January 24, 2024

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

Re: K231290
Trade/Device Name: Optilite Freelite Kappa Free Kit
Optilite Freelite Lambda Free Kit
Regulation Number: 21 CFR 866.5550
Regulation Name: Immunoglobulin (light chain specific) immunological test system
Regulatory Class: Class II
Product Code: DFH, DEH
Dated: December 21, 2023
Received: December 22, 2023

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 (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 located 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.

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 803) for devices or postmarketing safety reporting (21 CFR 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 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 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)*
K231290

Device Name
Optilite Freelite Kappa Free Kit
Optilite Freelite Lambda Free Kit

Indications for Use *(Describe)*
The Optilite Freelite Kappa Free Kit is intended for the quantitative in vitro measurement of Kappa free light chains in serum using the Binding Site Optilite analyser. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE), and aids in the evaluation of monoclonal gammopathy of undetermined significance (MGUS). Results of the free light chain measurements should always be interpreted in conjunction with other laboratory and clinical findings.

The Optilite Freelite Lambda Free Kit is intended for the quantitative in vitro measurement of Lambda free light chains in serum using the Binding Site Optilite analyser. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE), and aids in the evaluation of monoclonal gammopathy of undetermined significance (MGUS). Results of the free light chain measurements should always be interpreted in conjunction with other laboratory and clinical findings.

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.

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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:	K231290
Type of 510(k):	Original, Traditional 510(k)
Purpose of Submission:	Modification to a previously cleared device
Date of Preparation:	26 April 2023, revised on 23rd January 2024

1 SUBMITTER / APPLICANT:

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

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

2 DEVICE INFORMATION:

Proprietary Name:	Optilite® Freelite® Kappa Free Kit Optilite® Freelite® Lambda Free Kit
Measurand:	Kappa (κ) free light chains (FLC) Lambda (λ) free light chains (FLC)
Type of Test:	Quantitative, immunoturbidimetry

Regulatory information:

Regulation section:	21 CFR 866.5550, Immunoglobulin (light chain specific) immunological test system
Classification:	Class II
Product Code(s):	DFH – Kappa antigen, antiserum, control DEH – Lambda antigen, antiserum, control
Review Panel:	IM - Immunology (82)

3 PREDICATE DEVICES AND 510(k) NUMBERS

Freelite Human Kappa Free Kit for use on Roche Cobas Integra 400/400 plus - K070900
Freelite Human Lambda Free Kit for use on Roche Cobas Integra 400/400 plus - K070900

4 DEVICE DESCRIPTION

4.1 Test Principle

No modification is made to the principle of operation for the Optilite® Freelite® Kappa and Lambda Free Kits cleared in K150658.

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4.2 Special conditions for use statement(s):

Prescription use only.

The kappa free light chain results for a given specimen determined with assays from different manufacturers or on different systems can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the kappa free light chain assay used. Values obtained with different assays or systems cannot be used interchangeably. If, in the course of serially monitoring a patient, the assay or system used for determining kappa free light chain levels is changed, additional sequential testing should be carried out. Prior to changing assay or system, the laboratory MUST confirm baseline values for patients being serially monitored.

Interpretation of results:
Guidelines and consensus recommendations for the diagnosis and monitoring of multiple myeloma and AL amyloidosis have been published by the International Myeloma Working Group (IMWG) and National Cancer Comprehensive Network (NCCN). Evaluation of patients with MGUS is also included in guidelines published by IWMWG and NCCN. These include FLC testing, with the reference values for FLC based on Freelite® assay results. Guidelines are included in the product IFU bibliography; however, clinicians should refer to the most current versions available as they may be updated.

Limitations:
• Turbidimetric assays are not suitable for measurement of highly lipaemic or haemolyzed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

• Diagnosis cannot be made and treatment must not be given on the basis of kappa free light chain measurements alone. Clinical history and other laboratory findings must be taken into account.
• This assay has not been established for use with the paediatric population.

4.3 Special Instrument requirements

Optilite® Analyser

4.4 Kit Reagents and composition

The devices in this submission have not materially changed since originally cleared under K150658.

Materials provided in the Optilite Freelite Kappa Free kit:

- Optilite Kappa Free Reagent
- Optilite Kappa Free Calibrator
- Optilite Kappa Free High Control
- Optilite Kappa Free Low Control

Materials provided in the Optilite Freelite Lambda Free kit:

- Optilite Lambda Free Reagent
- Optilite Lambda Free Calibrator
- Optilite Lambda Free High Control
- Optilite Lambda Free Low Control

Reagents composition:

- *Latex Reagent*: Consisting of polyclonal monospecific antibody coated onto polystyrene latex. Supplied in stabilised liquid form. Preservatives: 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine, 0.05% ProClin.
- *Calibrator and Controls*: Pooled human serum, supplied in stabilised liquid form. Containing 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.
- *Reaction Buffer*: Containing 0.099% sodium azide as a preservative.

Note: In Optilite Freelite kits, the latex reagent and reaction buffer are supplied in a single wedge with a chamber for each fluid. They are therefore labelled as a single component *Optilite Kappa Free Reagent* or *Optilite Lambda Free Reagent*.

5 INTENDED USE/ INDICATIONS FOR USE:

5.1 Intended use:

Same as Same as indications for use.

5.2 Indications for use:

The Optilite® Freelite® Kappa Free Kit is intended for the quantitative in vitro measurement of Kappa free light chains in serum using the Binding Site Optilite® analyser. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE), and aids in the evaluation of monoclonal gammopathy of undetermined significance (MGUS). Results of the free light chain measurements should always be interpreted in conjunction with other laboratory and clinical findings.

The Optilite® Freelite® Lambda Free Kit is intended for the quantitative in vitro measurement of Lambda free light chains in serum using the Binding Site Optilite® analyser. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE), and aids in the evaluation of monoclonal gammopathy of undetermined significance (MGUS). Results of the free light chain measurements should always be interpreted in conjunction with other laboratory and clinical findings.

5.3 Summary and Explanation:

Immunoglobulin molecules consist of two identical heavy chains (α, μ, γ, δ or ε) which define the immunoglobulin class and two identical light chains (κ or λ). Each light chain is covalently linked to a heavy chain and the two heavy chains are linked covalently at the hinge region. In healthy individuals, the majority of light chain in serum exists in this form, bound to heavy chain. However, low levels of free light chain (FLC) are found in serum of normal individuals due to the over-production and secretion of FLC by the plasma cells. Whilst the molecular weight of both light chains is ≈22.5kD, in serum κ free light chain
(κ- FLC) exists predominantly as monomer and λ free light chain (λ-FLC) as a covalently linked dimer with a molecular weight of ≈45kD. This will lead to a differential glomerular filtration rate for κ-FLC and λ-FLC and may explain the observed ratio of κ-FLC to λ-FLC of 0.625 in serum compared to the ratio of bound κ to λ of 2.0.
Elevated serum levels of monoclonal FLC are associated with malignant plasma cell proliferation (e.g. multiple myeloma), AL amyloidosis and, light chain deposition disease and MGUS. Raised serum levels of polyclonal FLC may be associated with autoimmune diseases such as SLE.

6 TECHNOLOGICAL CHARACTERISTICS:

Both the subject and predicate devices contain the same intended use with respect to the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE). The purpose of this submission is to extend these claims to add an aid in evaluation of Monoclonal Gammopathy of Undetermined Significance (MGUS) to the intended use of the Optilite® Freelite® Kappa and Lambda Free Kits. Although, the predicate devices cited in this submission do not contain the evaluation of

MGUS claim in their instructions for use, such is supported by the body of peer revied literature as relevant extension to the clinical application of the Freelite assays; and it is considered appropriate to support the determination of substantial equivalence with an assessment of clinical performance study. Refer to section 6.3 for the summary of the clinical performance study pertinent to the MGUS extension claim. The extension of the clinical claims for the Optilite® Freelite® Kappa and Lambda Free Kits does not affect the safety and effectiveness of the devices relative to the predicate.

6.1 Similarities and Differences to the Predicate:

A comparison of the similarities and differences between the proposed Optilite Freelite Kappa and Lambda Free Kits and the predicate Freelite Human Kappa and Lambda Free Diagnostic test Kits for use on Roche Cobas Integra 400/400 plus, provided in Table 1 as follows:

Table 1. Technological similarities and differences.

Similarities		
Item	**(Proposed Device)** **Optilite Freelite Kappa and Lambda Free Kits (K150658)**	**(Predicate Device)** **Freelite Human Kappa and Lambda Free Kits for use on Roche Cobas Integra 400/400 plus (K070900)**
Assay type	Quantitative	same
Test method	Turbidimetry	same
Detection antibody	Kappa: Polyclonal sheep anti-human Kappa antibody coated onto latex particles Lambda: Polyclonal sheep anti-human Lambda antibody coated onto latex particles	same
Open Vial Stability	3 months	same
Adult Reference Interval	Kappa: 3.30 – 19.40mg/L Lambda: 5.71 – 26.30mg/L Ratio: 0.26 – 1.65mg/L	same
Specimen Type	Serum	same
Calibrator Traceability	Internal Reference Master Calibrator	same
Sample dilutions (Kappa)	1+1, 1+9, 1+99, 1+999, 1+4999	same (First 4 dilutions only)
Sample dilutions (Lambda)	1+1, 1+7, 1+79, 1+799, 1+7999	same
Measuring range (Kappa)	0.6 – 25.3mg/L (1+1) 2.9 – 127mg/L (1+9) 29 – 1270mg/L (1+99) 290 – 12700mg/L (1+999) 1450 – 63500mg/L (1+4999)	same (First 4 dilutions only)
Measuring range (Lambda)	1.3 – 34.7mg/L (1+1) 5.2 – 139mg/L (1+7) 52 – 1390mg/L (1+79) 520 – 13900mg/L (1+799) 5200 – 139000mg/L (1+7999)	same

Differences		
Item	**(Proposed Device)** **Optilite Freelite Kappa and Lambda Free Kits (K150658)**	**(Predicate Device)** **Freelite Human Kappa and Lambda Free Kits for use on Roche Cobas Integra 400/400 plus (K070900)**
Intended use	**Kappa:** The Optilite **Freelite** Kappa Free Kit is intended for the quantitative *in vitro* measurement of Kappa free light chains in serum using the Binding Site Optilite analyser. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE), and aids in the evaluation of monoclonal gammopathy of undetermined significance (MGUS). Results of the free light chain measurements should always be interpreted in conjunction with other laboratory and clinical findings. **Lambda:** The Optilite **Freelite** Lambda Free Kit is intended for the quantitative *in vitro* measurement of Lambda free light chains in serum using the Binding Site Optilite analyser. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE), and aids in the evaluation of monoclonal gammopathy of undetermined significance (MGUS). Results of the free light chain measurements should always be interpreted in conjunction with other laboratory and clinical findings.	**Kappa:** This kit is intended for the quantitation of kappa free light chains in serum on the Roche Cobas Integra 400, 400plus and 800. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings. **Lambda:** This kit is intended for the quantitation of Lambda free light chains in serum on the Roche Cobas Integra 400 / 400plus and 800. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.
On-board stability	30 days	3 months
Instrument	Optilite analyser	Roche Cobas Integra
Top Dilution (Kappa)	1+4999	1+9999
Measuring range (Kappa)	(Top Dilution) 1450 – 63500mg/L (1+4999)	(Top Dilution) 2900 – 127000mg/L (1+9999)

This submission is to add a claim for evaluation of MGUS to the intended use statement. The differences between the predicate and proposed device do not result in a change to the safety and efficacy when used according to the product labeling.

6.2 Performance Data:

Performance characteristics data is provided for the extended indication for evaluation of Monoclonal Gammopathy of Undetermined Significance (MGUS). Refer to submission K150658 for previously documented analytical performance studies:

- Precision/Reproducibility
- Linearity/assay reportable range
- Traceability
- Stability
- Detection Limit
- Analytical Specificity / Interferences
- Antigen excess/Prozone detection
- Reference Interval/ Expected values, and clinical cut off
- Method Comparison studies with predicate device which included samples with relevant admission diagnosis to the intended use (including multiple myeloma, Waldenström's Macroglobulinemia, lymphocytic neoplasms and systemic lupus erythematosus).

6.3 Performance data for evaluation of Monoclonal Gammopathy of Undetermined Significance (MGUS)

MGUS is a plasma cell dyscrasia characterised by the presence of a monoclonal protein (M-protein) in the serum of asymptomatic individuals who do not meet the diagnostic criteria for multiple myeloma (MM), AL amyloidosis, Waldenström's macroglobulinaemia (WM), lymphoproliferative disorders, plasmacytoma or related conditions. [1]

The expansion of the claims to add an aid in the evaluation of Monoclonal Gammopathy of Undetermined Significance (MGUS) to the Optilite Freelite Kappa and Lambda Free Kits is supported by the body of peer reviewed literature and investigated in the retrospective clinical performance studies carried out by The Binding Site Ltd.
The clinical performance studies included retrospective testing of residual samples from patients with clinically confirmed MGUS and from disease control subjects (non-MGUS patients) with the Optilite® Freelite® Kappa Free Kit, and the Optilite® Freelite® Lambda Free Kit, and assessing the concordance of the test results with the clinical truth of the patient. The testing investigated the clinical/diagnostic performance (sensitivity and specificity) of Freelite test results in MGUS and disease controls (non-MGUS) at single time points (Study 1); and the monitoring performance of Freelite test results measured on serial samples from patients with stable and progressive MGUS (Study 2).

6.3.1 Clinical Performance: Sensitivity and Specificity (Study 1)

6.3.1.1 <u>Sensitivity</u>

Freelite Kappa:Lambda Ratio Sensitivity in MGUS

A retrospective study was performed using a total of 234 MGUS samples from patients with clinically confirmed MGUS. The clinical diagnostic criteria that the clinicians used to establish the clinical truth of all samples included in the study as 'MGUS positive' was confirmed with each site. The diagnostic criteria and classification for MGUS and related plasma-cell disorders, as practiced clinically, fulfilled, but was not limited to, the criteria outlined by the 'International Myeloma Working Group (IMWG)' consensus. The diagnostic criteria in the

clinical study are aligned with actual clinical practices widely accepted and used in the U.S. for the target patient population. All samples were tested for free light chain (FLC) kappa and lambda levels with the Optilite Freelite Kappa and Lambda Free kits on the Optilite Analyser. The kappa:lambda ratios were calculated for each sample. The result of the device was compared to the clinical diagnosis for each sample.

Results have been categorized as clinical positive or negative based on the clinical diagnosis and evaluated as test positive and negative based on the results of the Freelite testing performed during the study. The test result for MGUS positive or negative were based on the following definitions:

- Test positive (Freelite Positive = Abnormal): FLC kappa:lambda ratio was outside the reference interval (0.26-1.65) for intact immunoglobulin MGUS; the FLC kappa:lambda ratio was outside the reference interval (0.26-1.65) and the involved FLC level was above the reference interval (kappa 3.30-19.40 mg/L and lambda 5.71-26.30 mg/L) for LC-MGUS.

- Test negative (Freelite Negative = Normal): FLC kappa:lambda ratio was within the reference interval (0.26-1.65).

Light chain MGUS (LC-MGUS), is a subset of MGUS in which the monoclonal protein produced consists of only immunoglobulin free light chains. The published definition of LC-MGUS is *"an abnormal FLC ratio with complete lack of IgH expression, plus elevation in the appropriate involved FLC"* [2], and was evaluated as part of this study to demonstrate the capability of the Freelite Kappa and Lambda Free assays to detect this subgroup of MGUS patients.

Sensitivity with 95% confidence interval has been summarized in Table 2 below:

Table 2. Summary Table - Freelite kappa:lambda Ratio Sensitivity.

Disease group	N =	Obtained Sensitivity (%) [95% CI]
All MGUS positive	234	59.4 [53.0 – 65.5]
Light chain MGUS	12	100.0 [75.8 – 100]
Non-light chain only MGUS	222	57.2 [50.6 – 63.5]

The pre-defined acceptance criterium for sensitivity in MGUS was set to at least 30 % which was met:

- The calculated sensitivity for all MGUS samples in the study was 59.4% (95% CI: 53.0 – 65.5%). This was the % of all MGUS samples determined as positive based on Optilite Freelite FLC testing, i.e., show a FLC kappa:lambda ratio outside the reference interval (0.26-1.65) for intact immunoglobulin MGUS; or a FLC kappa:lambda ratio outside the reference interval (0.26-1.65) with the involved FLC level above the reference interval

(kappa 3.30-19.40 mg/L and lambda 5.71-26.30 mg/L) for LC-MGUS.

- The sensitivity for the 12 LC-MGUS samples was 100% (95% CI: 75.8 – 100%).
- The sensitivity for the 222 non-LC-MGUS samples was 50.6% (95% CI: 50.6 – 63.5%).

Freelite Kappa:Lambda Ratio sensitivity in MGUS by isotype

The cohort of 234 MGUS samples included: 174 non-IgM MGUS, 24 IgM MGUS, 24 biclonal,12 light chain MGUS. The distribution of the cohort with confirmed M component isotype in MGUS samples and the number and percentage of test positive samples are summarised in Table 3 as follows:

Table 3. MGUS isotype, number in study and abnormal by Freelite®.

MGUS type	N	n (n/N%) Freelite positive
Non-IgM MGUS (all)	**174**	**101 (58.0%)**
IgG κ	74	52 (70.3%) >1.65
IgG λ	62	19 (30.6%) <0.26 5 (8.1%) >1.65 24 (38.7%) total FLC abnormal
IgA κ	21	15 (71.4%) >1.65 1 (4.8%) <0.26 16 (76.2%) total FLC abnormal
IgA λ	15	9 (60.0%) <0.26
IgD κ	0	0
IgD λ	0	0
λ band (not free light chain) with no obvious corresponding heavy chain*	2	0
IgM MGUS (all)	**24**	**10 (41.7%)**
IgM κ	19	9 (47.4%) >1.65
IgM λ	5	1 (20.0%) <0.26
LC-MGUS (all)	**12**	**12 (100%)**
κ	8	8 (100%)
λ	4	4 (100%)
Biclonal	**24**	**3 (12.5%) <0.26** **13 (54.2%) >1.65** **16 (66.7%) total abnormal**
Total	**234**	**139 (59.4%)**

* These samples have been categorized as non-IgM MGUS based on associated IFE and FLC results generated for these samples.

6.3.1.2 Specificity

Freelite Kappa:Lambda Ratio Specificity in Disease Controls (non-MGUS)

A retrospective study was performed using a total of 140 samples. These samples were from patients with polyclonal hypergammaglobulinemia confirmed by study testing (total IgG/IgA/IgM and serum IFE), with supporting clinical information for example, diagnosis of hepatitis, systemic lupus erythematosus. All samples were tested for free light chain (FLC) kappa and lambda levels with the Optilite Freelite Kappa and Lambda Free kits on the Optilite Analyser. The kappa:lambda ratios were calculated for each sample. The result of the device was compared to the clinical truth for each sample.

A specificity of 86.4% (95% CI: 79.8 – 91.1) for non-MGUS samples was determined; refer to Table 4 as follows:

Table 4. Freelite kappa:lambda Ratio Specificity.

Disease group	N =	Obtained Specificity (%) [95% CI]
Disease Controls (Non-MGUS)	140	86.4 [79.8 – 91.1]

The pre-defined acceptance criterium for specificity for non-MGUS study was set to at least 85 % which was met:

- The calculated specificity for the disease controls (non-MGUS samples) in the study was 86.4% (95% CI: 79.8 – 91.1%). This was the percentage of disease control samples determined as negative based on Optilite Freelite FLC testing, i.e., demonstrate an FLC kappa:lambda ratio within the reference interval (0.26-1.65).

6.3.2 Evaluation of MGUS progression (Study 2)

Another retrospective study was performed using a total of 185 samples from 49 MGUS patients with clinically determined stable or progressive status. Up to 4 individual sample draws, taken at various time intervals, were tested from each patient for the stable cohort and up to 6 for the progressive MGUS cohort. All samples were tested for free light chain (FLC) kappa and lambda levels with the Optilite Freelite Kappa and Lambda Free kits on the Optilite analyser. The kappa:lambda ratios were calculated for each sample. The result of the device was compared to the clinical diagnosis for each sample. The study population consisted of 45 patients with clinically stable MGUS diagnosis (stable cohort) and 4 patients that demonstrated a progressive clinical status by converting from MGUS to MM (progressive cohort).

Evaluation criteria: There are no published guidelines relating to the interpretation of consecutive FLC results for MGUS patients. Therefore, for the purpose of the device evaluation only, results were evaluated as stable and progressive MGUS based on the clinical diagnosis provided with the samples and Optilite Freelite FLC testing performed during the study, per definitions as follows:

- FLC stable: Stable MGUS defined as < 25% increase in the concentration of the involved FLC in two assessments taken at least 6 months +/- 2 months apart where possible. This analysis included MGUS patients with and without abnormal FLC kappa:lambda ratio.

- FLC progressive: Progressive MGUS defined as the FLC kappa:lambda ratio outside of the reference interval of 0.26-1.65, and an increase of ≥ 25% in the concentration of the involved light chain at or preceding the diagnosis of MM, compared to a previous sample taken at least 6 months +/- 2 months where possible.

Free light chain results criteria, specifically the 25% change, was adapted from IMWG guidelines for multiple myeloma [3].

6.3.2.1 MGUS stable and progressive

Results have been categorized as clinical positive or negative based on the clinical diagnosis and evaluated as test positive or negative based on the results of the Freelite testing performed during the study. The percentage of patients who are considered stable or progressive based on their FLC results among the patients with clinically stable or progressive disease was calculated as follows:

Stable MGUS patients:

((Number of stable patients test positive / total number of stable patients) x 100)

Table 16. Study 2, stable MGUS test positive cases

Disease group	No. of patients	Test positive patients (%)
Stable MGUS	45	93.3

The acceptance criteria for correct categorization of clinically stable MGUS by FLC testing for this study was set to at least 80 %. The acceptance criterium was met.

Progressive MGUS patients:

((Number of progressive patients test positive / total number of progressive patients) x 100)

Table 17. Study 2, progressive MGUS test positive cases

Disease group	No. of patients	Test positive patients (%)
Progressive MGUS	4	50.0

The acceptance criteria for correct categorization of clinically progressive MGUS by FLC

testing for this study was set to at least 30 %. The pre-defined acceptance criterium was met.

The distribution of the confirmed M component isotype (by IFE) in the stable and progressive MGUS samples are shown in Table 18.

Table 18. MGUS isotype, number and abnormal result by Freelite® in study 2

Isotype breakdown		
MGUS type	**N**	**n (n/N%) Freelite positive**
Non-IgM MGUS (all) abnormal	**39**	**22 (56.4%)**
IgG κ	20	15 (75%) >1.65
IgG λ	14	5 (35.7%) <0.26
IgA κ	2	2 (100%) >1.65
IgA λ	3	0 (0%) <0.26
IgM MGUS (all) abnormal	**6**	**3 (50%)**
IgM κ	4	3 (75%) >1.65
IgM λ	2	0 (0%) <0.26
Biclonal	**4**	3 (75%) >1.65 0 (0%) <0.26
Total	**49**	**28 (57.1%)**

7 Conclusion:

The completed clinical performance studies demonstrate that the Optilite Freelite Kappa and Lambda Free Kits assays can be used as an aid to evaluate Monoclonal Gammopathy of Undetermined Significance (MGUS). The studies generated successful results where all pre-defined acceptance criteria were met, therefore, supporting the extension of the claim to aid in evaluation of MGUS, and the products safety and effectiveness in this regard.

8 Bibliography

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END OF SUMMARY