510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

k150526

B. Purpose for Submission:

New Device

C. Measurand:

IgG4 subclass Antibody

D. Type of Test:

Quantitative, turbidimetric

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Optilite® IgG4 Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5510 – Immunoglobulins A, G, M, D, 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(s):

   The Optilite IgG4 kit is intended for the quantitative *in vitro* measurement of IgG4 in serum using the Binding Site Optilite analyser. Measurement of this immunoglobulin is an aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. The test result should be used in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Optilite analyzer (k141100)

I. Device Description:

The device consists of the following: polyclonal monospecific sheep anti-IgG4 antisera coated onto polystyrene latex in liquid form in the presence of preservatives; IgG4 calibrator and Controls (Low, High and Elevated levels) in stabilized liquid form with preservatives; and Reaction Buffer (with 0.099% sodium azide as preservative).

J. Substantial Equivalence Information:

1. Predicate device names:

   Binding Site Human IgG and IgG subclass (IgG1, IgG2, IgG3, IgG4) liquid reagent kits for use on the SPAplus

2. Predicate 510(k) number:

   k072889

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Assay type	Quantitative	Same
Detection Method	Turbidimetric immunoassay	Same
Sample matrix	Serum	Same
Antibody	Polyclonal monospecific sheep anti-human IgG4 ($F(ab)_2$) fragment bound to latex particles	Same
Traceability	Standardized against ERM-DA470k European Reference Material (previously CRM470)	Same
Open vial stability	3 months	Same
On board vial stability	30 days	Same
Reference Ranges (mg/L) (95% percentile range)	Adults: 39.2–864	Same

Differences		
Item	Device	Predicate
Intended Use	Quantitative measurement of IgG4 in serum	Quantitative measurement of Human IgG and IgG subclasses IgG1, IgG2, IgG3, IgG4 in serum
Calibrator	One Optilite IgG4 Calibrator	One calibrator for each: IgG, IgG1, IgG2, IgG3, IgG4
Controls	Low, High and Elevated levels, liquid, ready-to-use	Low and high IgG and IgG subclasses: IgG1, IgG2, IgG3, IgG4
Instruments	Optilite® Analyzer	SPA_{PLUS}™ Analyser
Measuring range (mg/L)	2.96–216 (1+1 dilution) 37–2700 (1+24 dilution) 179.1–13068 (1+120 dilution) 888–64800 (1+599 dilution)	3–85 (1:2 dilution) 30–850 (1:10 dilution) 120–3400 (1:40 dilution)

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

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition
CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
CLSI EP07-A2: Interference Testing in Clinical Chemistry
CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation
CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

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 photo iodide 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 (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The within-run, between-run, between-day, between-lot and between-instrument precision were determined by testing ten serum samples over 21 days with two runs per day and two replicates per run on three different reagent lots on four analysers. Results are summarized below.

Precision Summary													
	Mean	Within run		Between run		Between day		Between lot		Between instrument		Total	
Sample	(mg/L)	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	23.6	0.9	3.6	0.0	0.0	0.9	3.5	0.2	1.0	0.8	3.3	1.2	5.1
2	31.2	0.5	1.4	0.8	2.5	0.9	2.9	0.7	2.3	0.3	0.9	1.3	4.1
3	48.8	0.5	1.0	1.4	2.8	2.1	4.1	1.0	2.0	0.6	1.2	2.5	5.1
4	53.9	2.9	4.8	2.6	4.4	5.2	8.7	3.8	7.1	0.5	1.0	6.5	10.9
5	124.0	1.9	1.5	2.2	1.7	5.7	4.3	0.7	0.5	3.7	3.0	6.5	4.9
6	611.6	9.1	1.4	12.0	1.9	21.2	3.4	7.3	1.2	11.7	1.9	26.0	4.1
7	1148.3	16.4	1.4	25.8	2.1	42.0	3.5	14.3	1.2	28.7	2.5	51.9	4.3
8	1473.6	20.8	1.3	27.8	1.8	51.7	3.3	11.2	0.8	38.7	2.6	62.2	4.0

Precision Summary													
9	2152.5	23.0	1.1	77.7	3.6	40.1	1.9	39.5	1.8	22.7	1.1	90.4	4.2
10	4371.3	88.3	1.9	86.7	1.8	206.2	4.4	85.4	2.0	123.3	2.8	240.5	5.1

b. Linearity/assay reportable range:

A linearity study was performed following CLSI document Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. The linearity of this assay has been confirmed using serially diluted serum samples to cover the range 2.8–240.8 mg/L for Optilite analyzer dilution 1+1; 34.5–3010.4 mg/L for Optilite analyzer dilution 1+24; 169.4–14568.4 mg/L for Optilite analyzer dilution 1+120 and 828.0–72249.6 mg/L for Optilite analyzer dilution 1+ 599 with deviation from linearity ≤ 10%.

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

Traceability: An Internal Reference standard (IR) was assigned by comparison with the European Reference Material ERM-DA470k. Value assignment from total IgG to IgG4 was performed following the protocol outlined in Williams et al. [1].

Stability:

A real-time stability study of unopened kits was performed on three lots of Optilite IgG4 kit with testing time intervals at day 0, 3 months and 6.5 months. Data support a shelf life claim of 6 months at 2–8° C. Real time stability study is on-going.

Open-vial stability was performed on three lots of Optilite IgG4 kit with testing time intervals at day 0, 1, 2, and 3 months. Data support the open vial stability claim of 3 months at 2–8° C.

On-board stability was performed on three lots of Optilite IgG4 kit with testing time intervals at 0, 8, 15, 21, and 32 days. Data support the on-board stability claim of 30 days at 8–12° C, provided that the power is left switched on as stated in the product insert.

All stability results were within the sponsor's acceptance criteria.

d. Detection limit:

The analytical sensitivity was determined in accordance with CLSI EP17-A. The Limit of Blank (LoB) was based on 60 determinations of a blank sample and was

[1] Williams, D. R, Wilson, C. I. and Carr-Smith, H.D. Assignment of IgG Subclass Values to the Protein Reference Preparation DA470k. Clin Chem 2009;55(6) Supplement:A132

estimated at 95% percentile of the distribution. The Limit of Detection (LoD) was calculated according to the equation: the LoB + 1.645(SDs) where SDs, the standard deviation, was based on 12 determinations of 5 samples with analyte level near the lower limit of the reportable range. The LoQ was calculated and the total error (0.367 mg/L) was within the maximum allowable total error. The tabulated summary is shown below:

LoB	LoD	LoQ
0.000mg/L	0.299mg/L	2.960mg/

e. Analytical specificity:

Interference:
Interferences were assessed according to CLSI EP7-A2 by testing five serum samples with different IgG4 concentration ranges: (1) an IgG4 deficient sample, target level 20 mg/L; (2) a sample close to the close to the medical decision point (MDP) at the lower limit of the reference range, target 37 mg/L, (3) a sample with a normal level of IgG4, target level 135 mg/L; (4) a sample close to the upper limit of the reference range, target 845; and (5) a sample with an elevated IgG4 level, target value > 4000 mg/L. Each sample was spiked with interfering substances and tested in triplicate. The manufacturer's acceptance criteria were that the mean results from the spiked samples must be within ±10% of the mean of the control samples.

No significant assay interference effects were observed when the five samples were tested with bilirubin at 200mg/L, hemoglobin at 5g/L, or triglyceride at 1000mg/dL. Intralipid at 250 mg/dL showed signs of interference and lipemic samples are known to interfere in this assay. A warning is included in the product insert that this assay is not suitable for use with lipemic samples.

Drug Interference results: The data demonstrated that the assay was not affected by the 14 commonly used drugs tested at the concentrations given below when tested in the5 samples described above.

Drug	Concentration tested
Acetaminophen	1324 µmol/L
Acetylsalicylic Acid	3.63 mmol/L
Amoxicillin	206 µmol/L
Ascorbic Acid	342 µmol/L
Caffeine	308 µmol/L
Cefotaxime	673 µmol/L
Cefuroxime	1416 µmol/L
Chloramphenicol	155 µmol/L
Cimetidine	79.2 µmol/L
Digoxin	7.8 nmol/L
Fluconazole	245 µmol/L

Drug	Concentration tested
Ibuprofen	2425 µmol/L
Penicillin	75 mg/L
Phenytoin	198 µmol/L

Antigen Excess Detection

The Optilite's ability to detect antigen excess was evaluated with a sample of 2857 mg/L run neat on three different lots of reagents. This is equivalent to running a sample with a concentration of 71,425 mg/L at the standard measuring dilution of 1:26 (e.g., approximately 26 times the top of the calibration curve). Antigen excess was correctly detected and flagged by the analyser in all cases.

The product insert states that: "Antigen excess check warning protection ('antigen limit low' flags) may be seen against IgG4 results" and "Potential occurrences of antigen excess cannot be completely excluded; in rare cases samples with monoclonal IgG4 present may give falsely low results due to antigen excess. Where this is possible or suspected it is recommended that the sample is re-assayed at a higher dilution to confirm the result."

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study between the Optilite IgG4 Kit and the predicate device was performed by analyzing 567 serum samples (26 normal donors and 541 clinical samples, of which 322 were pediatric samples). Of the 567 total samples, 541 were clinical samples which included 199 samples with diagnosis and 342 samples with unavailable clinical information. The 199 samples with diagnoses includes: 45 confirmed hypogammaglobulinaemia; 23 hypergammaglobulinaemia; 1 suspected hypogammaglobulinaemia; 9 suspected hypergammaglobulinaemia; 1 anaphylaxis; 14 endocrine and autoimmune conditions were tested including 1 SLE (pediatric); 3 thyroid related disease (all pediatric); 2 diabetes (all pediatric); 8 obesity (all pediatric); 38 AIP; 5 benign conditions (all pediatric); 2 malignant conditions; 13 developmental conditions (all pediatric); 35 other clinical conditions (e.g. hyperkalemia, implanted device management, fracture, etc).

Passing-Bablok regression analysis generated the following result: $y = 0.91x + 8.09$ (mg/L) with correlation coefficient $r = 0.999$.

The package insert states that "Levels of IgG4 in normal adults may be undetectable;

therefore, low IgG4 alone is insufficient evidence of an antibody deficiency disorder requiring Immunoglobulin replacement. Clinical history and other laboratory findings must be taken into account in the diagnosis of antibody deficiency disorder".

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and specificity:

Not applicable

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

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference Interval:

The product insert states the following:
The ranges provided have been obtained from a limited number of samples and are intended for guidance purposes only. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population and, if necessary, determine its own reference interval.

Adult serum range
This range was obtained by measuring the subclass content of sera provided by the Birmingham Blood Transfusion Service taken from healthy UK adult donors and generated using Binding Site's BN™II subclass kits and verified using 50 US normal samples on the Optilite IgG4 Kit. Forty-eight of the 50 samples tested were within the 95 percentile range accepted criteria.

	Number (n)	Mean (mg/L)	Median (mg/L)	95 Percentile Range (mg/L)
IgG4	30	280	215.3	39.2–864

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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