October 17, 2025

The Binding Site Group Ltd
Jolanta Wolff
Regulatory Affairs Project Manager
8 Calthorpe Road
Edgbaston
Birmingham, B15 1QT
United Kingdom

Re: K250159
Trade/Device Name: Immunoglobulin Isotypes (GAM) for the EXENT Analyser; EXENT Analyser
Regulation Number: 21 CFR 866.5510
Regulation Name: Immunoglobulins A, G, M, D, And E Immunological Test System
Regulatory Class: Class II
Product Code: SGG, OTA
Dated: September 16, 2025
Received: September 16, 2025

Dear Jolanta Wolff:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ying Mao -S

Ying Mao, Ph.D.
Branch Chief
Division of Immunology and
Hematology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K250159

Device Name
Immunoglobulin Isotypes (GAM) for the EXENT Analyser
EXENT Analyser

Indications for Use *(Describe)*
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. The device is designed for professional in vitro diagnostic use only and it is not a device for self-testing.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

Binding Site
part of Thermo Fisher Scientific

510(k) SUMMARY (as per 21 CFR 807.92)

This 510(k) Summary of Safety and Effectiveness information is being submitted in accordance with the requirements of the Safe Medical Device Act 1990 and 21 CFR 807.92.

510(k) Number: K250159

Type of 510(k): Original, Traditional 510(k)

Purpose of Submission: New Device: Immunoglobulin Isotypes (GAM) for the EXENT®

Analyser, and the EXENT® Analyser

Date of Preparation: 17 January 2025, updated on October 17th, 2025

1 SUBMITTER / APPLICANT

The Binding Site Ltd
8 Calthorpe Road
Edgbaston
Birmingham, B15 1QT, GB

Correspondent/ Contact: Jolanta Wolff
Regulatory Affairs Project Manager
Phone: +44 121 456 9500

2 DEVICE INFORMATION

Proprietary Name: Immunoglobulin Isotypes (GAM) for the EXENT® Analyser, EXENT® Analyser,

Measurand: Monoclonal Immunoglobulins A, G, M,

Type of Test: semi-quantitative measurement of monoclonal immunoglobulins A, G, M and isotyping of monoclonal immunoglobulins A, G, M, Mass-spectrometry

Regulatory information:

Table 1. Regulatory information.

Proprietary Name	Regulation Section	Product Code	Classification	Review Panel
Immunoglobulin Isotypes (GAM) for the EXENT® Analyser	21 CFR 866.5510, Immunoglobulins A, G, M, D, and E immunological test system	SGG, Mass Spectrometric, Immunoglobulins (G, A, M, D, E)	Class II	Immunology (82)
EXENT® Analyser	21 CFR 862.2570, Instrumentation for clinical multiplex test systems	OTA, Mass Spectrometer for Clinical Multiplex Test Systems	Class II	Clinical Chemistry (75)

3 PREDICATE DEVICES AND 510(K) NUMBERS

The predicate devices chosen are included in Table 2 below:

Table 2. List of Predicate devices.

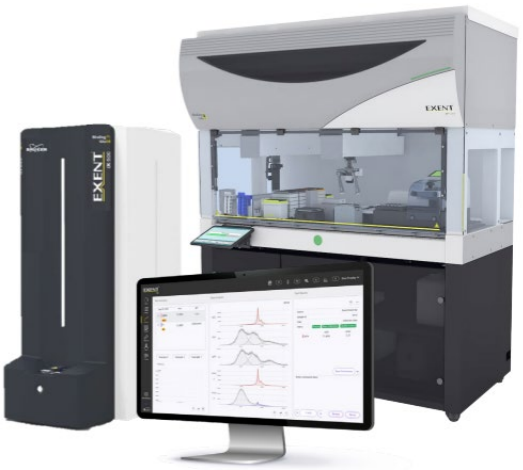
Measurement of Monoclonal immunoglobulins IgG, IgA, IgM					
Proprietary Name	**510(k)**	**Regulation section**	**Product Code**	**Classification**	**Panel review**
Human IgG Subclass Liquid Reagent Kits for Use on the SPAPLUS Analyser	K072889	21 CFR 866.5510, Immunoglobulins A, G, M, D, and E immunological test system	CFN, Method, Nephelometric, Immunoglobulins (G, A, M)	Class II	IM - Immunology (82)
Optilite IgA Kit	K191985				
Optilite IgM Kit	K191635				
Kappa (κ) and Lambda (λ) isotyping of Monoclonal immunoglobulins IgG, IgA, IgM					
Proprietary Name	**510(k)**	**Regulation section**	**Product Code**	**Classification**	**Panel review**
*HYDRAGEL Immunofixation	K960669	21 CFR 866.5510, Immunoglobulins A, G, M, D, and E immunological test system	CFF, immunoelectrophoretic, immunoglobulins, (G, A, M)	Class II	IM - Immunology (82)

*Manufacturer: Sebia; type: IFE (immunoelectrophoretic); Other name as per 510(k) database: HYDRAGEL IF, 6 IF, 12 IF PENTA KITS/HYDRAGEL IF, DOUBLE IF, 2 IF, & 4 IF KITS

Clinical performance studies included the comparison of EXENT results to clinical truth (diagnostic sensitivity and specificity), as well as the comparison of M-protein isotype and M protein quantification results obtained by EXENT with those obtained by SPE (serum protein electrophoresis) and IFE (immunofixation electrophoresis).

4 DEVICE DESCRIPTION

The system consisting of the EXENT® Analyser and the EXENT® assays are intended for the in vitro measurements of analytes in human body fluids. It is designed to provide automation and integration of all the analytical steps (including liquid handling and MALDI-ToF mass spectrometry). 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 device is designed for professional use only and it is not a device for self-testing.

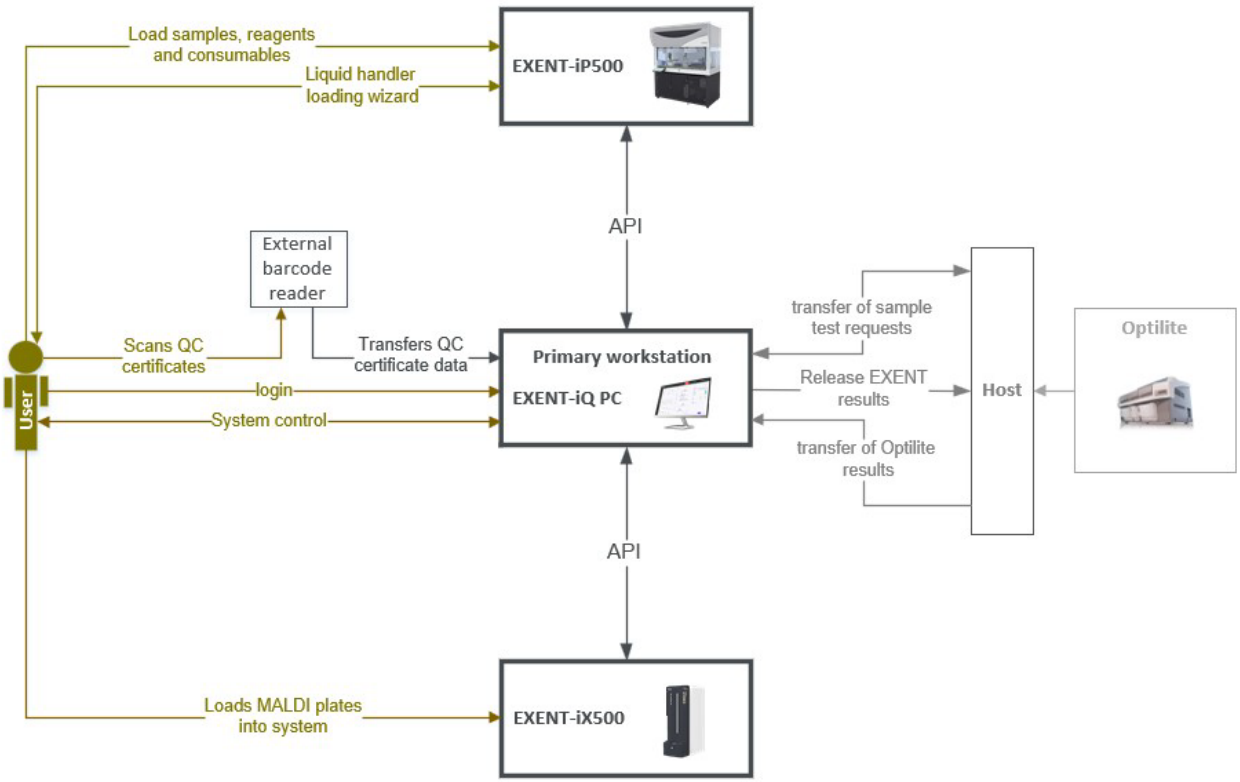


The EXENT Analyser combines automated immunoassay with readout by MALDI-ToF mass spectrometry. It is a modular analyser, and the major components are described in Table 3 and illustrated schematically in Figure 1.

Table 3. A description of the major components of the EXENT Analyser.

Component	Description	Function
EXENT-iP® 500	Automated liquid handler	Preparation of patient samples by magnetic bead immunoprecipitation assays for subsequent analysis by MALDI-ToF Mass spectrometry
EXENT-iX® 500	MALDI-ToF mass spectrometer	Analysis of prepared patient samples by MALDI-ToF mass spectrometry
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.

Figure 1. A schematic overview of the EXENT Analyser. User interactions are illustrated in brown. Components of the EXENT Analyser are depicted in black. Interactions with the Optilite are depicted in grey.



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 isotype-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.

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.

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.

4.1 Test Principle

Immunoglobulin Isotypes (GAM) for the EXENT® Analyser:

Patient samples are automatically prepared on the EXENT-iP®500 liquid handler using paramagnetic bead-mediated immuno-precipitation. Patient serum samples are divided into 5 equal aliquots which are mixed independently with antibody-coated paramagnetic beads (anti human-IgG, -IgA, -IgM, - total Kappa and -total Lambda) specific to the isotype of interest. The specific immunoglobulins bind to the immobilised 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 disulphide bonds reduced to dissociate the light chains from the heavy chains. The resulting eluates for each individual specificity contain a mixture of the heavy chains and light chains. Eluates are subsequently mixed with a MALDI matrix compound, spotted onto MALDI plates and allowed to co-crystallise on the plate surface. Sample spots are then analysed automatically using the EXENT-iX®500 MALDI-TOF mass spectrometer. The immunoglobulin light chain spectral peaks (doubly charged ions at m/z 10,900 to 13,600) are identified and then semi-quantified indirectly in combination with Optilite immunoturbidimetry.

The EXENT Analyser: uses quantitative IgG, IgA and IgM results, generated on a patient sample with the Optilite IgG, IgA and IgM assays imported automatically via the Laboratory Information System (LIS) or entered manually by the user, to deliver a result for the M-protein, derived from an area under the curve (AUC) calculation. Refer to Section 4.4, Figure 2.

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, IgM values derived from Optilite assays. These are provided in the EXENT® Immunoglobulin Isotypes (GAM) Control Pack.

4.2 Special Conditions for Use Statement(s)

Prescription use only.

Immunoglobulin Isotypes (GAM) for the EXENT® Analyser:

Interpretation of results:

The results of this assay should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings including previous EXENT results if available.

Results are obtained in combination with Optilite immunoglobulin measurements (see section 4.4). Refer to the Optilite Operator Manual, supplied with the analyser for further details.

The agreement between M-protein concentrations determined by serum protein electrophoresis and the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay depends on a number of factors, such as sample M-protein concentration and isotype, electrophoresis method (gel vs capillary electrophoresis) and peak integration methodology (perpendicular drop vs tangential skim).

M-proteins are expected to be more frequently present in patients with other haematological malignancies, especially B-cell lymphomas, compared to healthy subjects or other disease conditions [Ref. 1, 2]. In a cohort of 97 patients with leukaemia and (primarily B cell) lymphoma, an M-protein ≥ isotype specific cutoff was present in 29.9% of the samples.

Limitations:

This assay has not been established for use with the paediatric population.

The system is able to determine and report calculated concentrations of IgG, IgA and IgM monoclonal proteins. Absence of IgG, IgA, or IgM M-proteins does not exclude the presence of, IgD. IgE, or free light chain-only M-proteins. Alternative methods (such as immunofixation electrophoresis, serum free light chain concentration measurement, or urine studies) should be used if IgD, IgE, or light chain-only disease is suspected.

In rare instances the system may incorrectly flag one of the monoclonal proteins in a biclonal sample as antibody-independent binding (AIB). This occurs when the two heavy chains (e.g., a biclonal IgG and IgM) are associated with the same light chain (same isotype and m/z).

M-protein values generated by SPE/densitometry and the Immunoglobulin Isotypes (GAM) for the EXENT® Analyser are not interchangeable.

4.3 Special Instrument Requirements

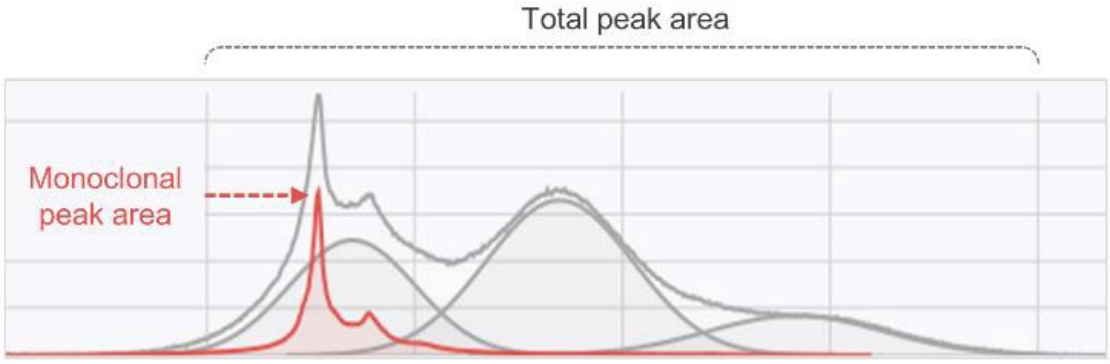
EXENT® Analyser

Optilite® Analyser

4.4 Calculation of Examination Results

Measurement of monoclonal protein peaks detected by the EXENT Analyser is performed automatically. The algorithm for this equates the modelled total light chain peak area detected for either IgG, IgA, or IgM using the EXENT assay to the corresponding total IgG, IgA, or IgM concentration by Optilite. It then expresses the modelled monoclonal peak area as a fraction of that concentration. Refer to Figure 2 as follows:

Figure 2. Graphical representation of the calculation and examination of results.



$$\text{Monoclonal protein (g/L)} = \frac{\text{Monoclonal peak area}}{\text{Total peak area}} \times \text{Total Immunoglobulin value by Optilite (g/L)}$$

4.5 Kit Reagents and Composition

4.5.1 Materials provided in the Immunoglobulin Isotypes (GAM) for the EXENT® Analyser

EXENT IgG Reagent
EXENT IgA Reagent
EXENT IgM Reagent
EXENT Total Kappa Reagent
EXENT Total Lambda Reagent

Reagents composition:

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.

4.5.2 Materials provided in the EXENT® Immunoglobulin Isotypes (GAM) Control Pack

EXENT Immunoglobulin (GAM) Control
EXENT Immunoglobulin (GAM) Control 2

Controls Composition:

Pooled human serum, supplied in stabilised liquid form. Containing 0.099 % (w/v) Sodium Azide, 0.1 % (w/v) E-amino-n-caproic acid (EACA), and 0.01 % (w/v) Benzamidine as preservatives.

4.5.3 Accessories required for the EXENT® Analyser

Accessories are described in Table 4 as follows:

Table 4. A list of the accessories for the EXENT® Analyser.

Reagent	Format	Purpose
EXENT Diluent 1	EXENT Diluent 1 vials ready to use	Dilution of patient sample
EXENT Elution Buffer 1	EXENT Elution Buffer 1 Solvent vials EXENT Elution Buffer 1 Compound vials	Recovery of measurand from the capture antibodies. Disassociation of the heavy & light chains from the captured immunoglobulins. Contains protein standard (Aprotinin) that is used as process control and within-sample mass calibrator.
EXENT HCCA MALDI Matrix Pack	EXENT HCCA MALDI Matrix Solvent vials EXENT HCCA MALDI Matrix Compound vials	This compound is mixed with the prepared patient sample when spotted on to the MALDI plate to assist with ion formation during MALDI-TOF mass spectrometric analysis. This reagent also serves as Detector Check Control.
EXENT Mass Calibration Standard 1	EXENT Mass Calibration Standard 1 – lyophilized protein mixture EXENT Mass Calibration Standard 1 Aliquot – empty barcoded aliquot vials	A mixture of standard proteins (cytochrome C, myoglobin and trypsinogen) that is used to create a mass calibration curve to ensure mass accuracy.
EXENT Disposable MALDI Plate Pack	EXENT Disposable MALDI Biotarget plates.	MALDI plate upon which the prepared samples are spotted prior analysis
EXENT Wash Solution 1	EXENT Wash Solution 1 buffer concentrate in a container	It is used to wash antibody-coated paramagnetic beads bound to the analyte of interest using the analyser on-board plate washer

5 INTENDED USE/ INDICATIONS FOR USE

5.1 Intended 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® Immunoglobulin Isotypes (GAM) Control Pack:

The EXENT Immunoglobulin Isotypes (GAM) Control Pack is intended to be used with the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay.

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. The device is designed for professional *in vitro* diagnostic use only and it is not a device for self-testing.

5.2 Indications for Use

Same as the Intended Use above.

5.3 Summary and Explanation

Immunoglobulin Isotypes (GAM) for the EXENT®Analyser:

Immunoglobulin molecules consist of two identical heavy chains (α, δ, ε, γ, or μ) which define the immunoglobulin isotype and two identical light chains (κ, Kappa or λ, Lambda). Each light chain is linked to a heavy chain and the two heavy chains are linked covalently at the hinge region. When immunoglobulins immunopurified from serum have their inter-chain disulphide bonds reduced, they are dissociated into their constituent heavy and light chains.

The analysis of these by mass spectrometry resolves the heavy and light chains into distinct molecular mass-to-charge (m/z) distributions. The software analyses only the light chain component, which manifests as a polyclonal distribution representing the diversity of light chain sequences. Because each light chain originates from a corresponding heavy chain, the light chain distribution is proportional to the total immunoglobulin content corresponding to the heavy chain isotype.

The presence of distinct m/z peaks within these polyclonal molecular mass distributions may represent the presence of monoclonal immunoglobulins which can be indicative of an underlying abnormality such as monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, smouldering multiple myeloma, AL amyloidosis, or

Waldenström's macroglobulinaemia. The Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay provides calculated measurements of monoclonal IgG, IgA, and IgM immunoglobulins. The assay can also type these monoclonal immunoglobulins as associated with either the Kappa or Lambda light chain.

The International Myeloma Working Group (IMWG) Mass Spectrometry Committee has recommended the use of MALDI-TOF mass spectrometry as an alternative to immunofixation electrophoresis (IFE) in the clinical assessment of patients, the assessment of patients in clinical trials [3].

6 TECHNOLOGICAL CHARACTERISTICS

6.1 Similarities and Differences to the Predicate

The similarities and differences between Immunoglobulin Isotypes (GAM) for the EXENT® Analyser assay compared to Human IgG Subclass Liquid Reagent Kits for Use on the SPAPLUS Analyser, Optilite IgA, and Optilite IgM kit assays are summarised in Table 5 below.

Table 5. Technological similarities and differences - Immunoglobulin Isotypes (GAM) for the EXENT® Analyser assay compared to SPAPLUS and Optilite assays. (Measurement of Monoclonal immunoglobulins IgG, IgA, IgM)

Similarities		
Item	(Proposed Device) Immunoglobulin Isotypes (GAM) for the EXENT® Analyser (Measurement of Monoclonal immunoglobulins IgG, IgA, IgM)	(Predicate Device) Human IgG Subclass Liquid Reagent Kits for Use on the SPAPLUS Analyser Optilite IgA kit, Optilite IgM kit
Units of measure	g/L	same
Sample preparation	Automatic	same
End User	Professional, prescription use only	same
Sample Type	Serum	same
Controls	2 levels of serum control material	same
Differences		
Item	(Proposed Device) Immunoglobulin Isotypes (GAM) for the EXENT® Analyser	(Predicate Device) Human IgG Subclass Liquid Reagent Kits for Use on the SPAPLUS Analyser, Optilite IgA, Optilite IgM
Intended Use	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.	These kits are intended for quantifying human IgG and IgG subclasses 1, 2, 3 and 4 in serum using the SPAPLUS analyser. Measurement of these immunoglobulins is an aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents in conjunction with other laboratory and clinical findings. The Optilite IgA Kit is intended for the quantitative in vitro measurement of IgA in serum, lithium heparin or EDTA plasma using the Binding Site Optilite analyser. Measurement of IgA aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. This test should be used in conjunction with other laboratory and clinical findings. The Optilite IgM Kit is intended for the quantitative in vitro measurement of IgM in human serum, lithium heparin or EDTA plasma using the Binding Site Optilite analyser. Measurement of IgM aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious

	Assay results should be used in conjunction with other laboratory and clinical findings.	agents. The test results are to be used in conjunction with other clinical and laboratory findings.
Test Method	Mass spectrometry	Turbidimetry
Analyte(s)	clonal IgG clonal IgA clonal IgM (+ Kappa, Lambda isotyping)	total IgG total IgA total IgM
Reagent Composition	Monospecific polyclonal sheep anti-human IgG, IgA, IgM, total kappa and total lambda antibodies conjugated to paramagnetic beads, supplied in liquid form.	Polyclonal sheep anti-human IgG, IgA, IgM
Mass Calibration	Protein standard containing a mix of proteins with known m/z values	N/A
Instrument	EXENT Analyser	SPAPLUS Analyser Optilite Analyser
Reported results	Semi-quantitative g/L value for the monoclonal element of the sample, m/z value, and isotype of the monoclonal protein	Quantitative g/L value for the total immunoglobulin present in the sample
Analytical measuring range	IgG: 0.308 – 88.9 g/L IgA: 0.073 – 65.9 g/L IgM: 0.054 – 74.2 g/L	Standard dilution ranges: IgG: 1.65 − 35 g/L IgA: 0.20 − 7.00 g/L IgM: 0.2 − 7.5 g/L All dilution ranges: IgG: 0.165 − 140 g/L IgA: 0.02 − 70.00 g/L IgM: 0.1 − 150 g/L
Reference Interval/ isotype-specific cut-off values	Isotype specific cut-off values: IgG: 0.359 g/L (95% CI: 0.000−0.866) IgA: 0.325 g/L (90% CI: 0.133−2.320) IgM: 0.197 g/L (95% CI: 0.123−0.829)	Reference Interval: IgG: 6.103 − 16.16 g/L IgA: 0.845 – 4.990 g/L IgM: 0.35 – 2.42 g/L (95 Percentile Range)

Similarities regarding Kappa/Lambda isotyping of monoclonal immunoglobulins IgG/IgA/IgM are listed in Table 6, including the technological differences between both devices.

Table 6. Technological similarities and differences - Kappa (κ) and Lambda (λ) isotyping of monoclonal immunoglobulins IgG, IgA, IgM.

Similarities		
Item	(Proposed Device) Immunoglobulin Isotypes (GAM) for the EXENT® Analyser (Kappa (κ) and Lambda (λ) isotyping of Monoclonal immunoglobulins IgG, IgA, IgM)	(Predicate Device) HYDRAGEL Immunofixation
Test Type	Kappa (κ)/ Lambda (λ) isotyping of monoclonal IgG/IgA/IgM	same
Process of sample assessment	detection	same
Evaluation by end user	Kappa (κ)/ Lambda (λ) isotyping is defined by the presence of a monoclonal protein peak in the kappa or lambda spectra with a peak m/z value corresponding to a monoclonal peak in either the IgG, IgA or IgM spectra. Peaks are identified automatically by EXENT software, no manual interpretation required	The electropherograms are evaluated visually for the presence of specific reactions with the suspect monoclonal proteins.
Analyte(s)	Kappa and Lambda (+ IgG, IgA, IgM)	same (+ IgG, IgA, IgM)

Indications for Use	Isotyping of monoclonal immunoglobulins (IgG, IgA, IgM + Kappa (κ)/ Lambda (λ)) can 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.	Identifying abnormal bands in serum and urine protein electrophoregrams, primarily those in the beta globulin and gamma globulin zones, are always suspect of being monoclonal proteins (M-proteins, paraproteins, monoclonal immunoglobulins) and therefore, an indication of monoclonal gammopathies.
End user	Professional, prescription use only	same
Sample Type	serum	same (+ urine)
Differences		
Item	(Proposed Device) Immunoglobulin Isotypes (GAM) for the EXENT® Analyser (Qualitative total Kappa/Lambda monoclonal Ig)	(Predicate Device) HYDRAGEL Immunofixation
Test Method	Immunoprecipitation Mass spectrometry	Immunofixation electrophoresis
Reagent Composition	Polyclonal monospecific anti-human kappa and anti-human lambda sheep antibody conjugated to paramagnetic beads, supplied in liquid form	Mammalian immunoglobulins anti-human kappa, and lambda, (free and bound) light chains (ready to use); Antisera segments (ready to use)
Instrument	EXENT Analyser	Hydrasys, Hydrasys 2
Reported results	The presence of monoclonal kappa or lambda peaks associated by mass (m/z) with monoclonal IgG, IgA, or IgM peaks is indicated on the instrument screen.	Qualitative detection for the total immunoglobulin presents in the sample - no results are reported by the device. Results are visually interpreted by a trained operator.
Sample preparation	automatic	manual
Controls	Control fluids containing two levels of human kappa and lambda immunoglobulins in a human serum background are required and supplied.	Controls recommended but not supplied

The differences between the predicate and proposed device do not raise safety or effectiveness questions when used according to the product labelling. Clinical performance testing is part of the submission to support the clinical claims. The submission is supported by successful analytical and clinical performance studies carried out by The Binding Site Ltd.

6.2 Performance Data

6.2.1 Analytical performance

6.2.1.1 Precision/Reproducibility

Precision: The precision was evaluated according to CLSI guideline CLSI EP05-A3:2014 by running five sample levels per analyte across the assay measuring intervals. The 20-day precision study comprised 2 runs per day, with samples run in duplicate, for 20 days using one analyser and one reagent lot (total 80 replicates per sample). The results are summarized in the table below:

Table 7. Repeatability and within-laboratory precision

Precision Summary										
Analyte	Level	N	Mean (g/L)	Within Run		Between Run		Between Day		Total	
				SD	CV%	SD	CV%	SD	CV%	SD	CV%
IgG	1	80	0.271	0.0253	9.3	0.000	0.0	0.0170	6.3	0.0304	11.2
	2	80	0.358	0.0286	8.0	0.000	0.0	0.0223	6.2	0.0362	10.1
	3	80	1.40	0.0711	5.1	0.0439	3.1	0.0716	5.1	0.110	7.9
	4	80	30.6	0.651	2.1	0.411	1.3	0.951	3.1	1.22	4.0
	5	80	102	4.13	4.0	2.83	2.8	5.76	5.6	7.63	7.4
IgA	1	80	0.101	0.00431	4.3	0.00419	4.1	0.00674	6.7	0.00903	8.9
	2	80	0.321	0.0127	4.0	0.0126	3.9	0.0140	4.3	0.0227	7.1
	3	80	0.974	0.0273	2.8	0.0338	3.5	0.0296	3.0	0.0526	5.4
	4	80	27.9	0.728	2.6	0.552	2.0	1.08	3.9	1.41	5.1
	5	80	57.2	2.54	4.4	1.00	1.8	1.48	2.6	3.11	5.4
IgM	1	80	0.0942	0.00458	4.9	0.00319	3.4	0.00799	8.5	0.00975	10.3
	2	80	0.179	0.0108	6.0	0.0114	6.4	0.000	0.0	0.0157	8.7
	3	80	1.73	0.0480	2.8	0.0417	2.4	0.0674	3.9	0.0927	5.4
	4	80	31.2	0.550	1.8	0.226	0.7	0.983	3.2	1.15	3.7
	5	80	71.2	2.93	4.1	2.25	3.2	3.11	4.4	4.83	6.8

Reproducibility: between site, and between lot precision was evaluated according to CLSI guideline CLSI EP05-A3:2014 by running five sample levels per analyte across the assay measuring intervals. 3 runs per day (1 run for each of 3 lots/sites), for 5 days were performed, giving 15 runs in total. 5 replicates of each sample were used per run. (Note: for some samples in the between instrument study, more than 75 replicates are included in the final dataset. Additional replicates were assayed and are valid results and as such have been included in the dataset). The results are summarized in the **Table 8** below:

Table 8. Between-site and between-lot precision

Level	N	Mean (g/L)	SD	%CV
IgG between site precision				
1	75	0.352	0.0358	10.2
2	75	0.452	0.0557	12.3
3	80	1.25	0.151	12.0
4	78	29.7	2.12	7.1
5	75	80.5	7.12	8.8
IgA between site precision				
1	75	0.114	0.0095	8.4
2	75	0.368	0.0299	8.1
3	80	0.82	0.0723	8.8
4	78	24.8	3.55	14.3
5	75	56.2	2.63	4.7
IgM between site precision				
1	75	0.126	0.0098	7.7
2	75	0.172	0.0093	5.4
3	77	1.47	0.0950	6.3
4	80	31.0	1.58	5.1
5	75	67.6	6.84	10.1
IgG between lot precision				
1	75	0.309	0.0109	3.5
2	75	0.405	0.00930	2.3
3	75	1.09	0.0875	8.0
4	75	27.0	1.94	7.2
5	75	85.2	0.0777	0.9
IgA between lot precision				
1	75	0.110	0.0143	12.7
2	76	0.339	0.0200	5.9
3	75	0.743	0.0762	10.3
4	75	22.7	2.24	9.9
5	75	60.9	2.54	4.3
IgM between lot precision				
1	75	0.098	0.00338	1.9

2	75	0.177	0.0070	4.0
3	75	1.33	0.0898	6.7
4	75	28.3	2.86	10.1
5	75	71.9	0.000	0.0

6.2.1.2 Linearity/assay reportable range

The linearity was evaluated in accordance to CLSI EP06 ED2:2020 by testing a series of 11 dilutions per isotype created using high and low M-protein pools. Each level was run in 4 replicates for each isotype. The absolute or relative deviation from the linear regression analysis was used to assess the non-linearity at each point in each dilution series. Analysis confirms the results demonstrate linearity over the following ranges:

Table 9. Linearity range.

Analyte	Linear Range (g/L)
IgG	0.014 – 98.4
IgA	0.011 – 69.1
IgM	0.011 – 80.5

6.2.1.3 Detection Limit

6.2.1.3.1 Analytical sensitivity – LLMI

The analytical sensitivity of the assay (LLMI) was determined according to CLSI guideline C62A:2014 which advises testing lower limit of measuring interval (LLMI) using the study design for Limit of Quantitation laid out in EP17-A2:2012. 4 samples for each monoclonal immunoglobulin (IgG, IgA and IgM) were assayed 4 times per run over 5 days using 2 lots of reagents (i.e., 80 replicates per lot).

The statistical test for the study was functional sensitivity, i.e., the lowest level that could be reported with a %CV that met the pre-defined acceptance criteria of ≤20% for IgG, ≤20% for IgA and ≤20% for IgM.

The LLMI for IgG, IgA and IgM M-proteins was defined in serum samples with a normal concentration level of involved polyclonal immunoglobulins, as follows:

Table 10. LLMI in serum samples with a normal concentration level of polyclonal immunoglobulins (IgG, IgA, and IgM).

Isotype	LLMI (g/L)
IgG	0.308
IgA	0.073
IgM	0.054

6.2.1.4 Analytical specificity - Interference

Interference from endogenous or exogenous substances was assessed according to CLSI guidelines EP07 ED3:2018, EP37 ED1:2018, C62-A:2014, and CLSI EP12 ED3:2023, by testing serum samples with or without M-protein spiked with either the interferent or with the corresponding blank solution. Interference testing was conducted by paired-difference testing: samples with the interferent (spiked) and without the interferent (blank) were measured, and the concentration difference or presence/absence of M protein was determined.

No significant interference was observed by the following substances:

Table 11. List and concentration of interferents tested.

Interfering Substance	Interferent Concentration
Triglyceride	1500 mg/dL
Intralipid	2000 mg/dL
*Rheumatoid factor	500 IU/mL for IgA and IgM and 200 IU/mL for IgG
Albumin	60 g/L
Bilirubin unconjugated	400 mg/L
Haemoglobin	10 g/L
Acetaminophen	156 mg/L
Ascorbic acid	52.5 mg/L
Caffeine	108 mg/L
Cimetidine	30 mg/L
Cyclophosphamide monophosphate	55 mg/dL
Penicillin	80 mg/L
Theophylline	60 mg/L
Acetylsalicylic acid	30 mg/L
Bortezomib	0.0612 mg/dL
Digoxin	0.039 mg/L
Ibuprofen	219 mg/L
Phenytoin	60 mg/L
Pomalidomide	100 ug/mL
Prednisolone	1.2 mg/L

*A sample with 1.3 g/L of monoclonal IgG showed >0.2 g/L interference by 500 IU/mL rheumatoid factor (RF). Information in the instructions for use was included: sample with an RF concentration greater than 200 IU/mL should be retested with an alternative method.

6.2.1.5 Interference - therapeutic monoclonal antibodies (tmAbs)

The tmAb interference testing was performed according to CLSI guidelines EP07 ED3:2018, and C62-A:2014. Four individual IgG samples were used for the testing of the therapeutic monoclonal antibodies (daratumumab, isatuximab, and elotuzumab), giving 12 unique IgG samples. The patient samples containing monoclonal IgG were chosen that had higher and lower m/z's compared to the therapeutic antibodies and were as close in m/z to the therapeutic antibodies as possible.

The study demonstrated that the minimum required separation between daratumumab, isatuximab, elotuzumab and patient clone is 19.6 m/z, 22.9 m/z, and 28.3 m/z, respectively.

Table 12. Interference summary of results.

tmAb (interferent)	Sample	tmAb concentration	m/z difference
Daratumumab	IgGk	0.5 g/L	-19.6
Isatuximab	IgGk	0.5 g/L	22.9
Elotuzumab	IgGk	0.5 g/L	28.3

6.2.2 Clinical Performance

Residual serum samples from patients with monoclonal gammopathies relevant to clinical claims in the intended use of the assay, from patients without the disease of interest (disease control patients), and from apparently healthy subjects were tested at three sites. In addition, samples from a group of patients with leukaemia/lymphoma (primarily B cell lineage malignancies) and from patients with infections were tested for cross-reactivity. The clinical studies aimed to establish the clinical sensitivity and specificity performance of the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay, as well as to establish expected results in the normal population and to assess cross-reactivity. Isotype agreement and quantitative agreement for M protein concentrations between the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay and those reported by routine patient care (based on serum protein electrophoresis, SPE, and immunofixation electrophoresis, IFE) were also assessed.

6.2.2.1 Clinical Sensitivity and Specificity

Clinical sensitivity and specificity were assessed on a cohort of 1343 patients (after exclusions), which consisted of 688 patients with monoclonal gammopathies (MGUS, SMM, MM, WM and AL amyloidosis), and 655 disease controls. Clinical sensitivity and specificity were based on clinical diagnosis as truth. The composition of the monoclonal gammopathy cohort was reflective of the demographics (age, gender, ethnicity), prevalence and isotype distribution of the various monoclonal gammopathies in the general population. The median age (range) in the monoclonal gammopathy cohort was 67 (31-94) years, with a female:male ratio of 340:348. The isotype distribution (considering all monoclonal gammopathies) included 387 IgG, 115 IgA, 110 IgM, and 66 biclonal patients. Samples from patients with IgD and light chain only disease were excluded from the calculations. The clinical composition of the cohorts are shown in **Table 13** and **Table 14**.

Table 13. Diagnostic Sensitivity - Composition of the monoclonal gammopathy group.

Monoclonal gammopathy	Number	%
MM (multiple myeloma)	180	26.2
SMM (smouldering multiple myeloma)	140	20.3
MGUS (monoclonal gammopathy of undetermined significance)	227	33.0
WM* (Waldenström's macroglobulinaemia)	64	9.3
AL amyloidosis	77	11.2
Total	**688**	**100.0**

*Including one smouldering WM patient

Table 14. Composition of the disease control group used for the diagnostic specificity calculations.

Disease Control	Number	%
Bone pain / bone disease	40	6.1
Cancer	52	7.9
Chronic inflammation	65	9.9
Chronic liver disease	30	4.6
Cardiovascular disease	80	12.2
Diabetes	54	8.2
Fatigue	10	1.5
Haematological disease / anaemia	59	9.0
Hypertension	37	5.6
Immune system / Autoimmune disease	56	8.5
Kidney disease	98	15.0
Neurological disorder	46	7.0
Other	28	4.3
Total	**655**	**100**

Results were categorized as clinical positive or negative based on the clinical diagnosis (monoclonal gammopathy or other diagnosis), and as test positive or negative based on Immunoglobulin Isotypes (GAM) for the EXENT® Analyser assay results. A positive test result was defined as an IgG, IgA or IgM M-protein at a concentration of ≥ clinical cut-off.

- **Diagnostic sensitivity** was calculated as: Sensitivity = TP/(TP+FN)

Diagnostic sensitivity was: 92.1% (87.8-94.9) for MGUS, 98.6% (94.9-99.6) for SMM, 94.4% (90.1-97.0) for MM, 98.4% (91.7-99.7) for WM, and 89.6% (80.8-94.6) for AL.

- **Diagnostic specificity** was calculated as: Specificity= TN/(FP + TN)

The diagnostic specificity of the Immunoglobulin Isotypes (GAM) for the EXENT® Analyser was 87.8% (85.1 – 90.1).

Results are presented in **Table 15**.

Table 15. Diagnostic sensitivity and specificity.

Monoclonal gammopathy (n)	Diagnostic sensitivity % (95% CI)	Diagnostic specificity % (95% CI)
MGUS (n=227)	92.1 (87.8-94.9)	**All:** 575/655 87.8% (85.1−90.1%) **SPE/FLC(+)**: 57/80 71.3% (60.5−80.0%) **SPE/FLC(−)**: 270/294 91.8% (78.7−84.1%)
SMM (n=140)	98.6 (94.9-99.6)	
MM (n=180)	94.4 (90.1-97.0)	
WM (n=64)	98.4 (91.7-99.7)	
AL amyloidosis (n=77)	89.6 (80.8-94.6)	
Total (n=688)	**94.3 (92.3-95.8)**	

Based on the clinical performance study the device demonstrates same or better diagnostic sensitivity compared to standard electrophoretic methods.

6.2.2.2 Cross-reactivity

The cross-reactivity cohort included patients with leukaemia/lymphoma (primarily B cell lineage malignancies) and from patients with infectious etiologies. B-cell malignancies and diseases associated with the stimulation of the immune system (infections) are expected to have a higher M-protein positivity rate than apparently healthy subjects or patients with other conditions, even though they do not have monoclonal gammopathy.

Table 16. The clinical composition of the cross-reactivity cohort.

Cross-reactivity cohort	Number	%
Infectious etiologies	47	32.6
Leukaemias/Lymphomas	97	67.4
Total	**144**	**100**

Positivity rate by the Immunoglobulin Isotypes (GAM) for the EXENT® Analyser assay (presence of intact immunoglobulin M proteins that are ≥ isotype-specific cut-off) is shown in **Table 17**.

Table 17. M-protein positivity rate in the cross-reactivity cohort.

Cross-reactivity cohort	Number	(%)
Leukaemia/lymphoma (primarily B cell) (n=97)	29	29.9
Infectious etiologies (n=47)	4	8.5
Total (n=144)	**33**	**22.9**

6.2.2.3 Comparison of M protein isotype with current standard of care (IFE)

Isotype of M proteins determined by the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay was compared to M-protein isotypes reported as part of routine patient care in the monoclonal gammopathy cohort. Only intact immunoglobulin M proteins that were ≥ each isotype-specific cut-off by the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay were considered for isotype comparison; biclonal samples were excluded.

M protein isotype comparison was studied in 531 samples with detectable M-protein with routine SPE. Total agreement was calculated for each isotype (IgG, IgA, IgM), for each disease group separately, and on the pooled data. Results are shown in Table 18.

Table 18. M protein isotype agreement between Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay results and current standard of care in samples with detectable M-protein with routine SPE, all diagnosis groups combined.

Number of samples	% Total Agreement
All samples (n=580)	95.3
IgG (n=376)	97.1
IgA (n=101)	96.0
IgM (n=102)	89.2
MM (all isotypes, n=156)	98.7
MGUS (all isotypes, n=188)	92.0
SMM (all isotypes, n=116)	97.4
WM (all isotypes, n=60)	95.0
AL amyloidosis (all isotypes, n=60)	93.3

In addition, a separate analysis was performed on all samples (SPE positive and SPE negative, n=618). The isotype concordance (total agreement) on all samples was 93.7%.

6.2.2.4 Comparison of M protein concentration with current standard of care (SPE)

The comparison study included samples with detectable and quantifiable IgG, or IgA or IgM M-proteins with routine SPE. M protein concentrations generated by the Immunoglobulin Isotypes (GAM) for the EXENT® Analyser assay (only M-proteins with concentration ≥ isotype specific cut-off) was compared to M protein concentrations by SPE using Passing Bablok regression for each M protein isotype and each diagnosis group, with concentration reported by routine patient care as reference.

In addition, predicted bias was calculated at the following concentrations: at the Lower Limit of the Measuring Interval (LLMI), at the isotype specific clinical cut-off, at 10 g/L and 30 g/L and at the Upper Limit of the Measuring Interval (ULMI) for each isotype.

Results for M protein quantitative comparisons are presented in **Table 19** and **Table 20.**

Table 19. Summary of M protein concentration comparison.

	Number of data points	Passing Bablok	
		Slope (95% CI)	Intercept (95% CI)
All samples	571	1.13 (1.08 to 1.17)	-0.80 (-1.21 to -0.52)
IgG	361	1.10 (1.06 to 1.15)	-0.86 (-1.25 to -0.46)
IgA	88	0.91 (0.84 to 1.05)	-0.48 (-1.45 to 0.19)
IgM	93	1.54 (1.42 to 1.73)	-1.16 (-2.80 to -0.48)
MM (all isotypes)	152	1.12 (1.01 to 1.20)	-1.55 (-2.94 to -0.24)
SMM (all isotypes)	112	1.09 (1.00 to 1.18)	-1.83 (-3.54 to -0.52)
MGUS (all isotypes)	165	1.30 (1.18 to -1.42)	-1.21 (-1.90 to -0.74)
WM (IgM)	58	1.57 (1.43 to 1.80)	-1.97 (-4.54 to -0.19)
AL amyloidosis (all isotypes)	55	1.07 (0.95 to -1.18)	-0.49 (-1.24 to 0.42)

Table 20. Predicted bias at medical decision levels.

Isotype	At concentration (g/L)	Predicted bias (%)	Predicted bias (g/L)
IgG	0.308	N/A	-0.833
	0.359	N/A	-0.827
	10.0	1.6%	N/A
	30.0	7.4%	N/A
	88.9	9.3%	N/A
IgA	0.073	N/A	-0.490
	0.325	N/A	-0.512
	10.0	-13.6%	N/A
	30.0	-10.4%	N/A
	65.9	-9.5%	N/A
IgM	0.054	N/A	-1.127
	0.197	N/A	-1.049
	10.0	42.9%	N/A
	30.0	50.6%	N/A
	74.2	52.9%	N/A

Immunonephelometry and immunoturbidimetry produces a concentration for monoclonal IgM that is known to be systematically higher than M protein values determined by SPE and densitometry [4, 5]. EXENT measurements of M proteins uses Optilite total immunoglobulin values in the calculation algorithm, therefore, it is expected that a similar bias is seen between the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay IgM M protein and SPE.

6.2.2.5 Cut-off

A multi-ethnic population of 364 apparently healthy subjects (176 female, 188 male, median age 57 years) was tested to determine the clinical cut-off for IgG, IgA and IgM M proteins.

All M proteins detected by the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay in this cohort were taken into account, and the isotype specific cut-off values were established at the 95th percentile reference limit for IgG and at the 99th percentile reference limit for IgA and IgM, with 90% or 95% Confidence Interval (CI), using the non-parametric quantile method. The 95th percentile for IgG and the 99th percentile for IgA and IgM were chosen based on published literature, consistently reporting about 70% prevalence for IgG MGUS, with the rest consisting of IgM, IgA and bi-clonal MGUS [6-9].

Table 21. Isotype specific cut-off values.

Isotype	Limit	Cut-off Value (95% CI) (g/L)
IgG	95th percentile	0.359 (0.000-0.866)
IgA	99th percentile	0.325 (0.133-2.320)*
IgM	99th percentile	0.197 (0.123-0.829)

*90% CI

IgG, IgA or IgM M proteins with a concentration of ≥ the cut-off are considered a positive finding in the setting of confirming positive screening (SPE and/or FLC) test results.

6.2.2.6 Expected results in the normal population

An M protein (any type and concentration) was detected by the Immunoglobulin Isotypes (GAM) for the EXENT Analyser assay in 216 out of 364 apparently healthy subjects (59.3%): one M protein in 118 (32.4%), and more than one in 98 (26.9%) subjects. The majority of these M proteins were below the LLMI or below the isotype-specific cut-off value.

Samples with one or more M proteins ≥ the isotype specific cut-off are listed in **Table 22**.

Table 22. Prevalence of samples with positive Immunoglobulin Isotypes (GAM) for the EXENT® Analyser assay result and their isotype distribution in the apparently healthy cohort (n=364).

Isotype breakdown	Number (%) of samples
IgG	15 (4.1%)
IgG + IgM	1 (0.3%)
IgG + IgG	1 (0.3%)
IgA	3 (0.8%)
IgM	3 (0.8%)
Total	**23 (6.3%)**

The expected result in the normal population is no monoclonal protein detected. Using the isotype-specific cut-off values as described in **Table 21**, the number (and %) of apparently healthy subjects with at least one intact immunoglobulin M protein above the cut-off or total Kappa and total Lambda was 23 out of 364 (6.3%).

7 CONCLUSION

The completed analytical and clinical performance studies support substantial equivalence, the clinical claims of the device, and its safety and effectiveness in this regard when used according to product labeling.

8 BIBLIOGRAPHY

1. Cox, M. C., Esposito, F., Postorino, M., Venditti, A. & Dl Napoli, A. 2023. Serum Paraprotein Is Associated with Adverse Prognostic Factors and Outcome, across Different Subtypes of Mature B-Cell Malignancies-A Systematic Review. Cancers (Basel), 15.
2. Witzig, T. E., Maurer, M. J., Habermann, T. M., Link, B. K., Micallef, I. N., Nowakowski, G. S., Ansell, S. M., Colgan, J. P., Inwards, D. J., Porrata, L. F., Markovic, S. N., Johnston, P. B., Lin, Y., Thompson, C., Gupta, M., Katzmann, J. A. & Cerhan, J. R. 2014. Elevated monoclonal and polyclonal serum immunoglobulin free light chain (FLC) as prognostic factors in B- and T-cell non-Hodgkin lymphoma. Am J Hematol.
3. Murray DL, Puig N, Kristinsson S, Usmani SZ, Dispenzieri A, Bianchi G, Kumar S, Chng WJ, Hajek R, Paiva B, Waage A, Rajkumar SV, Durie B. Mass spectrometry for the evaluation of monoclonal proteins in multiple myeloma and related disorders: an International Myeloma Working Group Mass Spectrometry Committee Report. Blood Cancer J. 2021;11(2):24
4. Murray, D.L. *et al.* (2009) Quantitation of serum monoclonal proteins: relationship between agarose gel electrophoresis and immunonephelometry. *Clin Chem* 55, 1523-1529. clinchem.2009.124461 [pii] 10.1373/clinchem.2009.124461
5. Riches, P.G. *et al.* (1991) Overestimation of monoclonal immunoglobulin by immunochemical methods. *Ann Clin Biochem* 28 (Pt 3), 253-259
6. Kyle, R.A. et al. (2018) Long-term follow-up of monoclonal gammopathy of undetermined significance. N Engl J Med 378, 241-249. 10.1056/NEJMoa1709974

7. Rajkumar, S.V. et al. (2005) Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. Blood 106, 812-817. 2005-03-1038 [pii] 10.1182/blood-2005-03-1038;
8. Landgren, O. et al. (2019) Association of Immune Marker Changes With Progression of Monoclonal Gammopathy of Undetermined Significance to Multiple Myeloma. JAMA Oncol 5, 1293-1301. 10.1001/jamaoncol.2019.1568
9. Vachon, C.M. et al. (2022) Prevalence of Heavy Chain MGUS by Race and Family History Risk Groups Using a High Sensitivity Screening Method. Blood Adv. 10.1182/bloodadvances.2021006201

END OF SUMMARY