision studies.

A study of lot-to-lot precision was based on CLSI EP05-A2. Three different serum samples were tested for 21 days with 2 runs per day; each sample was run in duplicate within each run for a total of 84 replicates per sample. This was carried out using three reagent lots (batches). Each batch was tested every three days over the course of the study (Day 1, Batch 1; Day 2 Batch 2, etc.) so that each batch was tested a total of seven times. The tested samples represented low, mid-range, and high concentrations across the assay range.

Quality control procedures were followed during the study by running control samples during each run. The analyzer was recalibrated if the controls did not meet their predetermined acceptance criteria.

IgA Kappa	Batch 1 Mean (g/L)	Batch 2 Mean (g/L)	Batch 3 Mean (g/L)	Inter-Batch Mean (g/L)	SD (g/L)	CV (%)
Low (2.92 g/L)	2.89	2.99	2.88	2.92	0.06	2.0
Medium (11.26 g/L)	11.46	11.21	11.09	11.26	0.19	1.7
High (24.05 g/L)	23.65	24.81	23.68	24.05	0.66	2.8

IgA Lambda	Batch 1 Mean (g/L)	Batch 2 Mean (g/L)	Batch 3 Mean (g/L)	Inter-Batch Mean (g/L)	SD (g/L)	CV (%)
Low	1.34	1.40	1.42	1.39	0.04	3.1

(1.38 g/L)						
Medium (5.91 g/L)	5.94	5.77	6.02	5.91	0.13	2.2
High (10.83 g/L)	10.32	11.20	10.98	10.83	0.46	4.2

b. *Linearity/assay reportable range:*

See k082823

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

See k082823

The calibrator and the controls are processed human sera with the target analyte levels given below; an Internal Reference (IR) used to control and validate calibration between batches is traceable to reference material ERM-DA470k:

	IgA kappa (g/L)	IgA lambda (g/L)
Calibrator	5.5	2.8
Low Control	4.0	2.0
High Control	16.0	8.0

An increase in the claimed sample stability to 21 days at 2 – 8°C from 48 hours at 2 – 8°C in k082823 was supported by a study that demonstrated less than 15% difference between the Day 0 and Day 21 samples. The sponsor refers users to WHO document “Use of Anticoagulants in Diagnostic Laboratory Investigations” (WHO/DIL/LAB/99.1 Rev. 1) for advice on long term storage of samples.

d. *Detection limit:*

See k082823

e. *Analytical specificity:*

See k082823

f. *Assay cut-off:*

See k082823

2. Comparison studies:

a. *Method comparison with predicate device:*

See k082823

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity and specificity:*

Purpose of study:

In multiple myeloma (MM) the production of a monoclonal immunoglobulin is frequently associated with reduced production of polyclonal immunoglobulins. For example, in a myeloma patient with an elevated concentration of IgA κ produced by their tumor the concentration of IgA λ will be frequently suppressed below the normal level. Therefore, the determination of the IgA κ /IgA λ ratio in a MM patient (and its comparison with a normal range) may provide an indication of monoclonal IgA production.

Differences between the κ/λ ratio obtained at baseline and during patient follow-up may provide an indication of patient response to treatment, and the results might be used to assist in determining patients' response criteria to treatment in a way similar to international response criteria.

The current standard of practice for monitoring responses and relapses in multiple myeloma involve serum protein electrophoresis (SPEP) and immunofixation (to determine complete response). International guidelines such as the National Comprehensive Cancer Network Clinical Practice Guidelines for Multiple Myeloma (NCCN) use reductions of monoclonal protein by SPEP and normalization of IFE to stratify response.

The aim of the study was to evaluate the performance of Hevylite IgA κ/λ ratios in monitoring MM. Hevylite κ/λ ratios and other criteria were used to assess response based on a set of criteria developed in a cut-off study. The response was then compared to the NCCN-determined response which is based on a set of reference techniques used to assess the clinical status of previously diagnosed MM patients during treatment.

Study design:

This was a retrospective study, utilizing serial sequential samples from IgA MM patients. These samples were divided into a cut-off study and a pivotal study. Samples were collected from clinical trials taking place at three sites: Centre René Gauducheau, Nantes/St. Herblain, France, Wilhelminenspital Der Stat Wien, Vienna, Austria, and University of Birmingham, Birmingham, United Kingdom. Final total enrollment was 97 patients; 509 sequential samples were evaluated.

Patients/samples were included in the study if they met these criteria:

- Diagnosed with MM as defined by the International Multiple Myeloma Working Group.
- Samples collected in accordance with the protection of subjects described in "Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are not Individually Identifiable."
- Appropriate quantities of archived serum samples stored either at 4°C for less than 4 weeks or at -20°C for less than 20 years were available.

- Subject samples with a baseline sample and at least one follow-up sample available and at least 3 weeks between each sample.
- Appropriate records of laboratory assessments available for each sample time point.

Patients/samples were excluded from the study if they met these criteria:

- Patients with biclonal disease (identified by the presence of two different classes of monoclonal protein identified by IFE at presentation)
- Oligosecretory patients (identified by the presence of <10 g/L of monoclonal protein at presentation)
- Nonsecretory patients, or light chain MM at presentation
- Missing data at baseline, no follow-up samples, or if there was >364 days between presentation and the first follow-up sample.
- Hemolysed, contaminated or excessive lipemic samples

Samples were analyzed at the clinical sites and at The Binding Site's laboratories. Results were classified into NCCN v1.2011 response criteria categories using available reference techniques data, and into HLC response categories using the ratio cut-offs described in the next section. Concordance was assessed using cross tabulation and estimates of sensitivity/specificity. Agreement between the classifications was evaluated using quadratic weighted kappa and associated boot strap analysis. In addition, individual assessment charts were provided for each subject.

Establishment of cut-offs:

An analysis was performed to establish cut-off values for classifying clinical response to treatment in MM by Hevylite IgA κ/λ ratios. Sixty samples from 21 patients were analyzed (14 IgA κ and 7 IgA λ). These patients were randomly selected from the samples from the Nantes and Vienna studies. The patients/samples used to establish the cut-off values were not used in the pivotal study.

The cut-offs were developed as follows:

- The results were classified according to the international response criteria.
- The results were then ranked according to % change in HLC ratio (smallest to largest).
- The HLC cut-off points were set in order to give the greatest number of concordant results in the complete data set.
- The precise values of HLC cut-offs were calculated by taking the mid-point of the two samples between which the cut-off point has been set.

Responses were categorized in accordance with NCCN Guidelines v.1.2011 by using the percentage (%) change in SPEP or total IgA from baseline. Responses were characterized as progressive disease (PD), stable disease (SD), partial response (PR), very good partial response (VGPR) and complete response (CR):

Table 1: Comparison of Treatment Response Classification – NCCN v1.2011 and Hevylite IgA kappa/lambda ratio (HLC ratio)

Response	NCCN v1.2011	Disease Monitoring Using HLC IgA
Complete Response (CR)	Negative IFE on the serum and urine and disappearance of any soft tissue plasmacytomas and $\leq 5\%$ plasma cells in bone marrow	HLC ratio within the normal range (IgA κ /IgA λ 0.80-2.04) and negative urine immunofixation and $\leq 5\%$ plasma cells in bone marrow (where available)
Very Good Partial Response (VGPR)	Serum and urine M protein detectable by IFE but not SPEP or $\geq 90\%$ reduction in serum M protein level plus urine M protein level < 100 mg per 24 hours	>94% reduction of HLC ratio from baseline and urine M protein level < 100 mg per 24 hours.
Partial Response (PR)	$\geq 50\%$ reduction of serum M protein and reduction in 24 hour urinary M protein by $\geq 90\%$ or to < 200 mg per 24 hours.	Reduction of HLC ratio from baseline between 60 – 94% and reduction in 24 hour urinary M protein by $\geq 90\%$ 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 $< 24\%$ increase but $< 60\%$ reduction.
Progressive Disease (PD)	Increase of $\geq 25\%$ from baseline in 1 or more: <ul style="list-style-type: none"> • Serum M-component and/or (the absolute increase must be ≥ 0.5 g/dL) • Urine M component and/or (the absolute increase must be ≥ 200mg/24hr) • Bone marrow plasma cell percentage: the absolute percentage must be $\geq 10\%$ • 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 	$\geq 24\%$ increase in HLC ratio from baseline (the absolute increase in involved IgA must be ≥ 5 g/L) or a $\geq 25\%$ increase in urine M-component from baseline (the absolute increase must be ≥ 200 mg/24hr)

Pivotal Study:

Study Design:

Samples were obtained from the two clinical sites above and the University of Birmingham, Birmingham, UK. Seventy-six (76) IgA MM patients (48 IgA κ and 28 IgA λ) from the three clinical sites generated 428 observations.

Demographic information is detailed in Table 2.

Table 2: Demographic Characteristics of Patients and Samples Enrolled in Hevylite IgA Pivotal Trial

IgA Demographics		Vienna	Nantes	Birmingham	Total
Patients (n)		30	31	15	76
IgA isotype	Kappa	19	18	11	48
	Lambda	11	13	4	28
Age median (range)		62 (32 – 81)	NR*	59.8 (49 – 63)	60 (32 – 81)
Sex	Male	15	18	7	40
	Female	10	13	8	31
	NR	5	0	0	5
ISS at diagnosis	Stage 1	9 (30%)	9 (29%)	6 (40%)	24 (32%)
	Stage 2	14 (47%)	13 (42%)	5 (33%)	32 (42%)
	Stage 3	7 (23%)	8 (26%)	4 (27%)	19 (25%)
	NR	0	1 (3%)	0	1 (1%)
# sequential samples		83	171	195	449
# samples/patient median (range)		3 (1- 4)	4 (1 – 17)	11 (1 – 31)	
Median days monitoring (range)		216 (99 – 536)	336 (32 -1909)	1014 (107 – 2722)	

*NR = not recorded

Assignment of classification was based on the criteria detailed in Table 1, using all assay data available. In some cases, complete datasets were not available for all patients. For example, serum analysis data were available for all 76 patients but urine analysis data were only available for 48/76 patients. Bone marrow analysis was available for 7 of the 40 patients whose IFE had been interpreted as negative or oligoclonal.

M-immunoglobulin could not be accurately quantified at in the initial sample by SPEP in 23/76 patients. In these cases, as recommended by NCCN, total IgA was used as a surrogate marker for M immunoglobulin. When assigning response to sequential samples, if protein could not be detected by SPEP, or if there was a $\geq 90\%$ decrease in total IgA, classification between VGPR and CR was distinguished by serum IFE (negative = CR, positive = VGPR). If serum IFE data was not reported, the physician's classification was assigned; where no physician response was available VGPR was assigned.

Because bone marrow data is an integral component of the NCCN assignment of CR but was not complete in many cases, two approaches to the assignment

of CR to the reference method were taken: 1) CR was assigned in the absence of bone marrow data by the clinician based on other test results; and 2) CR was only assigned when the response was confirmed with bone marrow data. This produced two datasets, whose results are shown below.

Data Analysis: Weighted Kappa Analysis

The first analysis took each sample from every patient and compared them to the baseline measurement to obtain information for the weighted kappa analysis. This method assessed response criteria at every time point and is not standard in the assessment of patient response in clinical practice. Two different approaches to the analysis were taken. The first analysis was a 'traditional' analysis where all data points were analyzed in a single analysis. This analysis makes the assumption that all data points were independent of each other, which may not be satisfied in this instance, as there were multiple measurements from most patients. Therefore, an alternative approach using a bootstrapping approach was employed. Rather than use a traditional bootstrap approach, where individual measurements were selected at random for each bootstrap sample, the selection was based on selecting individual patients. Each bootstrap sample selected patients at random, with replacement, from the dataset as a whole. If an individual patient was selected, then all individual data points from that patient were included in the sample.

Agreement is calculated as samples Responses are grouped as either in agreement (i.e. PD vs. PD), in minor disagreement (i.e. PD vs. SD), or in major disagreement (i.e. PD vs. PR).

Weighted kappa analysis: CR assigned without confirmatory bone marrow required

		Predicate Response					Total
		PD	SD	PR	VGPR	CR	
HLC Response	PD	21	6	0	0	0	27
	SD	56	80	33	2	0	121
	PR	0	18	77	22	0	117
	VGPR	0	0	25	49	16	90
	CR	1	0	0	14	79	94
Total		28	104	135	87	95	449
Agreement		75%	77%	57%	56%	83%	

Traditional weighted kappa (95% CI): 0.88 (0.78 – 0.97)

Boot strapping (10000 samples) weighted kappa (95% CI): 0.87 (0.83 – 0.91)

Weighted kappa analysis (w/ bone marrow): Assignment of CR requires BM biopsy information, otherwise maximal response is VGPR

		Predicate Response					Total
		PD	SD	PR	VGPR	CR	
HLC Response	PD	15	6	0	0	0	21
	SD	6	81	33	2	0	122
	PR	0	20	80	24	0	124
	VGPR	0	0	26	126	2	154
	CR	1	0	0	0	27	728
Total		22	107	139	152	29	449
Agreement		68%	76%	58%	83%	93%	

Traditional weighted kappa (95% CI): 0.85 (0.75 – 0.94)

Boot strapping (10000 samples) weighted kappa (95% CI): 0.84 (0.78 – 0.89)

Data Analysis: Sensitivity and Specificity

The data set was dichotomized into samples that were assigned a PR, VGPR or CR (response) or SD or PD (no response) by the NCCN determination and HLC ratio in the weighted kappa analysis. A 2x2 cross table was produced, and sensitivity and specificity were calculated:

		NCCN Determination		
		No Response	Response	Total
HLC determination	No Response	108	35	143
	Response	21	285	306
	Total	129	320	449

Statistics	Traditional analysis (95% CI)	Bootstrap analysis (95% CI)
Sensitivity	89% (85% - 92%)	89% (83% - 94%)
Specificity	84% (76% - 90 %)	84% (71% - 92%)

Of the discordant samples, three (3) of 449 samples (0.7%) had major disagreement between the HLC ratio response determination and the NCCN response determination. In one sample, the NCCN assigned response was progressive disease (PR), but Hevylite IgA HLC classified the response as complete (CR). In this patient, a relapse from CR after 594 days was identified by a re-emergence of the disease and an increase in monoclonal immunoglobulin by >5g/L (Anderson et al, JNCCN, 2011, 9, 1146-1183). At this time the patient had an abnormal Hevylite IgA κ/λ ratio, however an increase of 4 g/L in the involved immunoglobulin concentration did not meet

the relapse from CR criteria. The sponsor reports that the re-emergence of the clone did not coincide with symptomatic disease and therapy was not started until a later time point (719 days) when the patient had additional treatment to control the disease. At that time, both NCCN classification and Hevylite classification showed progressive disease.

In two samples, the NCCN assigned response was VGPR, but Hevylite IgA HLC classified the response as stable disease (SD). Both samples were from the same patient. This patient's M-immunoglobulin had decreased by 91% after 91 days and a VGPR was assigned by NCCN criteria. In contrast, while the patient's IgA κ/λ ratio had increased by 44% after 91 days, the total IgA concentration did not meet the criteria for progressive disease, and SD was assigned. Likewise, in the next patient sample (119 days after baseline) the patient's M-immunoglobulin had continued to decrease, a VGPR was assigned by NCCN criteria. Meanwhile, the patient's IgA κ/λ ratio had decreased by 53%, but the total IgA concentration did not meet the criteria for progressive disease, and SD was assigned. Subsequent sequential samples (n = 16) from this patient showed good agreement.

b. Other clinical supportive data (when a. is not applicable):

4. Clinical cut-off:

See discussion above.

5. Expected values/Reference range:

The assay has been previously cleared under k082823. In that study the normal range was found as follows:

	Mean	Median	95 th Percentile Sample Range
IgA κ	1.24	1.19	2.19 – 10.70
IgA λ	1.00	0.98	0.36 – 1.98
IgA κ/λ ratio	1.28	1.27	0.80 – 2.04

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.