510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K190686

B. Purpose for Submission:

Previously cleared analyte on a previously cleared instrument

C. Measurand:

IgM

D. Type of Test:

Quantitative, Immunoturbidimetry

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Optilite IgM CSF Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5510, Immunoglobulins A, G, M, D, and E immunological test system

2. Classification:

   Class II

3. Product code:

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

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   The Optilite IgM CSF Kit is intended for the quantitative *in vitro* measurement of IgM in cerebrospinal fluid (CSF) using the Optilite analyser.

2. Indication for use:

   Same as intended use.

3. Special conditions for use statement:

   For prescription use only.

4. Special instrument requirements:

   The Binding Site Optilite analyser

I. Device Description:

The Optilite IgM CSF Kit comprises the following reagents:

Latex Reagent: Supplied in stabilised liquid form. Preservatives: 0.025% sodium azide, 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: Contains 0.099% sodium azide as a preservative.

J. Substantial Equivalence Information:

1. Predicate device name:

   The Binding Site Group Ltd Human IgM CSF Kit

2. Predicate 510(k) number:

   K120750

3. Comparison with predicate:

Similarities		
Item	Device Optilite IgM CSF Kit	Predicate The Binding Site Group Ltd Human IgM CSF Kit
Intended Use	The Optilite IgM CSF Kit is intended for the quantitative *in vitro* measurement of IgM in cerebrospinal fluid (CSF) using the Optilite analyser.	Human IgM CSF kit for use on SPAPLUS is intended for the quantitative measurement of human IgM in cerebrospinal fluid (CSF) samples using the SPAPLUS analyser. Measurement of this immunoglobulin aids in the assessment of the body's lack of ability to resist infectious disease in conjunction with other clinical and laboratory findings.
Analyte	IgM	Same
Traceability	ERM-DA470k/IFCC	Same
On-board stability	30 days	Same
Reference interval	< 1.3 mg/L	Same
Assay type	Quantitative	Same
Antibody	Sheep anti-human IgM	Same
Method	Turbidimetric	Same

Differences		
Item	Device Optilite IgM CSF Kit	Predicate The Binding Site Group Ltd Human IgM CSF Kit
Calibrator	Liquid stabilized human serum	Lyophilized
Open-vial stability	Three months	Two months
Measuring range	0.11 – 4 mg/L (1+0) 1.0 – 40 mg/L (1+9)	0.3 – 7 mg/L (1+0) 3.0 – 70 mg/L (1+9)
Instrument	Binding Site Optilite	Binding Site SPAplus
Specimen type	CSF	CSF

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

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

CLSI EP07-A2 Interference Testing in Clinical Chemistry, Approved Guideline – Second Edition

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP05-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition

L. Test Principle:

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.

M. Performance Characteristics:

1. Analytical performance:

The results of all the studies met the Manufacturer's pre-specified acceptance criteria.

a. Precision/Reproducibility:

The precision study was performed over five working days, in duplicate and with two runs per day. The study was conducted using three instruments and three reagent lots (for levels 2, 3 and 4 of sample concentrations). Level 1 sample was tested at a later date using one reagent lot. Level 1 sample was contrived by adding native pooled CSF sample (from 19 different clinical samples) to a mock CSF matrix. Level 2 sample is a native CSF sample. Level 3 sample was contrived by spiking native CSF sample with 0.2% of affinity-purified IgM from normal human sera. Level 4 sample was contrived by spiking native CSF sample with 0.6% of affinity-purified IgM from normal human sera.

Results are summarized below:

Within-Laboratory Imprecision:

Level	N	Mean (mg/L)	Within-Run		Between-Run		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	**20**	0.20	0.01	3.9	0.00	1.5	0.01	5.9	0.01	7.2
2	**20**	0.44	0.01	2.8	0.01	2.5	0.02	4.6	0.03	5.9
3	**20**	1.20	0.03	2.2	0.03	2.6	0.02	1.3	0.04	3.6
4	**20**	3.02	0.07	2.3	2.9	2.9	0.06	2.0	0.13	4.2

Instrument-to-Instrument and Lot-to-Lot Imprecision:

Level	Mean (mg/L)	Between lot		Between instrument	
		SD	%CV	SD	%CV
1	0.20	N/A	N/A	0.01	5.07
2	0.44	0.02	4.32	0.01	2.57
3	1.20	0.02	1.86	0.00	0.28
4	3.02	0.03	1.07	0.07	2.36

b. Linearity/assay reportable range:

The linearity study was conducted using mock CSF samples that covered the range of the standard sample dilution (1+0) from 0.04 mg/L to 4.28 mg/L. Mock CSF samples were contrived by spiking in purified IgM into a mock CSF matrix. The analyte concentrations of the high and low mock CSF samples were selected in order to cover at least 10% beyond the limits of the measuring range of the assay (1.1 – 4.4 mg/L). A dilution series with 11 levels was prepared by blending the high pool and low pool. Three replicates of each level of the dilution series were run and the mean value calculated. Data were analyzed using linear regression and deviation from linearity according to CLSI guideline EP6-A. The results demonstrate that the assay is linear over the range of 0.11 – 4.0 mg/L at the standard 1+0 sample dilution. This confirms the analytical measuring interval is linear over the range 0.11 – 4 mg/L (neat, 1+0).

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

Traceability:

The calibration of the assay is traceable to ERM-DA470k/IFCC.

Stability:

Real-time stability: The purpose of this study was to validate the stability of the Optilite IgM CSF kit when stored at the recommended storage temperature of 2–8°C for the duration of the kit shelf life. The study was performed with one master calibrator (662 mg/L), low control (1.87 mg/L) and high control (3.94 mg/L) using three reagent lots at time points of 0, 3, 6.5, 10, 13 and 19 months. The Optilite IgM CSF Reagent, Calibrator and Controls have a shelf life of up to 18 months.

Open-vial stability: The purpose of this study was to validate the open-vial stability of the Optilite IgM CSF kit reagents when stored at 2–8°C for three months as stated in the product insert. Two samples were used for this study: a low control (1.87 mg/L), and high control (3.94 mg/L), which were run on days 0, 33, 61 and 111 with three lots. The Optilite IgM CSF Reagent, Calibrator and Controls can be stored opened at 2–8°C for up to three months.

On-board stability: The purpose of this study was to confirm the stability of the Optilite IgM CSF kit, once opened and stored ‘on-board’ the Optilite analyzer for a period of at least 30 days. Two samples were used for this study: a low control (1.87 mg/L), and high control (3.94 mg/L), which were run on days 0, 7, 14, 21, 28 and 35 for lot 1, on days 0, 7, 18, 21, 28 and 35 for lot 2, and on days 0, 4, 7, 14, 21, 28 and 35 for lot 3. The Optilite IgM CSF Reagent can be stored on-board the Optilite Analyser for up to 30 days.

d. Detection limit:

The purpose of this analytical sensitivity study was to estimate the Limit of Blank (LoB) and Limit of Detection (LoD) and to validate the Limit of Quantitation (LoQ) of the Optilite IgM CSF Kit, to ensure that these meet the pre-defined criteria. The study was carried out using mock CSF samples designed to mimic patient samples as closely as possible.

LoB: The LoB study was carried out using four samples of mock CSF matrix. Samples were run five times each per day, over three days to give a total of 60 replicates. The LoB results were ranked from lowest to highest concentration, and the LoB was estimated to be the result equivalent to the concentration at the 95th percentile. The LoB for the assay was determined to be 0 mg/L.

LoD: Four mock CSF sample matrices spiked with purified IgM were used to calculate the LoD. Samples were run five times each per day, over three days. Samples were targeted so that they were close to the bottom of the measuring range (0.11 mg/L) for the neat (1+0) dilution. The LoD for the assay was determined to be 0.015 mg/L.

LoQ: Four mock CSF pooled samples were diluted with mock CSF matrix targeting the concentrations close to the bottom of the standard measuring range (0.11 mg/L). These samples were run five times over three days. The LoQ was validated and set to be the bottom of the measuring range, 0.11 mg/L.

e. Analytical specificity:

Interference:

Two samples were tested: mock CSF matrix spiked with purified IgM (1.296 mg/L, and 2.978 mg/L), with clinically relevant concentrations to evaluate the effects of hemoglobin, bilirubin, acetaminophen and acetylsalicylic acid on the results of the assay. The two contrived samples were spiked separately with the interferents and with appropriate blank material to create ‘negative’ control samples. No significant assay interference effects were observed in CSF when tested with hemoglobin (1 g/L), bilirubin (80 mg/L), acetaminophen (1324 μmol/L) or acetylsalicylic acid (3.62 mmol/L).

Antigen Excess:

Turbidimetric assays can be susceptible to antigen excess with high concentration samples resulting in a falsely low result. This study is designed to investigate the antigen excess capacity of the Optilite IgM CSF kit, demonstrating that the native antigen excess capacity is robust enough to ensure that very high concentration samples flag correctly as “high activity”. This error flag means the sample is in antigen excess. The antigen excess capacity is defined as the concentration at which

the resultant change in Optical Density (OD) is not reported lower than the OD given for the standard top calibrator. Antigen excess capacity was determined at neat dilution. A calibration curve was extended with the addition of three extra calibrator points; at 5 mg/L, 5.50 mg/L and 6.00 mg/L. The additional calibrator points were manufactured using IgM calibrator material diluted in the calibrator diluent. This curve spanned a measuring range of 0.1053 – 6.00 mg/L. The absorbance value for each calibrator point was analyzed to determine the antigen excess capacity of the assay. The concentration at which the absorbance value is increasing and there is no hook effect, whilst still above the standard calibrator value, is the proven antigen excess capacity of the assay. This study showed that the Optilite IgM CSF kit has sufficiently robust antigen excess capacity up to at least 5.5 mg/L at the 1+0 sample dilution, which is equivalent to 1.375 times the top point of the standard calibration curve.

f. Assay cut-off:

Refer to expected values

2. Comparison studies:

a. Method comparison with predicate device:

Initially, a total of 219 native CSF samples from patients with various diagnoses were tested in this study. Additionally, 20 patient samples were spiked with purified IgM in order to cover the upper end of the measuring range as it was not possible to source native CSF samples with sufficiently high concentrations of IgM. Samples were stored at −20°C before testing and in accordance with the recommendations in the product insert. Of the tested samples, 155 were within the measuring range and could be included in the study. A comparison study was performed by analyzing 155 CSF samples using the Optilite IgM CSF Kit and in comparison to the predicate.

Passing Bablok regression analysis generated the following results:

Analyte	N	Sample Range	Passing & Bablok	Slope 95% CI	Intercept 95% CI
IgM	155	0.251–39.35 mg/L	y = 1.02x – 0.07	0.99 to 1.04	0.04 to 0.09

The measured correlation coefficient was 0.997.

All samples were analyzed in singlicate on the new device and the predicate device. Overall, the results of the method comparison study demonstrate that the test device correlates well with the predicate in terms of overall concordance. Concordance analysis demonstrated 100% positive agreement (95% CI = 95.1 – 100), and 97.6% negative agreement (95% CI = 91.8 – 99.7%) between samples. Overall, 98.7% of samples gave concordant results between test and predicate assays. Concordance was based on the clinical cutoff of 1.3 mg/L.

For additional information on the clinical samples used in the method comparison

study, see “Other clinical supportive data” section below.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Individual sample demographic information and admission diagnosis for the samples used in the method comparison study, where available, are summarized below:

Age (years) – available on 125/136		**Gender – available on 128/136**		
Range	**Median**	**Male**	**Female**	**Unknown**
21–90	45.5	69	59	8

Admission diagnosis information:

Group	**Admission Diagnosis**	**Number**
Neurological	Intrathecal immunoglobulin synthesis	34
	Blood-brain barrier dysfunction	31
	Neuroborreliosis	3
	CNS disorders	16
	Multiple Sclerosis	3
Other		10
Unknown		39

4. Clinical cut-off:

Refer to expected values

5. Expected values/Reference range:

   The reference range of <1.3 mg/L was transferred from literature as was done for the predicate device[1,2].

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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

[1] Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci 2001;184:101-22.
[2] Felgenhauer K. Laboratory diagnosis of neurological diseases. In Thomas L (Ed.) Clincal laboratory diagnosis, TH-books, Frankfurt/Main 1998; 1308-26.