

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k132555

B. Purpose for Submission:

New assay

C. Measurand:

Immunoglobulin IgG Kappa (combined α heavy and κ light chain) and
Immunoglobulin IgG 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 IgG Kappa Kit for use on Siemens BN™ II Systems
Hevylite™ Human IgG 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 2

3. Product code:

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 use(s):

Hevylite Human IgG Kappa is a quantitative in vitro assay performed on the Siemens BN II nephelometer for the measurement of IgG kappa (IgG heavy chain and lambda 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.

Hevylite Human IgG Lambda is a quantitative in vitro assay performed on the Siemens BN II nephelometer 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. 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 IgG 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 IgG 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 IgG 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 IgG 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 IgG 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 IgG 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 IgG Kappa and IgG Lambda Kits contain vials of ready-to-use polyclonal monospecific sheep anti-IgG antisera against combined α γ heavy and κ light chain or combined γ 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 IgG (k083445).

Similarities		
Item	Device Hevylite™ IgG Kappa and IgG Lambda Kit	Predicate Hevylite IgA Kappa and IgA Lambda kit
Method	Nephelometric	Same
Instrument	Siemens BN™ II	Same
Antibody	Sheep anti-human combined Hevylite™	Same
Control	Binding Site High and Low Control	Same
Sample Matrix	Serum	Same

Differences		
Item	Device	Predicate
Intended Use	Quantitative in vitro assay for the measurement of IgG kappa (IgG heavy chain and lambda light chain intact immunoglobulin) and IgG lambda (IgG heavy chain and lambda 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	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.

Differences		
Item	Device	Predicate
	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 Lambda	IgA Kappa and Lambda
Measuring Range	<p>At standard 1/100 dilution: IgG Kappa: 1.72 - 27.5 g/L IgG Lambda: 0.88 - 14.00 g/L</p> <p>Extended Range for IgG Kappa: 1/5 dilution: 0.086 – 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</p> <p>Extended Range for IgG Lambda: 1/5 dilution: 0.04 – 0.70 g/L 1/20 dilution: 0.18 – 2.80 g/L 1/400 dilution: 3.50 – 56.0 g/L 1/2000 dilution: 17.5 - 280 g/L</p>	<p>At standard 1/100 dilution: IgA Kappa: 0.35 - 11.2 g/L IgA Lambda: 0.33 - 10.4 g/L</p> <p>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</p> <p>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</p>
Calibrator	Single level Binding Site Hevylite Calibrator autodiluted by BN II to five different	Single level Binding Site Hevylite Calibrator autodiluted by BN II to six different

Differences		
Item	Device	Predicate
	concentrations	concentrations
Reference Interval	IgG Kappa: 4.03 - 9.78 g/L IgG Lambda: 1.97 - 5.71 g/L IgG Kappa / IgG Lambda ratio: 0.98 - 2.75	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
Capture antibody	Sheep anti-human IgG combined	Sheep anti-human IgA combined

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

CLSI EP05-A2 “Evaluation of Precision Performance of Quantitative Measurement Methods”

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 IgG Kappa (IgGκ) or IgG Lambda (IgGλ)) to a solution containing the appropriate antibody (Anti-IgGκ or Anti-IgGλ) 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:*

The study 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. The 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.

IgG Kappa	Within Run		Between Run		Between Day		Total	
	SD	CV	SD	CV	SD	CV	SD	CV
Low (2.92 g/L)	0.13	4.5%	0.06	2.0%	0.14	4.8%	0.20	6.8%
Medium (11.26 g/L)	0.56	5.0%	0.41	3.6%	0.46	4.1%	0.83	7.4%
High (24.05 g/L)	0.71	2.9%	0.67	2.8%	0.94	3.9%	1.35	5.6%

IgG Lambda	Within Run		Between Run		Between Day		Total	
	SD	CV	SD	CV	SD	CV	SD	CV
Low (1.38 g/L)	0.09	6.3%	0.02	1.6%	0.09	6.4%	0.13	9.1%
Medium (5.91 g/L)	0.15	2.5%	0.20	3.4%	0.16	2.6%	0.29	4.9%
High (10.83 g/L)	0.21	2.0%	0.20	1.9%	0.42	3.9%	0.51	4.7%

The study above 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.

IgG 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

IgG 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 (1.38 g/L)	1.34	1.40	1.42	1.39	0.04	3.1
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:

Linearity of the Hevylite IgG assays was demonstrated by testing a low concentration sample and an elevated sample for each assay. The samples were diluted as described in CLSI EP06-A. Each dilution was tested in triplicate. Each sample dilution series was tested in four separate assay lots. Samples that were outside the range of the standard 1/100 dilution were automatically re-run at a higher or lower dilution as per standard instrument protocol. Each lot yielded very similar regression statistics; the analyses below from Lot 4 are representative.

IgG Kappa Sample	Regression Analysis	Sample Range (g/L)
Low	$y = 1.000x + 0.001$, $R^2 = 0.999$	0.133 – 1.333
Elevated	$y = 1.051x - 0.649$, $R^2 = 0.997$	2.168 – 43.367

IgG Lambda Sample	Regression Analysis	Sample Range (g/L)
Low	$y = 0.980x - 0.015$, $R^2 = 0.996$	0.067 – 0.669
Elevated	$y = 1.017x + 0.215$, $R^2 = 0.997$	1.760 – 35.200

High dose hook effect

The possibility of antigen excess affecting the assay results was investigated using seven IgG kappa multiple myeloma samples and seven IgG lambda multiple myeloma samples. No antigen excess effect was seen up to 100 g/L for IgG kappa and up to 77 g/L for IgG lambda.

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

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:

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

Kit stability studies show that the kits are stable for 3 months after opening when stored between 2-8°C. Real-time stability results demonstrate kits are stable for 16 months when stored at 2-8°C.

Sample stability studies were conducted. Samples may be stored at 2-8°C for up to 21 days, but for prolonged storage they should be kept frozen at -20°C or below. 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:

The detection limit was determined by testing a blank sample to determine limit of blank (LoB), a sample with value close to the blank sample was used to determine the limit of detection (LoD), and the lowest calibrator was used to determine the limit of quantitation (LoQ). Each sample was tested 60

times. The study and statistical analysis was performed following CLSI EP17-A.

The limit of blank (LoB) was tested using instrument diluent and was determined to be the average of the 58th and 59th sample by rank; it was calculated as 0.002 g/L for IgG Kappa and 0.001 g/L for IgG Lambda at minimum sample dilution (1/5).

The LoD was defined as the LoB +1.645(Standard Deviation of the LoD sample); it has been calculated as 0.008 g/L for IgG Kappa and 0.003 g/L for IgG Lambda at minimum sample dilution (1/5).

The LoQ was defined as the lowest calibrator concentration tested undiluted (1/1) divided by the minimal sample dilution. LoQ samples were calibrator diluted with instrument diluent. The LoQs are 0.0890 g/L for IgG Kappa and 0.0435 g/L for IgG Lambda

e. *Analytical specificity:*

Endogenous Interferents:

Interference by endogenous interferents was tested using IgG kappa samples and IgG lambda samples near the bottom of the normal range, near the top of the normal measuring range, and near the top of the measuring range. Each sample was spiked with hemoglobin (5.0 g/L), bilirubin (200 mg/L), or chyle (1460 FTUs for the IgG kappa assay, 1200 FTUs for the IgG lambda assay). Each interferent at each sample concentration was tested at least in triplicate pairs of sample without interferent and sample plus interferent. Less than or equal to 10% difference was seen in any of the unaltered/spiked pairs.

Cross Reactivity:

The cross-reactivity of both assays was assessed by testing a panel of samples from IgG kappa and IgG lambda multiple myeloma patients undergoing treatment by plasmaphoresis. Samples were screened for the presence of monoclonal proteins using serum protein electrophoresis (SPEP) and free light chain tests and were classified according to immunoglobulin measurements. IgG kappa multiple myeloma samples (n = 20) did not cross react with the Hevylite IgG Lambda assay; likewise, IgG lambda multiple myeloma samples (n = 16) did not cross react with the Hevylite IgG Kappa assay.

In another experiment, a base pool of normal sera with an analyte level (IgG kappa or IgG lambda) at the upper end of the reference range was spiked separately with immunoglobulins of differing isotypes from multiple myeloma samples which did not contain detectable levels of the analyte of interest (IgG kappa or IgG lambda). Results were compared with the analyte level measured in the control base pool. There was less than 11% reduction in all samples tested, suggesting that the presence of immunoglobulins of differing isotypes does not result in significant cross reaction.

f. *Assay cut-off:*

The cut-off values are the 95th percentile ranges for the normal population. See description of study below.

2. Comparison studies:

a. *Method comparison with predicate device:*

The Hevylite IgG kappa and IgG lambda assays were compared to three predicate devices:

Comparison with total IgG quantitative measurement:

To demonstrate the relationship between Total IgG levels and the summated Hevylite IgG assays, IgG kappa and Hevylite IgG lambda levels in 359 samples (143 blood donor samples and 121 IgG kappa MM and 95 IgG lambda MM patient samples). Samples ranged from 6.7 – 15.8 g/L by the predicate method. Passing-Bablok analysis of all samples yielded a regression equation of $y = 0.90x + 0.55$ (slope 95% CI: 0.87-0.93).

Comparison with Serum Protein Electrophoresis (SPEP) semi-quantitative measurement:

One hundred sixty (160) samples with monoclonal protein from the clinical studies were analyzed by Sebia SPEP densitometry and the Hevylite IgG kappa and IgG lambda assays. Passing-Bablok analysis gave a regression equation of $y = 0.94x - 0.74$, with a systemic bias of 6% (95% CI: 0 – 14%).

Comparison with Immunofixation Electrophoresis (IFE):

One hundred sixty- one (161) samples from two clinical trial sites (see below) that were previously characterized by IFE were compared to the levels of IgG kappa and IgG lambda quantitative measurements. One hundred ten (110) samples were identified as IgG kappa myelomas; fifty-one (51) samples were identified as IgG lambda myelomas. All samples gave the expected result.

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 IgG kappa produced by their tumor the concentration of IgG lambda will be frequently suppressed below the normal level. Therefore, the

determination of the IgG kappa/IgG lambda ratio in a MM patient (and its comparison with a normal range) may provide an indication of monoclonal IgG production.

Differences between the kappa/lambda 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 IgG kappa/lambda ratios in monitoring MM. Hevylite kappa/lambda 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 IgG MM patients. These samples were divided into a cut-off study and a pivotal study. Samples were collected from clinical trials taking place at two sites: Centre René Gauducheau, Nantes/St. Herblain, France, and Wilhelminenspital Der Stat Wien, Vienna, Austria. Final total enrollment was 127 patients; 428 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 <10g/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 IgG kappa/lambda ratios. Eighty-five samples from 25 patients were analyzed (14 IgGκ and 7 IgGλ). 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 National Comprehensive Cancer Network Guidelines Version 1. 2011 by using the percentage (%) change in SPEP or total IgG 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 v1.2001 and Hevylite IgG kappa/lambda ration (HLC ratio)

Response	NCCN v1.2011	Disease Monitoring Using HLC 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 normal range (IgGκ/IgGλ 0.98-2.04) and negative urine immunofixation.

Response	NCCN v1.2011	Disease Monitoring Using HLC IgG
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	$> 91\%$ reduction of HLC ratio from baseline and 90% or greater reduction in serum M protein level plus 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 47 – 91% 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 $< 32\%$ increase but $< 47\%$ 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 	$\geq 32\%$ increase in HLC ratio from baseline (the absolute increase in involved IgG 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:

One hundred twenty-seven (127) IgG MM patients (87 IgGk and 40 IgGl) 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 IgG Pivotal Trial

IgG Demographics		Vienna	Nantes	Total
Patients (n)		35	92	127
IgG isotype	Kappa	22	65	87
	Lambda	13	27	40
Age median (range)		67 (45 – 85)	NR	NR
Sex	Male	13	50	63
	Female	12	39	51
	NR	10	3	13
ISS at diagnosis	Stage 1	12 (34%)	31 (36%)	43 (34%)
	Stage 2	11 (31%)	44 (48%)	55 (43%)
	Stage 3	12 (34%)	17 (18%)	29 (23%)
	NR	0	0	0
# sequential samples*		180	283	418
# samples/patient median (range)*		5 (1 – 9)	3 (1 - 3)	3 (1 – 9)
Median days monitoring (range)		286 (56 – 714)	211 (44 – 274)	216 (44 – 714)

NR = not recorded

* excluding presentation samples

Five additional individual progressive disease samples from patients treated at the University of Birmingham, United Kingdom, and five additional individual complete response samples from the Wilhelminenspital Der Stat Wien were included to supplement the number of responses in those categories. These 10 samples are not included in the demographic information above.

Assignment of classification was based on the criteria detailed in Table 1, using all assay data available. In some cases, complete data sets were not available for all patients. For example, serum analysis data was available for all 127 patients but urine analysis data was only available for 113 patients. Bone marrow analysis was available for five patients. When assigning response to sequential samples, if protein could not be detected by SPEP, 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 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

		Clinical Response					Total
		PD	SD	PR	VGPR	CR	
HLC Response	PD	4	0	0	0	0	4
	SD	5	67	24	0	0	96
	PR	0	26	151	6	0	183
	VGPR	0	0	44	29	7	80
	CR	0	0	11	32	22	65
Total		9	93	230	67	29	428
Agreement		44%	72%	66%	33%	76%	62%

Traditional weighted kappa (95% CI): 0.75 (0.66 – 0.84)

Boot strapping (10000 samples) Weighted Kappa (95% CI): 0.75 (0.70 – 0.80)

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

		Clinical Response					Total
		PD	SD	PR	VGPR	CR	
HLC Response	PD	4	0	0	0	0	4
	SD	5	67	24	0	0	96
	PR	0	26	151	6	0	183
	VGPR	0	0	55	79	4	138

	CR	0	0	0	0	7	7
Total		9	93	230	85	11	428
Agreement		44%	72%	66%	93%	64%	72%

Traditional weighted kappa (95% CI): 0.78 (0.68 – 0.87)

Boot strapping (10000 samples) Weighted Kappa (95% CI): 0.78 (0.72 – 0.82)

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 predicate assay and HLC ratio in the weighted kappa analysis. A 2x2 cross table was produced, and sensitivity and specificity were calculated:

		NCCN Determination		
		Response	No Response	Total
HLC determination	Response	76	24	100
	No Response	26	302	328
	Total	102	326	428

Statistics	Traditional analysis (95% CI)	Bootstrap analysis (95% CI)
Sensitivity	93% (89% - 95%)	93% (88% - 96%)
Specificity	75% (65% - 83%)	74% (64% - 85%)

Of the discordant samples, 11 of 428 samples (2.6%) had major disagreement between the HLC ratio response determination and the NCCN response determination. All 11 samples were categorized as complete response (CR) by the HLC ratio but were deemed ‘partial response’ by the NCCN criteria.

The following is a breakdown of the eleven samples:

- four samples were very close to the cut-off between PR and VGPR by the NCCN response determination and were in a region of the SPEP method noted for variability
- three samples classified as PR by comparison to the initial sample would have been maintained the classification of VGPR from the previous sample following the NCCN monitoring criteria. The increase in the concentration of protein did not meet the criteria for changing to PR.
- two samples from the same patient, the HLC ratio suggested an earlier indication of a CR than the NCCN criteria
- in two samples, both from the same patient, the discordance in the response was not obvious. Overall both HLC ratio and SPEP

identified that the patient responded to treatment, although there was discordance in both the timing and depth of response. Since the Intended Use requires other laboratory tests to confirm the CR response indicated by HLC ratio, the risk to the patient of inappropriate treatment should be minimized.

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

4. Clinical cut-off:

See discussion above.

5. Expected values/Reference range:

Adult normal range was assessed in 130 sera samples from healthy adult blood donors (age 18 – 65 years old, 66 male and 64 female) supplied by the UK Blood Transfusion Service. The assays were performed on the Dade Behring BN II analyzer. Samples evidencing abnormal SPEP results or abnormal serum free light chain ratios were not included in the normal range study. A non-parametric analysis of results was performed; all units are g/L.

	Mean	Median	SD	95 th Percentile Range	Sample Range
IgG Kappa (g/L)	6.90	6.85	1.47	4.03 – 9.78	2.19 – 10.70
IgG Lambda (g/L)	3.84	3.80	0.96	1.97 – 5.71	0.90 – 6.74
IgG Kappa/Lambda ratio	1.86	1.87	0.45	0.98 – 2.75	0.90 – 4.66

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.