



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY AND INSTRUMENT**

**I Background Information:**

**A 510(k) Number**

K250159

**B Applicant**

The Binding Site Group Ltd

**C Proprietary and Established Names**

Immunoglobulin Isotypes (GAM) for the EXENT Analyser  
EXENT Analyser

**D Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
SGG	Class II	21 CFR 866.5510 - Immunoglobulins A, G, M, D, and E Immunological Test System	IM - Immunology
OTA	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

**II Submission/Device Overview:**

**A Purpose for Submission:**

New device

**B Measurand:**

Monoclonal immunoglobulins (IgG, IgA, IgM)

**C Type of Test:**

Qualitative and semi-quantitative, Mass Spectrometry

### **III Intended Use/Indications for Use:**

#### **A Intended Use(s):**

See Indications for Use below.

#### **B Indication(s) for Use:**

Immunoglobulin Isotypes (GAM) for the EXENT Analyser:

The Immunoglobulin Isotypes (GAM) for the EXENT Analyser is a MALDI-TOF mass spectrometry immunoassay that is used in conjunction with the Binding Site Optilite IgG, IgA and IgM assays for the semi-quantitative in vitro measurement of monoclonal IgG, IgA, and IgM as a reflex test in serum for patients with a result suggestive of the presence of monoclonal immunoglobulins by serum protein electrophoresis (gel or capillary zone electrophoresis), or with an abnormal serum free light chain concentration and free light chain ratio result.

The assay is intended for use as an aid in the evaluation of monoclonal gammopathy of undetermined significance (MGUS); and as an aid in the diagnosis of smouldering multiple myeloma (SMM), multiple myeloma (MM), Waldenström's macroglobulinaemia, and AL amyloidosis.

Assay results should be used in conjunction with other laboratory and clinical findings.

EXENT Analyser:

The EXENT analyser is an automated analyser intended for the qualitative and quantitative in vitro measurement of analytes in human body fluids used in conjunction with the EXENT assays.

#### **C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

#### **D Special Instrument Requirements:**

EXENT Analyser  
Optilite Analyser

### **IV Device/System Characteristics:**

#### **A Device Description:**

The system consisting of the EXENT assay, i.e., Immunoglobulin Isotypes (GAM) for the EXENT Analyser, and is intended for the in vitro determination of analytes in human serum. It is designed to provide automation and integration of all the analytical steps (including liquid handling and MALDI-TOF mass spectrometry). Quantitative results are obtained in combination with Optilite immunoglobulin measurements.

Immunoglobulin Isotypes (GAM) for the EXENT Analyser contains the following materials:

- 1 × 5.6 mL EXENT IgG Reagent
- 1 × 5.6 mL EXENT IgA Reagent
- 1 × 5.6 mL EXENT IgM Reagent
- 1 × 5.6 mL EXENT Total Kappa Reagent
- 1 × 5.6 mL EXENT Total Lambda Reagent

Reagent Component: Polyclonal monospecific sheep antibody conjugated to paramagnetic beads, supplied in liquid form in buffered saline solution, 1 mM EDTA, 0.1% (w/v), E-amino-n-caproic acid (EACA), 0.01 % (w/v) benzamidine, 0.08 % (v/v) ProClin 300, 0.01 % (w/v) Polysorbate 20.

Materials required but not provided:

- EXENT Analyzer with EXENT Operation Manual
- Materials required for running the assay on EXENT Analyzer: EXENT Diluent 1, EXENT Elution Buffer 1, EXENT HCCA MALDI Matrix Pack, EXENT Mass Calibration Standard 1, EXENT Disposable MALDI Plate Pack, EXENT Wash Solution 1, Equipment for collection and preparation of test samples.
- EXENT Immunoglobulin Isotypes (GAM) Control Pack
- Valid Optilite results for IgG, IgA, and IgM obtained for patient samples using the Optilite IgG kit, Optilite IgA kit, and Optilite IgM kit, respectively on the Optilite Analyser

The EXENT Analyser is designed to be used solely in combination with EXENT assays to measure a variety of analytes depending on the reagents. The EXENT Analyser combines automated immunoassay with readout by MALDI-TOF mass spectrometry. It is a modular instrument consisting of the following major components:

- **EXENT-ip500:** Automated liquid handler - Preparation of patient samples by magnetic bead immunoprecipitation assays for subsequent analysis by MALDI-TOF mass spectrometry.

The EXENT-ip500 component is an automated liquid handler that prepares human body fluids using the EXENT assay specific reagents. The samples are prepared using magnetic beads that are coated with analyte specific antibodies. Any unbound material is washed away during the sample preparation process. The EXENT-ip500 also manages the transfer of the prepared patient sample to the MALDI plate.

- **EXENT-iX500:** MALDI-ToF Mass Spectrometer - Analysis of prepared patient samples by MALDI-ToF mass spectrometry

The EXENT-iX500 component is a MALDI-TOF mass spectrometer. Signals are produced by ionizing the compound or biological material under investigation and separating the resulting ions by means of an electrical and magnetic field according to their mass-to-charge ratios. The EXENT-iX500 is used to read samples prepared by the EXENT-ip500.

- **EXENT-iQ software:** Workflow and data management software - Management of the workflow between the EXENT-ip500 and EXENT-iX500 instruments. Data management including processing and results release.

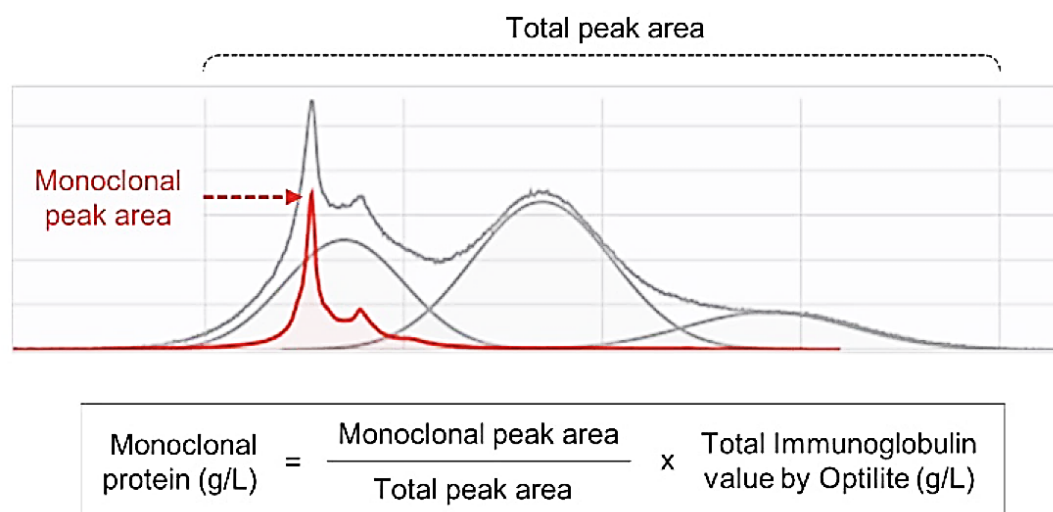
The EXENT-iQ software integrates sample preparation and MALDI-TOF mass spectrometry and is used for data storage and processing. It is the primary user interface used by the user to review and release results.

## B Principle of Operation:

Patient samples are automatically prepared on the EXENT-iP500 liquid handler using paramagnetic bead-mediated immunoprecipitation. Patient serum samples are divided into 5 equal aliquots which are independently mixed with antibody-coated paramagnetic beads specific to the analyte of interest: anti-human IgG, IgA, IgM heavy chains; and kappa (Igκ) and lambda (Igλ) light chains. The specific immunoglobulins bind to the immobilized antibodies on the bead surface. The beads are repeatedly washed using magnetic precipitation and buffer exchanges to remove non-specific sample components. The bound immunoglobulins are then simultaneously released from the paramagnetic bead and their disulfide bonds reduced to dissociate light from paired heavy chains. The resulting eluates for each individual specificity contain a mixture of heavy and light chains. Eluates are subsequently mixed with a MALDI matrix compound, spotted onto MALDI plates and co-crystallized on the plate surface. Sample spots are then automatically acquired by the EXENT-iX500 MALDI-TOF mass spectrometer.

Monoclonal immunoglobulin heavy and/or light chain spectral peaks, specifically 2+ ions at  $m/z$  10,900–13,600, are automatically identified by software, which calculates a proportional peak area ratio (PAR) for the monoclonal M-protein peak relative to the holoclonal immunoglobulin background repertoire, ranging from zero to one. IgG, IgA, and IgM results from the Optilite IgG, IgA, and IgM assays are imported automatically via the Laboratory Information System (LIS) or entered manually by the user, to deliver a semi-quantitative result for the M-protein in estimated (g/L) units by multiplying the PAR from  $m/z$  spectra with quantitative Optilite IgG, IgA, or IgM (g/L) units. Each monoclonal M-protein peak identified by software is accompanied by qualitative assignment of isotype: heavy chain IgG, IgA, or IgM in association with paired light chain Igκ or Igλ.

A schematic representation of the EXENT Immunoglobulin Isotypes calculation is depicted below:



Two levels of control material containing pooled normal human serum is to be used with each MALDI plate as process controls. The controls have assigned IgG, IgA, and IgM values derived

from Optilite assays. These are provided in the EXENT Immunoglobulin Isotypes (GAM) Control Pack.

## **C Instrument Description Information:**

### 1. Instrument Name:

EXENT Analyser

### 2. Specimen Identification:

Samples loaded into the sample runners can be labelled with a readable barcode. The barcode must be in one of the following primary formats:

- Code128 (recommended, with check digit, must be grade A, B, or C)
- Code39
- Codabar

The barcode must be a certain number of digits long as determined by individual site requirements and established at the time of installation of the EXENT Analyser. The barcode must be affixed to the sample tube and be oriented to the left in the sample runner such that the barcode is fully visible to the barcode reader. Samples may also be loaded without a barcode and manually input an identifier.

### 3. Specimen Sampling and Handling:

Samples should be obtained by venipuncture. Blood should be allowed to clot and the serum separated as soon as possible to prevent hemolysis. Sera may be stored at 2-8°C for up to 28 days, otherwise aliquot and freeze at -20°C or below and store for up to 6 months. Repeated freeze/thaw cycles should be avoided. Microbially contaminated, hemolyzed and lipemic serum samples should not be used. Samples containing precipitates should be centrifuged before performing the assay. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria.

### 4. Calibration:

EXENT Mass Calibration Standard 1 is used on each MALDI plate to calibrate the MALDI-TOF mass spectrometer. The material is a purified protein standard. Each MALDI plate is prepared via the liquid handler and contains a spot with this material prepared. This spot is then automatically measured on the EXENT-iX500 and calibrates mass-to-charge ratios ( $m/z$ ) for the subsequent measurements on the MALDI plate (quality controls and patient samples). Each quality control and patient sample is also assessed for the presence of a protein marker that is added during preparation on the liquid handler (Internal Process Control - IPC). The protein marker is then used to apply a further  $m/z$  calibration (Lock Mass) to each measured spectrum.

### 5. Quality Control:

EXENT Immunoglobulin Isotypes (GAM) Control Pack contains two concentrations of pooled human serum (2 levels of controls for each measurand IgG, IgA and IgM) that are used as quality controls per MALDI plate. These undergo the same immunoassay and preparation as patient samples. They are measured automatically on the EXENT-iX500 before patient samples on each MALDI plate and assessed against ranges provided in a quality control certificate (barcoded). Should a control measurement be out of range, samples on the affected MALDI plate will not be measured.

In addition to the Quality Control check, EXENT employs further automated status checks in order to ensure the validity of the sample results:

- 1) Preparation check: evaluated per sample preparation phase. Single or multiple sample results may be invalidated dependent on the preparation failure mode.
- 2) Mass Calibration check: evaluated per MALDI plate. All samples associated with the affected MALDI plate are invalidated.
- 3) IPC check: evaluated per mass spectrum. All samples associated with one or more IPC failures are invalidated.
- 4) Lock Mass check: evaluated per mass spectrum. All samples associated with one or more IPC failures are invalidated.

Each of these status checks is performed on a per sample basis. Results failing any of the above status checks are invalidated by EXENT Analyser and cannot be released.

## V Substantial Equivalence Information:

### A Predicate Device Name(s):

HYDRAGEL If, 6 If, 12 If Penta Kits/HYDRAGEL If, Double If, 2 If, & 4 If Kits  
Human IgG Subclass Liquid Reagent Kits for Use On the Spaplus Analyser  
Optilite IgM Kit  
Optilite IgA Kit

### B Predicate 510(k) Number(s):

K960669  
K072889  
K191635  
K191985

### C Comparison with Predicate(s):

Device & Predicate:	<u>K250159</u>	<u>K960669</u>
Device Trade Name	Immunoglobulin Isotypes (GAM) for the EXENT Analyser	HYDRAGEL If, 6 If, 12 If Penta Kits/HYDRAGEL If, Double If, 2 If, & 4 If Kits
<b>General Device Characteristic Similarities</b>		
Intended Use/ Indications For Use	The Immunoglobulin Isotypes (GAM) for the EXENT Analyser is a MALDI-TOF mass spectrometry immunoassay	Sebia's Hydragel IF Penta and Hydragel IF series of products are intended for use in the

Device & Predicate:	<u>K250159</u>	<u>K960669</u>
Device Trade Name	Immunoglobulin Isotypes (GAM) for the EXENT Analyser	HYDRAGEL If, 6 If, 12 If Penta Kits/HYDRAGEL If, Double If, 2 If, & 4 If Kits
	<p>that is used in conjunction with the Binding Site Optilite IgG, IgA and IgM assays for the semi-quantitative in vitro measurement of monoclonal IgG, IgA, and IgM as a reflex test in serum for patients with a result suggestive of the presence of monoclonal immunoglobulins by serum protein electrophoresis (gel or capillary zone electrophoresis), or with an abnormal serum free light chain concentration and free light chain ratio result.</p> <p>The assay is intended for use as an aid in the evaluation of monoclonal gammopathy of undetermined significance (MGUS); and as an aid in the diagnosis of smouldering multiple myeloma (SMM), multiple myeloma (MM), Waldenström's macroglobulinaemia, and AL amyloidosis.</p> <p>Assay results should be used in conjunction with other laboratory and clinical findings.</p>	<p>diagnosis of diseases collectively known as gammopathies. Specifically, the intended uses are as follows:</p> <p>All individual kits in Sebia's Hydragel IF Penta series of products are intended for screening of human sera for gammopathies. Immunofixation electrophoresis, using Pentavalent Antiserum, is employed to screen suspected sera in order to detect monoclonal components.</p> <p>All individual kits in Sebia's Hydragel IF series of products are intended for identification of gammopathies in human serum. Immunofixation electrophoresis using specific antisera is employed to detect and identify monoclonal components in suspected sera.</p>
Isotype determination	qualitative immunoprecipitation	qualitative immunofixation
Detected Analyte	IgG, IgA, IgM in combination with Igκ, Igλ light chains	same
<b>General Device Characteristic Differences</b>		
Sample preparation	automated	manual
Sample Type	human serum	human serum, human urine
Controls	2 levels of human immunoglobulins	recommended, not supplied
Detection Technology	matrix-assisted laser desorption and ionization / time-of-flight (MALDI-TOF) mass spectrometry	acid violet or amido black staining
Instrumentation	EXENT Analyser	Hydrasys, Hydrasys 2
Monoclonal resolution	<i>m/z</i> of 2+ ions	visual inspection by trained operator

Device & Predicate(s):	<u>K250159</u>	<u>K072889</u>	<u>K191635</u>	<u>K191985</u>
Device Trade Name	Immunoglobulin Isotypes (GAM) for the EXENT Analyser	IgG on the Spaplus Analyser	Optilite IgM	Optilite IgA
<b>General Device Characteristic Similarities</b>				
Intended Use/ Indications For Use	<p>The Immunoglobulin Isotypes (GAM) for the EXENT Analyser is a MALDI-TOF mass spectrometry immunoassay that is used in conjunction with the Binding Site Optilite IgG, IgA and IgM assays for the semi-quantitative in vitro measurement of monoclonal IgG, IgA, and IgM as a reflex test in serum for patients with a result suggestive of the presence of monoclonal immunoglobulins by serum protein electrophoresis (gel or capillary zone electrophoresis), or with an abnormal serum free light chain concentration and free light chain ratio result.</p> <p>The assay is intended for use as an aid in the evaluation of monoclonal gammopathy of undetermined significance (MGUS); and as an aid in the diagnosis of smouldering multiple myeloma (SMM), multiple myeloma (MM), Waldenström's macroglobulinaemia, and AL amyloidosis.</p> <p>Assay results should be used in conjunction with other laboratory and clinical findings.</p>			
Sample type	serum	same	same	same
Sample preparation	automated	same	same	same
Reagents	polyclonal sheep antisera	same	same	same
Traceability	to ERM-DA470K (through integral Optilite assays)	same	same	same
<b>General Device Characteristic Differences</b>				
Detection Method	Mass spectrometry	Turbidimetry		
Analyte(s)	Monoclonal M-protein (IgG, IgA, or IgM)	Total polyclonal IgG	Total polyclonal IgA	Total polyclonal IgM



Isotype determination	qualitative immunoprecipitation	not done		
Unit	Semi-quantitative (g/L)	quantitative (g/L)		
Calibration	protein standard for <i>m/z</i> parameter	six calibrators for immunoglobulin concentration (g/L)		
AMI(s)	IgG: 0.308–88.9 g/L IgA: 0.073–65.9 g/L IgM: 0.054–74.2 g/L	1.65–35 g/L	0.20–7.0 g/L	0.2–7.5 g/L
cut-off(s)	IgG: 0.359 g/L IgA: 0.325 g/L IgM: 0.197 g/L	RefInt <sup>1</sup> : 6.10–16.2 g/L	RefInt <sup>1</sup> : 0.85–4.99 g/L	RefInt <sup>1</sup> : 0.35–2.42 g/L

<sup>1</sup> RefInt: Reference Interval

## VI Standards/Guidance Documents Referenced:

Standard	Title
CLSI C62-A	Liquid Chromatography-Mass Spectrometry Methods
CLSI EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures
CLSI EP06, 2 <sup>nd</sup> Ed.	Evaluation of the Linearity of Quantitative Measurement Procedures
CLSI EP07, 3 <sup>rd</sup> Ed.	Interference Testing in Clinical Chemistry
CLSI EP09c, 3 <sup>rd</sup> Ed.	Measurement Procedure Comparison and Bias Estimation Using Patient Samples
CLSI EP12, 3 <sup>rd</sup> Ed.	Evaluation of Qualitative, Binary Output Examination Performance
CLSI EP15-A3	User Verification of Precision and Estimation of Bias
CLSI EP17-A2	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
CLSI EP25-A	Evaluation of Stability of In Vitro Medical Laboratory Test Reagents
CLSI EP25, 2 <sup>nd</sup> Ed.	Evaluation of Stability of In Vitro Medical Laboratory Test Reagents
CLSI EP37, 1 <sup>st</sup> Ed.	Supplemental Tables for Interference Testing in Clinical Chemistry
CLSI EP39, 1 <sup>st</sup> Ed.	A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of In Vitro Medical Laboratory Tests
IEC 61010-1 Ed. 3.1	Safety requirements for electrical equipment for measurement control and laboratory use - Part 1: General requirements
IEC 62304 Ed. 1.1	Medical device software – Software life cycle processes
ISO 14971 3 <sup>rd</sup> Ed.	Medical devices – Application of risk management to medical devices

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

Unless stated otherwise, all of the results of analytical performance for the Immunoglobulin Isotypes (GAM) for the EXENT Analyser (a.k.a., the EXENT Immunoglobulin Isotypes) described below met the manufacturer's pre-determined acceptance criteria.

# 1. Precision/Reproducibility:

## Within-Laboratory Precision:

To evaluate precision, panels of  $n=5$  serum samples per isotype (IgG, IgA, IgM) were tested by the EXENT Immunoglobulin Isotypes assay to represent the entirety of the analytical measuring interval (AMI) of each analyte. Individual samples were tested in duplicate/run  $\times$  2 runs/day  $\times$  20 days using one reagent lot, one EXENT Analyser instrument, and one Optilite instrument at a single laboratory site for a total of 80 datapoints per sample. The results are summarized as follows:

Monoclonal IgG repeatability and (20 $\times$ 2 $\times$ 2) within-laboratory precision									
Level	Mean (g/L)	Within-run		Between-run		Between-day		Within-lab	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.271	0.025	9.3	0.000	0.0	0.017	6.3	0.030	11.2
2	0.358	0.029	8.0	0.000	0.0	0.022	6.2	0.036	10.1
3	1.40	0.071	5.1	0.044	3.1	0.072	5.1	0.110	7.9
4	30.6	0.65	2.1	0.41	1.3	0.95	3.1	1.22	4.0
5	102.4	4.13	4.0	2.83	2.8	5.76	5.6	7.62	7.4

Monoclonal IgA repeatability and (20 $\times$ 2 $\times$ 2) within-laboratory precision									
Level	Mean (g/L)	Within-run		Between-run		Between-day		Within-lab	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.101	0.0043	4.3	0.0042	4.1	0.0067	6.7	0.0090	8.9
2	0.321	0.013	4.0	0.013	3.9	0.014	4.3	0.023	7.1
3	0.974	0.027	2.8	0.034	3.5	0.030	3.0	0.026	5.4
4	27.9	0.73	2.6	0.55	2.0	1.08	3.9	1.41	5.1
5	57.2	2.54	4.4	1.00	1.8	1.48	2.6	3.11	5.4

Monoclonal IgM repeatability and (20 $\times$ 2 $\times$ 2) within-laboratory precision									
Level	Mean (g/L)	Within-run		Between-run		Between-day		Within-lab	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.094	0.0045	4.9	0.0032	3.4	0.0080	8.5	0.0157	10.3
2	0.179	0.011	6.0	0.011	6.4	0.000	0.0	0.016	8.7
3	1.73	0.048	2.8	0.015	2.4	0.047	3.9	0.093	5.4
4	31.2	0.55	1.8	0.23	0.7	0.98	3.2	1.15	3.7
5	71.2	2.93	4.1	2.25	3.2	3.11	4.4	4.83	6.8

## Between-instrument/site Reproducibility:

For assessment of instrument variability upon semi-quantitative measurand results, panels of  $n=5$  serum samples per isotype (IgG, IgA, IgM) were tested by the EXENT Immunoglobulin Isotypes assay to represent the entirety of the AMI of each analyte. Individual samples were tested in 5 replicates/run  $\times$  1 run/day  $\times$  5 days  $\times$  3 laboratory sites  $\times$  1 instrument/site  $\times$  1 operator/site. All testing utilized a single reagent lot for a minimum of 75 datapoints per sample. Results for between-instrument/site comparisons are summarized in the following tables:

Monoclonal IgG (5×5×3) between-instrument/site reproducibility									
Level	Mean (g/L)	within-run		between-day		between-instrument/site		total reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.352	0.0205	5.8	0.00	0.0	0.0358	10.2	0.0412	11.7
2	0.452	0.0250	5.5	0.00	0.0	0.0557	12.3	0.0611	13.5
3	1.25	0.0725	5.8	0.013	1.0	0.151	12.0	0.1679	13.4
4	29.7	1.49	5.0	0.55	1.8	2.12	7.1	2.65	8.9
5	80.5	2.05	2.5	00.00	0.0	7.12	8.8	7.41	9.2

Monoclonal IgA (5×5×3) between-instrument/site reproducibility									
Level	Mean (g/L)	within-run/repeatability		between-day		between-instrument/site		total reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.114	0.0054	4.7	0.00	0.0	0.0095	8.4	0.0109	9.6
2	0.368	0.0169	4.6	0.00	0.00	0.0299	8.1	0.0344	9.3
3	0.82	0.035	4.3	0.00	0.0	0.0723	8.8	0.0803	9.8
4	24.8	2.80	11.3	0.00	0.0	3.55	14.3	4.523	18.2
5	56.2	1.12	2.0	0.35	0.6	2.63	4.7	2.88	5.1

Monoclonal IgM (5×5×3) between-instrument/site reproducibility									
Level	Mean (g/L)	within-run/repeatability		between-day		between-instrument/site		total reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.126	0.0089	7.1	0.0082	6.5	0.0098	7.7	0.0156	12.3
2	0.172	0.0147	8.5	0.0085	4.9	0.0093	5.4	0.0194	11.2
3	1.47	0.079	5.3	0.039	2.6	0.0950	6.3	0.130	8.6
4	31.0	0.89	2.9	0.45	1.5	1.58	5.1	1.87	6.0
5	67.6	3.04	4.5	0.00	0.0	6.84	10.1	7.48	11.1

#### Between-lot Precision:

For assessment of reagent lot variability upon semi-quantitative measurand results, panels of  $n=5$  serum samples per isotype (IgG, IgA, IgM) were tested by the EXENT Immunoglobulin Isotypes assay to represent the entirety of the AMI of each analyte. Individual samples were tested in 5 replicates/run × 1 run/day × 5 days for each reagent lot, at a single laboratory site, using one EXENT instrument and one Optilite instrument for a total of 75 datapoints per sample. The results are summarized as follows:

Monoclonal IgG (5×5×3) lot-to-lot reproducibility									
Level	Mean (g/L)	Within-run		Between-day		Between-lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.309	0.0199	6.4	0.0077	2.5	0.0109	3.5	0.024	7.8
2	0.405	0.0257	6.3	0.003	0.8	0.0093	2.3	0.0275	6.8
3	1.09	0.0797	7.3	0.0408	3.7	0.0875	8.0	0.1252	11.5
4	27.0	1.93	7.1	0.00	0.0	1.94	7.2	2.74	10.1
5	85.2	2.423	2.8	3.448	4.0	0.746	0.9	4.28	5.0

Monoclonal IgA (5×5×3) lot-to-lot reproducibility									
Level	Mean	Within-run		Between-day		Between-lot		Total	
	(g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.110	0.0048	4.3	0.00	0.0	0.0143	13.0	0.0151	13.7
2	0.339	0.0155	4.6	0.00	0.0	0.0200	5.9	0.0253	7.5
3	0.743	0.0465	6.3	0.00	0.0	0.0762	10.3	0.0893	12.0
4	22.7	1.49	6.6	0.00	0.0	2.24	9.9	2.69	11.9
5	60.9	1.73	2.8	1.38	2.3	2.59	4.3	3.41	5.6

Monoclonal IgM (5×5×3) lot-to-lot reproducibility									
Level	Mean	Within-run		Between-day		Between-lot		Total	
	(g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.098	0.0068	7.0	0.000	0.0	0.0034	3.4	0.0076	7.8
2	0.177	0.0147	8.3	0.000	0.0	0.0070	4.0	0.0163	9.2
3	1.33	0.058	4.4	0.090	6.7	0.090	6.7	0.107	8.0
4	28.3	2.11	7.5	0.00	0.0	2.86	10.1	3.55	12.6
5	71.9	4.33	6.0	2.75	3.8	0.000	0.0	5.13	7.1

m/z Precision: mass-to-charge ratios accompanying precision studies above were assessed separately; maximum imprecision observed across all variance components and samples did not exceed 0.011% CV.

## 2. Linearity:

To evaluate the linearity of the semi-quantitative analytes (IgG, IgA, IgM), two overlapping dilution series per isotype were created from targeted intermixing of serum pools containing high and low concentrations of individual monoclonal M-protein analytes. Each individual dilution series carried a minimum of 11 dilutions, tested in 4 replicates/dilution in 1 run. Weighted least squares regression methods were utilized to calculate the linear regression parameters described below for individual dilution series, as well as composite (high and low) series for each isotype:

Summary of linearity studies conducted for EXENT Immunoglobulin Isotypes							
Isotype	Series	Interval <sub>Expected</sub>	Slope (95% CI)		Intercept (95% CI)		Δ <sub>Max</sub>
IgG	low	0.014–1.10 g/L	1.08	(1.02–1.13)	0.000	(-0.004–0.001)	-0.082 g/L
IgG	high	0.018–98.4 g/L	1.02	(1.00–1.03)	0.000	(-0.001–0.001)	+4.2%
IgG	all	0.014–98.4 g/L	1.04	(1.02–1.07)	0.000	(-0.002–0.001)	-6.9%
IgA	low	0.011–0.92 g/L	1.06	(1.03–1.10)	0.000	(-0.001–0.001)	-0.057 g/L
IgA	high	0.019–69.1 g/L	0.97	(0.94–1.00)	0.000	(-0.001–0.002)	-6.0%
IgA	all	0.011–69.1 g/L	1.06	(1.04–1.08)	0.000	(-0.001–0.000)	-6.0%
IgM	low	0.011–1.04 g/L	1.06	(1.03–1.08)	-0.001	(-0.001–0.002)	-0.058 g/L
IgM	high	0.013–80.5 g/L	1.00	(0.98–1.02)	0.000	(-0.001–0.001)	+1.4%
IgM	all	0.011–80.5 g/L	1.03	(1.02–1.04)	0.000	(-0.001–0.000)	-3.4%

Linear behavior with <10% deviation from linearity is observed for the test system throughout the tested intervals. The results support the claimed AMIs of 0.308–88.9 g/L for IgG, 0.073–65.9 g/L for IgA, and 0.054–74.2 g/L for IgM.

### 3. Analytical Specificity:

#### Interference

The performance of the EXENT Immunoglobulin Isotypes test system was evaluated in the presence of common interfering factors. Testing was performed using three samples – negative, low, and high M-protein values for each isotype in five replicates per sample. The samples were assessed as native samples or interferent-spiked samples (<10% v/v, maximum concentrations tested with no interference listed below), in the presence or absence of each of potential interference compounds with concentrations recommended based on CLSI EP07, 3<sup>rd</sup> Ed. or EP37, 1<sup>st</sup> Ed.

Testing with endogenous and exogenous interferents determined no significant impact of interferents, tested to the following concentrations, upon semi-quantitative results from the EXENT Immunoglobulin Isotypes test system:

<b>Interferent</b>	<b>[Concentration]<sub>max</sub> (unit)</b>
albumin	60 g/L
bilirubin	400 mg/L
intralipid	2.0 g/dL
rheumatoid factor <sup>I</sup>	200 IU/mL
triglyceride	1.5 g/dL
bortezomib	61.2 µg/dL

<sup>I</sup> RF interference originally tested at 500 IU/mL and titered in 100 IU/mL increments to the threshold depicted

Exogenous and endogenous interferents were tested in pooled cocktail formats, determined to have no significant impact on EXENT results, tested at the following concentrations in the following arrangements:

<b>Cocktail 1</b>	
<b>Interferent</b>	<b>[Conc]<sub>max</sub> (unit)</b>
acetaminophen	156 mg/L
ascorbic acid	52.5 mg/L
caffeine	108 mg/L
cimetidine	30 mg/L
cyclophosphamide	55 mg/dL
theophylline	60 mg/L
penicillin	80 mg/L
hemoglobin	10 g/L

<b>Cocktail 2</b>	
<b>Interferent</b>	<b>[Conc]<sub>max</sub> (unit)</b>
prednisolone	1.2 mg/L
digoxin	39 µg/L
acetylsalicylic acid	30 mg/L
ibuprofen	219 mg/L
phenytoin	60 mg/L
pomalidomide	100 µg/mL

#### Therapeutic monoclonal antibodies (t-mAb) Interference:

Multiple immunotherapeutic monoclonal antibodies have been developed and approved for use among several of the target monoclonal gammopathy indications. The EXENT test system has been trained to detect these specific t-mAbs and flag their detection in samples for the user. To validate these detections, patient serum samples containing monoclonal M-proteins with isotype and  $m/z$  identities in close proximity to the  $m/z$  spectra associated with t-mAb detection flags. These samples were spiked with the following concentrations of the following t-mAbs, and (semi-)quantitative M-protein values determined. In these interference studies, the t-mAb detection flags identified (12/12) 100% of the t-mAb interferent.

The following table summarizes the minimum required separation between  $m/z$  of the tested t-mAb and target patient M-protein analyte for detection and flagging:

Therapeutic monoclonal antibody (t-mAb)			Sample	
Interferent	Test concentration	$m/z$	Isotype	Minimum $m/z$ difference
daratumumab	0.5 g/L	11691.2	IgG, $\kappa$	-19.6
isatuximab	0.5 g/L	11710.0	IgG, $\kappa$	22.9
elotuzumab	0.5 g/L	11745.0	IgG, $\kappa$	28.3

#### Cross-Reactivity:

A supplemental study was conducted to examine the performance of EXENT in select indications in which M-proteins are known to be encountered, but are not necessarily relevant differential diagnoses in the clinical workflow for the monoclonal gammopathy target indications. Specifically,  $N=97$  leukemias and lymphomas (L&L) – largely representing B-cell malignancies (e.g., B-cell acute lymphocytic leukemia, B-ALL), as well as  $N=47$  acute infectious diseases (ID). For purposes of comparative performance with L&L or ID with corresponding SPE or IFE results, summarized in the following table:

Disease category	( $N$ )	(n) EXENT(+) patient results		Total EXENT(+)	
	Total	SPE/IFE(+)	SPE/IFE(-)	(n)	(%)
leukemia and lymphoma	97	16	13	29	29.9%
infectious disease	47	1	3	4	8.5%

Zero samples from the five samples from which neither SPE nor IFE results were available showed M-protein peaks above cut-off from EXENT.

#### 4. Assay Reportable Range:

Isotype	Analytical Measuring Interval (AMI)
IgG	0.308–88.9 g/L
IgA	0.073–65.9 g/L
IgM	0.054–74.2 g/L

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

*Traceability:*

Total immunoglobulin concentrations for each isotype (IgG, IgA, IgM) are traceable to ERM-DA470K, through the integral Optilite total immunoglobulin test systems.

*Stability:*

Reagent/Kit Stability:

To test the stability of the EXENT Immunoglobulin Isotypes assay reagents and kit, seven serum samples were used to evaluate the performance of three lots, tested at eight timepoints of real-time storage plus time zero ( $T_0$ ) baseline in triplicates. Additional studies tested the stability of select reagents when stored onboard the EXENT Analyser, open vials returned to storage conditions, freeze-thaw for the mass calibrator, and the stability of reagents stored on MALDI plates. The Sponsor claims the following shelf-life and stability conditions for the components of the EXENT Immunoglobulin Isotypes assay:

Study Design	Applicable reagents	Storage Conditions	Stability Claim
Real-time	Elution Buffer	2-8°C	18 months
	all other reagents	2-8°C	24 months
On-board	beads, mass calibrator, controls, Diluent Buffer 1, Elution Buffer	on-board EXENT Analyser	21 hours
	Wash Solution 1		7 days
	matrix		4.5 hours
Open Vial	beads, control, mass calibrator	2-8°C	2 months
Freeze/Thaw	mass calibrator	-20°C / RT	4 F/T cycles

Sample Stability:

To test the stability of serum samples for use with the EXENT Immunoglobulin Isotypes test system,  $n=27$  sera, collectively representing all M-protein isotypes, and stored in conditions tested at 6 timepoints in addition to  $T_0$  baseline. Separate studies tested the stability of analyte in multiple cycles of freeze/thaw from -20°C. In addition, stability of samples spotted onto MALDI plates by the EXENT-iP500 component, before testing by the EXENT-iX500 MALDI-TOF were also tested. The Sponsor claims the following shelf-life and stability conditions for the components of the EXENT Immunoglobulin Isotypes assay:

Study Design	Storage Conditions	Stability Claim
Real-time	4°C	28 days
	-20°C	6 months
Freeze/thaw	-20°C / RT	3 F/T cycles
On-plate	on- or off-board	6 hours

6. Detection Limits:

Limits of Quantitation:

The Limit of Quantitation (LoQ) of each semi-quantitative analyte (IgG, IgA, IgM) were determined, following CLSI EP17-A2, based on a precision profile of 4 low-level

reconstituted sample pools of monoclonal M-proteins in normal human serum to a target threshold of 20% CV for 4 replicates/run  $\times$  5 days. A total of three reagent lots, utilizing two EXENT Analyser instruments, and one Optilite instrument were utilized, and the greatest values represent among these combinations of lot and instrument were selected as final LoQ claims, as follows:

Isotype	LoQ/LLMI
IgG	0.308 g/L
IgA	0.073 g/L
IgM	0.054 g/L

## 7. Assay Cut-Offs:

Cut-offs for each semi-quantitative analyte (IgG, IgA, IgM) were determined from the  $N=364$  reference population study cohort (see §VII.E below). Subjects with no detectable monoclonal peak were defined as [analyte] = 0.0 g/L for non-parametric rank purposes for each isotype. If multiple quantifiable monoclonal peaks that were detected in individual subjects, each peak was analyzed separately. The non-parametric percentile-rank targets for each isotype for all peaks (and non-detections, treated as 0.0 g/L) are summarized below:

Isotype	Percentile Threshold	Cut-off value
IgG	95 <sup>th</sup> percentile	0.359 g/L
IgA	99 <sup>th</sup> percentile	0.325 g/L
IgM	99 <sup>th</sup> percentile	0.197 g/L

## B Comparison Studies:

### 1. Method Comparison with Predicate Device:

#### Semi-quantitative method comparisons:

Semi-quantitative method comparisons to serum capillary protein electrophoretic methods (SPE) were performed on a total composite population of 520 test result pairs (EXENT versus SPE) across all three semi-quantitative isotype analytes, following exclusion of samples with test results that were not quantitatively valid – i.e., below the LoQ/LLMI of either assay, or above the ULMI of either assay. Passing-Bablok regression parameters for each analyte are summarized in the following table:

Isotype	<i>N</i>	Interval <sub>EXENT</sub>	Slope (95% CI)	Intercept (95% CI)
IgG	361	0.37–84.1 g/L	1.10 (1.06–1.15)	-0.86 (-1.25 – -0.46)
IgA	88	0.62–49.4 g/L	0.91 (0.84–1.05)	-0.48 (-1.45–0.19)
IgM	93	0.55–63 g/L	1.54 (1.42–1.73)	-1.16 (-2.80 – -0.48)

Bias predicted from these regression parameters at critical medical decision levels, as compared to the SPE comparator methods are summarized in the following table:

Isotype	Cut-off <sub>EXENT</sub>	Predicted bias (U)
IgG	0.359 g/L	-0.83 g/L
IgA	0.325 g/L	-0.51 g/L
IgM	0.197 g/L	-1.05 g/L



### Qualitative method comparisons:

Qualitative method comparisons compared the isotype assignments identified by the EXENT test system to those determined by an immunofixation electrophoresis (IFE) comparator. A total of 618 heavy chain isotype and light chain determinations from both methods (EXENT and IFE) are summarized in the following table:

Summary of qualitative comparison of isotype calls (paired heavy, light chains)											
EXENT Immunoglobulin Isotypes	IFE predicate comparator										
	IgGh <sup>1</sup>	IgG,κ	IgG,λ	IgAh <sup>1</sup>	IgA,κ	IgA,λ	IgMh <sup>1</sup>	IgM,κ	IgM,λ	none <sup>2</sup>	total
	IgGh <sup>1</sup>	0	1	0	0	0	0	0	0	0	1
	IgG,κ	0	225	1	0	0	0	0	1	0	227
	IgG,λ	0	1	144	0	0	0	0	0	0	145
	IgAh <sup>1</sup>	0	0	0	0	1	0	0	0	0	1
	IgA,κ	0	0	0	1	45	0	0	0	0	45
	IgA,λ	0	0	0	1	0	62	0	0	1	63
	IgMh <sup>1</sup>	0	0	0	0	0	0	3	3	0	6
	IgM,κ	0	0	0	0	0	1	64	1	0	65
	IgM,λ	0	0	0	0	0	1	0	30	0	30
	negative	0	6	9	0	3	2	1	2	3	35
	total	0	233	154	0	49	64	1	69	38	618
	% agree	–	96.6	93.5	–	91.8	96.9	0	92.8	78.9	93.7

**green shaded cells** depict heavy and light chain concordance  
**orange shaded cells** depict heavy chain concordance, but light chain discordance  
**violet shaded cells** depict heavy chain detection in the absence of light chain by the predicate and were not tabulated for paired agreement  
**red shaded cells** depict discordance of heavy and light chain isotype assignment

<sup>1</sup> detection of heavy chain M-protein (Igγ, Igα, Igμ) in the absence of associated light chain (Igκ, Igλ)

<sup>2</sup> the method comparison study population was selectively enriched for SPE-negative samples to provide an estimate of NPA

Agreement measures (PPA, with 95% CI) for paired heavy and light chain isotype assignments from the above table are summarized in the following 2×2 confusion matrix:

		Predicate Comparator		Total
		(+)	(–)	
EXENT Immunoglobulin Isotypes	≥c/o (+)	570	1	571
	≤c/o (–)	38	9	47
Total		608	10	618

PPA 93.8% (91.5–95.4%)

NPA 90.0% (59.6–98.2%)

Agreement measures (PPA, with 95% CI) for all calls of heavy or light chain isotypes (e.g., patients with multiple M-protein detections from either method have each M-protein assessed independently) from the above table are summarized in the following table:

Isotype	(n)/(N)	PPA (95% CI)
IgG	372/387	96.1% (93.7–97.6%)
IgA	110/115	95.7% (90.2–98.1%)
IgM	103/110	93.6% (87.4–96.9%)
Igκ	334/351	95.2% (92.4–97.0%)
Igλ	236/256	92.2% (88.2–94.9%)
<b>Total</b>	<b>1,155/1,219</b>	

Agreement measures (PPA, with 95% CI) for all total individual calls of heavy or light chain isotype assignments, irrespective of pairing from the above table are summarized in the following 2×2 confusion matrix:

		Predicate Comparator		Total
		(+)	(–)	
EXENT Immunoglobulin Isotypes	≥c/o (+)	1,155	1	1,156
	≤c/o (–)	64	9	73
	<b>Total</b>	<b>1,219</b>	<b>10</b>	<b>1,229</b>

PPA	94.8%	(93.4–95.9%)
NPA	90.0%	(59.6–98.2%)

## 2. Matrix Comparison:

Serum is the only claimed matrix for this assay.

## C Clinical Studies:

### 1. Clinical Sensitivity:

To assess the clinical performance of the EXENT Immunoglobulin Isotypes test system, a total study population of  $N=688$  patient subjects was assembled to represent the five target monoclonal gammopathy indications, enrolled by retrospective selection of banked serum samples of patients from four clinical sites, conforming to diagnoses in alignment with contemporary clinical guidelines. The distribution of target indications in the evaluable clinical sensitivity study population are summarized in the following table:

Diagnosis	(n)	Diagnostic Criteria	Diagnostic considerations
MGUS: Monoclonal gammopathy of undetermined significance	227	1	stable, untreated
MM: Multiple myeloma	180	1	newly diagnosed or relapsed
SMM: Smoldering multiple myeloma	140	1	stable, untreated
AL: Acute light chain amyloidosis	77	1	newly diagnosed or relapsed
WM: Waldenstrom's macroglobulinemia	64	2	newly diagnosed or relapsed
<b>Total</b>	<b>688</b>		

- 1: 2014 International Myeloma Working Group updated criteria
- 2: 2003 Consensus Panel Recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia

The clinical performance of the EXENT Immunoglobulin Isotypes test system was determined by testing the above patient samples; the resulting clinical performance of the test across all six analytes above the pre-specified cut-offs (§VII.A .7) is depicted in the tables for the individual monoclonal gammopathy indications, dividing true positive (*tp*) by the number of patient samples (*n*) represented in the disease study population:

<b>Diagnosis</b>	<b><i>tp</i>/(<i>n</i>)</b>	<b>Sensitivity (95% CI)</b>
MM	170/180	94.4% (90.1–97.0%)
SMM	138/140	98.6% (94.9–99.6%)
AL	69/77	89.6% (80.8–94.6%)
WM	63/64	98.4% (91.7–99.7%)
<b>Total</b>	<b>440/461</b>	<b>95.4% (93.1–97.0%)</b>

For MGUS, 209 out of 227 cases were tested positive by the EXENT Immunoglobulin Isotypes assay with a positive rate of 92.1% (95% CI: 87.8–94.9%).

Qualitative immunophenotype of the identified M-protein isotypes (i.e., pairings of heavy and light chain) determined by the EXENT Immunoglobulin Isotypes test system above isotype-specific cut-offs in this population are summarized in the following table:

Summary of qualitative isotype calls (paired heavy, light chains) by target diagnosis by EXENT

<b>Diagnosis</b>	<b>IgGh<sup>1</sup></b>	<b>IgG,κ</b>	<b>IgG,λ</b>	<b>IgAh<sup>1</sup></b>	<b>IgA,κ</b>	<b>IgA,λ</b>	<b>IgMh<sup>1</sup></b>	<b>IgM,κ</b>	<b>IgM,λ</b>	<b>poly<sup>2</sup></b>	<b>none</b>	<b>total</b>
MGUS	0	90	52	1	15	9	4	27	6	5	18	227
MM	0	86	33	0	23	22	0	2	2	2	10	180
SMM	1	65	38	0	14	14	1	1	1	3	2	140
AL	0	7	28	0	1	26	1	0	3	3	8	77
WM	0	0	0	0	0	0	2	41	19	1	1	64
<b>Total</b>	<b>1</b>	<b>248</b>	<b>151</b>	<b>1</b>	<b>53</b>	<b>71</b>	<b>8</b>	<b>71</b>	<b>31</b>	<b>14</b>	<b>39</b>	<b>688</b>

<sup>1</sup> detection of heavy chain M-protein (Igγ, Igα, Igμ) in the absence of associated light chain (Igκ, Igλ)

<sup>2</sup> multiple clonal M-protein peaks detected above isotype-specific cut-offs

## 2. Clinical Specificity:

To assess the clinical specificity of the EXENT Immunoglobulin Isotypes test system, a total study population of *N*=655 patient subjects was assembled to represent relevant differential diagnoses corresponding to the intended population of the device – patients being evaluated for monoclonal gammopathy. Archived serum samples from *n*=589 patients representing a total of eight diagnostic categories other than monoclonal gammopathy were selected in a retrospective manner. An additional *n*=66 patient subjects were enrolled prospectively, based on routine ordering of serum protein electrophoresis (SPE) testing to enrich the reflex test positioning of the EXENT Intended Use.

Representative diseases, diagnoses, indications comprising these thirteen disease categories are described as below:

Representative diseases, diagnoses, indications among specificity population categories		
Diagnostic Category	(n)	Represented Diagnoses, Diseases, Indications
kidney disease	98	Chronic kidney disease, acute renal failure, end-stage renal disease (ESRD).
cardiovascular disease	80	Coronary artery disease, aortic stenosis, myocardial infarction, stroke, cardiovascular event, any heart failure, enlargement of the heart, arrhythmia, bradycardia.
chronic inflammation	65	Fibromyalgia, psoriasis, eczema, Crohn's disease, pancreatitis, polymyalgia rheumatica, hidradenitis, arthritis, bronchitis
hematological disease, anemia	59	Anemia, sickle cell disease, amyloidosis, hypogammaglobulinemia, thrombocytopenia, abnormal bleeding time, von Willebrand disease
immune, autoimmune disease	56	Still's disease, Sjögren's syndrome, myasthenia gravis, fibromyalgia, common variable immune deficiency (CVID), primary immunodeficiency, lupus erythematosus, rheumatoid arthritis
diabetes	54	Type 2 diabetes, pre-diabetes, diabetic complications such as neuropathy or ulcers
cancer	52	Solid tumor cancers, including metastatic <sup>1</sup>
neurological disease	46	Multiple sclerosis, neuropathy, carpal tunnel syndrome, paresthesia, Parkinson's, neuropathic pain, memory loss, Alzheimer's, seizures, psychosis
bone pain, bone disease	40	Osteoporosis, bone lesion, metabolic bone disease, osteopenia, skeletal pain or musculoskeletal pain, fractures, observations of low bone density
hypertension	37	Primary hypertensive disease <sup>2</sup>
chronic liver disease	30	Hepatitis, cirrhosis, transaminitis, hyperbilirubinemia
fatigue	10	Fatigue, insomnia, parasomnia, obstructive sleep apnea, other sleep and fatigue-related disorders
others	28	Hypermobility joints; irritable bowel syndrome; hypotension; Raynaud's disease; asthma; weakness; patients with rare, unusual disorders that do not seem to have a common link or could not be allocated to one of the above categories
<b>Total</b>	<b>655</b>	

<sup>1</sup> See §VII.A.3 for hematological malignancies, predominantly B-cell leukemias & lymphomas

<sup>2</sup> Hypertension was not selected as the primary category if the patient had other, more appropriate concurrent diagnoses, such as renal disease or cardiovascular disease

The clinical performance of the EXENT Immunoglobulin Isotypes test system was determined by testing the above patient samples; the resulting clinical performance of the test across all analytes below the pre-specified cut-offs is depicted for the individual differential diagnostic categories in the following table, dividing true negative (*tn*) by the number of patient samples (*n*) represented in the differential diagnostic category:

Diagnostic Category	<i>tn</i> /( <i>n</i> )	Specificity (95% CI)
kidney disease	86/98	87.8% (79.8–92.9%)
cardiovascular disease	69/80	86.3% (77.0–92.2%)
chronic inflammation	56/65	86.2% (75.7–92.5%)
hematological disease, anemia	48/59	81.4% (69.6–89.3%)
immune, autoimmune disease	52/56	92.9% (83.0–97.2%)

Diagnostic Category	tn/(n)	Specificity (95% CI)
diabetes	45/54	83.3% (71.3–91.0%)
cancer	43/52	82.7% (70.3–90.6%)
neurological disease	42/46	91.3% (79.7–96.6%)
bone pain, bone disease	37/40	92.5% (80.1–97.4%)
hypertension	35/37	94.6% (82.3–98.5%)
chronic liver disease	28/30	93.3% (78.7–98.2%)
fatigue	10/10	100% (72.3–100%)
others	28/28	100% (87.9–100%)
<b>Total</b>	<b>579/655</b>	<b>88.4% (85.7–90.6%)</b>

Additional analyses considered populations that fulfilled the Intended Use/Indications for Use condition as a “reflex test”, incumbent upon positive results from SPE or FLC methods. Results from this analysis are summarized in the following table:

	tn/(n)	Specificity (95% CI)
SPE OR FLC(+)	56/79	70.9% (60.1–79.8%)
SPE AND FLC(–)	271/295	91.9% (88.2–94.5%)
SPE/FLC Unavailable	248/281	88.3% (84.0–91.5%)

### 3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

#### **D Clinical Cut-Off:**

Cut-offs for the three semi-quantitative isotype analytes (monoclonal IgG, IgA, and IgM) were determined by non-parametric evaluation of a  $N=364$  population of apparently healthy subjects (see §VII.E). Non-detections of any monoclonal M-protein peaks were treated as 0.0 g/L. Results from these determinations are described below:

Isotype	Threshold	Cut-off value
IgG	95 <sup>th</sup> percentile	0.359 g/L 95% CI: (0.224–0.866)
IgA	99 <sup>th</sup> percentile	0.325 g/L 90% CI: (0.133–2.320)
IgM	99 <sup>th</sup> percentile	0.197 g/L 95% CI: (0.123–0.829)

#### **E Expected Values/Reference Range:**

Serum samples were tested by the EXENT Immunoglobulin Isotypes test system in a reference population consisting of  $N=364$  apparently healthy subjects. No M-proteins were detected in 148/364 (40.7%) of this population. Of the remaining 216 reference subjects in which M-protein peaks were detected (see §VII.D above), 118/364 (32.4%) displayed a single M-protein peak, whereas 98/364 (26.9%) displayed multiple (i.e., more than one) M-protein detection. Altogether, this represented a total of 346 peaks among 216 subjects. 206 of these peaks were above the respective LoQ/LLMI of respective isotypes; 25 total peaks were above the respective isotype-specific cut-offs. The 25 total M-protein peaks above cut-offs in the reference population represented 23 subjects – two subjects had more than one M-protein above isotype-specific cut-offs.

The distribution of isotype peaks (i.e., heavy chain assignment calls, irrespective of multiple detections in individual subjects) detected, above LoQ/LLMI, and above clinical cut-offs, are

summarized on the left columns [(n) clonal peaks] in the following table; in the right-side columns, peak detections per isotype above cut-off by reference subjects were normalized to the reference population (N=364) for isotype-specific positivity rates:

Isotype	(n) clonal peaks			positive subjects ( $\geq c/o$ )		
	detected	$\geq LoQ/LLMI$	positive ( $\geq c/o$ )	(n)	(% ÷ 364)	(95% CI)
IgG	45	20	18	17	4.67%	(2.94–7.35%)
IgA	38	15	3	3	0.82%	(0.28–2.39%)
IgM	263	225	4	4	1.10%	(0.43–2.79%)
<b>Total</b>	346	260	25	23		

**Reference Positive Rate:** 23/364: 6.32% (4.25–9.30%)

## VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

## IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.