

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

A. 510(k) Number:

k082823

B. Purpose for Submission:

New device

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 codes:

CFN - Method, Nephelometric, Immunoglobulins (G, A, M)

OPX - IgAK (Heavy and Light chain Combined). Antigen, antiserum, control

OPY - IgAL (Heavy and Light chain Combined). Antigen, antiserum, control

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

This kit is intended for the *in vitro* quantification of IgA Kappa (combined α heavy and κ light chain) concentration in human serum on the Siemens Behring Nephelometer™ II (BN™ II). The test result is to be used with previously diagnosed IgA multiple myeloma, in conjunction with other clinical and laboratory findings.

This kit is intended for the *in vitro* quantification of IgA Lambda (combined α heavy and λ light chain) concentration in human serum on the Siemens Behring Nephelometer™ II (BN™ II). The test result is to be used with previously diagnosed IgA multiple myeloma, in conjunction with other clinical and laboratory findings.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only. A black box warning stating: “Assay has not been established for diagnosis, monitoring or prognosis of IgA myeloma”.

4. Special instrument requirements:

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

I. Device Description:

The Hevylite™ Human IgA Kappa Kit and IgA Lambda Kit contains polyclonal monospecific sheep anti-IgA antisera against combined α heavy and κ light chain or combined α heavy and λ light chain, 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):

Sebia Hydragel 30 B1-B2 SPE Kit

Sebia Hydragel 4 IF Kit

Siemens Dade Behring Human IgA antisera, standards and controls

2. Predicate K number(s):

SPE: k960029

IFE: k960669

IgA: k042735

3. Comparison with predicate:

Differences				
Item	Device	Predicate		
	Hevylite	Serum Protein Electrophoresis (SPE)	Total IgA	Immunofixation Electrophoresis (IFE)
Intended Use	Quantification of IgA Kappa (combined α heavy and κ light chain) or IgA Lambda (combined α heavy and λ light chain) in human serum on the Siemens Dada Behring Nephelometer BN II. The result is to be used in previously diagnosed IgA multiple myeloma	Protein separation to assess for protein pattern abnormalities in serum and urine	Quantitative determination of Total IgA in human serum, heparinized and EDTA plasma in the BN systems	Identification of monoclonal immunoglobulins (IgG, IgA, or IgM Heavy Chain) and light chains (Kappa or Lambda Light Chain) in human serum and urine thereby aiding in the diagnosis of monoclonal gammopathies by using the Hydrasys system
Sample Matrix	Serum	Serum and urine	Serum, heparinized and EDTA plasma	Serum and urine

Differences				
Item	Device	Predicate		
	Hevylite	Serum Protein Electrophoresis (SPE)	Total IgA	Immunofixation Electrophoresis (IFE)
Detection Method	Nephelometry	Electrophoresis	Nephelometry	Immunofixation electrophoresis
Instruments	Siemens Dade Behring BNII	Sebia Hydrasys System	Siemens Dade Behring BN System	Sebia Hydrasys System
Controls	Binding Site Low and High levels liquid ready to use	Sebia Control Serum	Siemens Dade Behring Protein Controls and N Protein Standard	Normal and High levels liquid ready to use
Measuring range	IgA Kappa: 0.35 – 11.2 g/L IgA Lambda: 0.33 – 10.4 g/L	Semi-quantitative measurement: minimum detection approximately 21-44 mg/dL monoclonal protein	Dependent on the Siemens N Protein standard used.	Semi-quantitative measurement: minimum detection: 5.3-17.2 g/L monoclonal immunoglobulins (either Heavy Chains of IgG, IgA, IgM; or Light Chains of Kappa, or Lambda)
Reference Range	IgA Kappa: 0.48 – 2.82 g/L IgA Lambda: 0.36 – 1.98 g/L IgA Kappa/Lambda Ratio: 0.80 – 2.04	Absence of monoclonal proteins	0.7 – 4.0 g/L	Absence of monoclonal immunoglobulins
Controls	The Binding Site Low and High Controls	Sebia Control Serum	Siemens Dade Behring Protein Controls and N Protein Standard	Not applicable
Calibrator	Binding Site Hevylite Calibrator	Not applicable	N Protein standard	Not applicable
Antisera specificity	Sheep anti-human IgA combined	No antisera required	Rabbit anti-human IgA	Mammalian immunoglobulins

Differences				
Item	Device	Predicate		
	Hevylite	Serum Protein Electrophoresis (SPE)	Total IgA	Immunofixation Electrophoresis (IFE)
	alpha heavy and kappa light chain or combined alpha heavy and lambda light chain antiserum			anti-human alpha heavy chains, anti-human kappa light chains and anti-human lambda light chains
Result Interpretation	Quantitative measurement by nephelometry	Semi-quantitative results are calculated from Total Protein for each of five protein fractions (albumin, α -1 AT, α -2 Beta globulin, Gamma globulin) Qualitative results are visual interpretation of densitometric scan of each five protein fractions according to pattern symmetry by pathologist	Quantitative measurement by nephelometry	Qualitative visual interpretation of each six electrophoretic mobility of bands: (Total Protein, IgG IgA, IgM, Kappa and Lambda bands) by pathologist whether it is restricted monoclonal band or homogeneous polyclonal band

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

CLSI EP-5A: Evaluation of Precision Performance of Clinical Chemistry.

CLSI EP-17A: Determination of Limits of Detection and Limits of Quantitation.

L. Test Principle:

Evaluating the concentration of a soluble antigen by nephelometry involves the addition of the test sample (with either IgA Kappa (IgAK) or IgA Lambda (IgAL)) to a solution containing the appropriate antibody (Anti-IgAK or Anti-IgAL) 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. 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 (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The study was carried out by testing three samples with different concentrations of IgAK or IgAL using three different reagent lots on one analyzer. The study was performed over 21 working days, with two runs per day. Results are summarized below.

IgA Kappa Precision	Within run		Between-run		Between-day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low (mean 0.558 g/L)	0.02	3.2	0.01	1.0	0.01	1.3	0.02	3.6
Medium (mean 2.480 g/L)	0.05	1.8	0.06	2.3	0.12	4.6	0.14	5.5
High (mean 9.144 g/L)	0.16	1.8	0.04	0.5	0.44	4.8	0.47	5.1
IgA Lambda Precision								
IgA Lambda Precision	Within run		Between-run		Between-day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low (mean 0.500 g/L)	0.02	3.2	0.01	1.1	0.01	2.2	0.02	4.0
Medium (mean 1.926 g/L)	0.05	2.6	0.13	0.6	0.04	1.8	0.06	3.3
High (mean 8.445 g/L)	0.18	2.1	0.21	2.5	0.47	5.5	0.54	6.4

b. *Linearity/assay reportable range:*

Linearity across the assay ranges of IgAK (0.6 - 30.0 g/L) and IgAL (0.4-9.0 g/L) was confirmed by testing two pooled IgAK sera with high concentrations from 28.7-30.9 g/L and low concentrations from 0.496 – 0.555 g/L and two pooled IgAL sera with high concentrations from 8.9-9.4 g/L and low concentrations from 0.421 – 0.453 g/L. Testings were performed on three kit lots. The samples were serially diluted 13 times with saline (1:5) down to the lower measuring range (0.575 g/L IgAK and 0.368 g/L IgAL). All testing were performed three times. The regression plot equations where y is the measured level of IgA concentration and x the theoretical concentration were:

$$y = 1.015x + 0.232 \text{ (g/L), } r^2 = 0.996 \text{ for IgAK}$$

$$y = 0.997x - 0.173 \text{ (g/L), } r^2 = 0.997 \text{ for IgAL}$$

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

Value of an internal reference standard (Hevylite IR using affinity-purified IgAK and IgAL) was assigned by comparison with the CRM470 International Reference Material.

Stability: The expiration date claims are 7 months for both unopened kits when stored at 2-8°C.

d. *Detection limit:*

The detection limit was determined by testing a blank sample, the lowest calibrator, and a sample with value close to the blank sample (0.001 g/L both for IgAK and IgAL) tested 60 times each. The limit of detection represents the lowest measurable concentration of the analyte that can be distinguished

from zero; it has been calculated as 0.0075 g/L for IgAK and 0.0095 for IgAL. The limit of quantitation for IgAK and IgAL assays are 0.018 and 0.017g/L respectively which was calculated to be the lowest calibrator concentration divided by the minimal sample dilution.

e. *Analytical specificity:*

Interference by endogenous and other substances: A known IgAK (using 0.1g/L at a minimum 1/5 sample dilution) and a known IgAL (using 0.9 g/L at a minimum 1/5 sample dilution) sera were tested in triplicate with the following interferents: 4.8g/L hemoglobin, 200 mg/L bilirubin, 800 FTU of chyle. Minimal interference by these substances were observed (-1.8%; 3.9%; 7.1% on IgAK and -9.0%; 8.3%; 9.8% on IgAL respectively). The package insert states that “turbidimetric assays are not suitable for measurement of highly lipemic or hemolyzed samples, or samples containing high levels of circulating immune complexes due to the unpredictable degree of non-specific scatter these sample types might generate. Unexpected results should be confirmed using alternative assay method”.

Antigen excess effect:

The possibility of antigen excess occurring when using the devices on BN II Nephelometer was evaluated with eight serum samples with IgAK and seven IgAL concentrations above the assay range. No antigen excess effect up to 73 g/L of IgAK and 52 g/L IgAL were observed at the standard 1/100 sample dilution.

Cross reactivity:

For IgAK, the possibility of cross reactivity with other immunoglobulin heavy chain/light chain combinations was assessed by assaying normal serum samples to which known concentrations of pure IgGK, IgGL, IgMK, IgML, IgAL, Free Kappa and Free Lambda had been added. No significant (<5%) cross reactivity were observed.

For IgAL, the possibility of cross reactivity with other immunoglobulin heavy chain/light chain combinations was assessed by assaying normal serum samples to which known concentrations of pure IgGK, IgGL, IgMK, IgML, IgAK, Free Kappa and Free Lambda had been added. No significant (<5%) cross reactivity were observed.

f. *Assay cut-off:*

Refer to Expected values.

2. Comparison studies:

a. *Method comparison with predicate device:*

Analyses of the method comparisons were performed with each of the three predicate devices: Total IgA, SPE and IFE.

Comparison with Total IgA quantitative measurement:

Testing was performed on 138 normal adult sera and 94 previously diagnosed

IgA multiple myeloma patients (58 IgA Kappa and 36 IgA Lambda).

The table below shows the comparison of 232 sera tested by Dade Behring Total IgA and Hevylite IgAK and IgAL. Regression analysis of these samples is summarized below:

Regression equation	n	Slope	95% CI	Intercept	95% CI	R ²
Y = 1.04x-0.28 g/L	232	1.04	1.01 to 1.08	-0.28	-0.38 to -0.16	0.945

Comparison with SPE semi-quantitative measurement:

Forty normal and 94 previously diagnosed IgA multiple myeloma samples were compared by Sebia SPE semi-quantitative measurement and Binding Site Hevylite IgA Kappa and IgA Lambda quantitative devices. Results are shown in table below:

		Sebia SPE		
		positive	negative	Total
New Device	positive	91	4**	95*
	negative	0	39	39
	Total	91	43	134

*94 of 95 samples were IgA multiple myeloma patients.

**3 of 4 samples: SPE β or γ regions were not semi-quantifiable & 1 of 4 samples had slight elevation of IgA k (2.43 g/L; Ref. range: 0.44-2.36).

Positive percent agreement 100% (91/91)
 Negative percent agreement 91 % (39/43)
 Overall percent Agreement 97 % (130/134)

Comparison with 94 previously identified IgAK or IgAL by IFE:

The results of the IgA Kappa and IgA Lambda quantitative measurements were consistent in concentration levels with previously identified IgA Kappa or IgA lambda by IFE, except for one IgA Lambda myeloma which had an IgA Lambda concentration quantitative measurement of 0.777 g/L which was within the IgA Lambda reference range of 0.36 – 1.98 g/L.

- b. *Matrix comparison:*
Not applicable.
- 3. Clinical studies:
 - a. *Clinical Sensitivity and specificity:*
Not applicable.
 - b. *Other clinical supportive data (when a. is not applicable):*
Not applicable.
- 4. Clinical cut-off:
Not provided
- 5. Expected values/Reference range:
Adult normal range was assessed on a total 138 sera samples from healthy adult

blood donors (age 17-70 years old) supplied by the UK Blood Transfusion service. The assays were performed on the Dade Behring BN II™ analyser. A non-parametric analysis of specimens ranging from 0.48 – 2.82 g/L for the distribution of IgA Kappa was performed. The 95th percentile value of IgA Kappa was 2.292 (median = 1.19 g/L). A non-parametric analysis of specimens ranging from 0.36-1.98 g/L for the distribution of IgA Lambda was performed. The 95th percentile value of IgA Lambda was 1.812 (median = 0.98 g/L):

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.