

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM**

A. 510(k) Number:

K150412

B. Purpose for Submission:

New device

C. Measurand:

Human CH50, total complement activity

D. Type of Test:

Quantitative, Turbidimetric

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Optilite® CH50 Reagent
Optilite® CH50 Controls
Optilite® CH50 Calibrator

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5240, Complement components, immunological test system
21 CFR §862.1150, Calibrator
21 CFR §862.1660, Quality control material (assayed and unassayed)

2. Classification:

Class II (Assay and Calibrator)
Class I (Controls)

3. Product code:

DAE, Complement C9, Antigen, Antiserum, Control
JIX, Calibrator, Multi-analyte Mixture
JJY, Multi-analyte controls, All kinds (Assayed)

4. Panel:

Immunology (82)
Chemistry (75)

H. Intended Use:

1. Intended use(s):

The Optilite® CH50 reagents are intended for the quantitative *in vitro* determination of total classical complement activity (CH50) in human serum using the Binding Site Optilite turbidimetric analyser. Measurement of complement activity aids in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components. The test should be used in conjunction with other laboratory and clinical findings. This *in vitro* diagnostic device is intended for prescription use only and can only be used by professionals.

The Optilite CH50 calibrator is intended for use on the Optilite analyser in conjunction with the Binding Site Optilite CH50 Reagent (product code NK095.OPT) for the determination of total complement activity. This *in vitro* diagnostic device is intended for prescription use only and can only be used by professionals.

The Optilite CH50 controls are intended for use on the Optilite analyser in conjunction with the Binding Site Optilite CH50 Reagent (product code NK095.OPT) for the determination of total complement activity. This *in vitro* diagnostic device is intended for prescription use only and can only be used by professionals.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Binding Site Optilite analyzer (K141100)

I. Device Description:

The Optilite® CH50 reagent consists of: (a) liquid liposome reagent containing G6PDH, (b) CH50 substrate (lyophilized) containing anti-DNP antibody (goat), 24 mmol/L and 9 mmol/L NAD and (c) liquid substrate diluent containing 10 mmol/L maleate buffer, pH 5.0. The user reconstitutes the CH50 substrate prior to loading onto the Optilite instrument.

The Optilite® CH50 calibrator is supplied as a lyophilized powder to be reconstituted with distilled water by the user prior to use.

The Optilite® CH50 controls consist of three levels (low, high and elevated) of lyophilized powder that must be reconstituted by the user.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

CH50 reagent pack for use on the SPA_{PLUS}, CH50 calibrator set for use on the SPA_{PLUS}, CH50 controls for use on the SPA_{PLUS} (K113349)

2. Comparison with predicate:

Similarities – Reagent		
Item	New Device Optilite® CH50 Reagent	Predicate Binding Site Human CH50 reagent pack
Intended Use/Indications for Use	Measurement of complement activity aids in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components.	Same
Analyte	CH50	Same
Sample Matrix	Serum	Same
Type of Test	Quantitative	Same
Assay Type	Turbidimetric	Same
On-board Stability	30 days	Same
Antibody	Goat anti-DNP	Same
Reference Interval	41.68–95.06 U/mL	Same
Reagent	CH50 liposome reagent, substrate and substrate diluent	Same
Traceability	Value assigned using Internal Reference which is compared to the predicate Internal Reference	Same

Differences – Reagent		
Item	New Device Optilite® CH50 Reagent	Predicate Binding Site Human CH50 reagent pack
Measuring Range	12.5–100 U/mL	12–95 U/mL
Instrumentation	Optilite Analyzer	SPA _{plus} Analyzer
Sample dilution	1/2 instrument dilution	1/1 (neat) instrument dilution

Similarities – Calibrators and Controls		
Item	New Device Optilite® CH50 Calibrator and Controls	Predicate Binding Site Human CH50 calibrator set and controls
Intended Use, Calibrators	The Optilite CH50 calibrators are intended for use on the Optilite analyzer in conjunction with the Binding Site Optilite CH50 Reagent for the determination of total complement activity.	Same
Controls	One low, one high and one elevated	Same
Traceability	Value assigned using Internal Reference which is compared to the predicate Internal Reference	Same

Differences – Calibrators and Controls		
Item	New Device Optilite® CH50 Calibrator and Controls	Predicate Binding Site Human CH50 calibrator set and controls
Calibrators	Single Calibrator	Calibrator set consisting of 6 vials
Stability of reconstituted calibrators and controls	8 hrs	10 hrs
Intended Use, Controls	The Optilite CH50 controls are intended for use on the Optilite analyzer in conjunction with the Binding Site Optilite CH50 Reagent for the determination of total complement activity.	The Human CH50 controls are intended for use in conjunction with the Binding Site Human CH50 reagent pack for use on the SPA _{PLUS}

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

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods
- CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedure
- CLSI EP7-A2: Interference Testing in Clinical Chemistry
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
- CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in a Clinical Laboratory

L. Test Principle:

Complement acts in a number of ways to help clear invading organisms, with a major function being the lysis of bacteria through formation of the membrane attack complex (MAC). The reagent uses liposomes encapsulating glucose-6-phosphate dehydrogenase (G6PDH) to mimic an invading microorganism. Upon addition of sample, antibodies in the reagent combine with dinitrophenyl groups on the surface of the liposomes. The resultant complex activates complement in the sample, which lyses the liposome, releasing G6PDH to react with glucose-6-phosphate and NAD in the reagent. The change in absorbance can be measured and is proportional to the complement activity in the sample. Comparison to a calibration curve gives a value for the unknown patient sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Five levels of pooled serum (one at very low level but above LoD, one 25% below cut-off point, one at the cut-off, one 25% above cut-off and one normal sample within the reference range) were tested on an Optilite analyzer to establish intra-assay and inter-assay precision. A 21-day precision study was carried out by running the samples in duplicate with two runs per day, using three instruments and three reagent lots for a total of 84 runs. The sponsor's pre-determined acceptance criteria are as follows: within-run $\leq 5\%$ CV; between-run $\leq 8\%$; between-day $\leq 8\%$ CV, total precision $\leq 10\%$ CV. No specification was set for between-batch and between- instrument precisions. Results are summarized below:

Sample	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	19.23	0.92	4.8	0.64	3.3	1.12	5.8	1.59	8.3
2	30.22	1.12	3.7	1.18	3.9	0.67	2.2	1.76	5.8
3	40.34	0.59	1.5	0.75	1.9	1.49	3.7	1.77	4.4
4	51.35	0.71	1.4	0.65	1.3	1.51	2.9	1.79	3.5
5	78.72	1.64	2.1	1.76	2.2	2.44	3.10	3.43	4.4

Sample	Between-lot	Between-Instrument
--------	-------------	--------------------

	SD	%CV	SD	%CV
1	1.04	5.38	0.21	1.11
2	0.54	1.78	0.15	0.50
3	0.94	2.33	0.62	1.54
4	1.38	2.69	0.49	0.95
5	1.85	2.34	1.05	1.33

b. Linearity/assay reportable range:

Linearity was performed following CLSI EP6-A guidelines. A high pool and a low pool with target values 10% above and below the upper and lower limits of the measuring range were prepared to evaluate linearity across the measuring range of the assay (i.e. 9.35 – 110.75 U/mL). A series of dilutions was prepared by blending the high pool and low pool to give a total of 13 concentrations. Linearity was demonstrated at concentrations spanning the claimed measuring range (12.5 – 100 U/mL). Three replicates of each level of the dilution series were tested and the mean value calculated. Linearity was evaluated by calculating the percentage recovery at each concentration in the dilution series, and the %CV of the three replicates. All of the concentrations within the analytical measuring range met the sponsor's pre-determined acceptance criterion of %CV < 10%, and all concentrations met the sponsor's regression analysis acceptance criteria.

The observed values were graphed against the calculated values and a linear regression was performed. The regression plot equations where y is the measured level of CH50 concentration and x the theoretical concentration is as follows: $y = 0.977x + 0.240$ U/mL, $R^2 = 0.995$.

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

i) Traceability:

Calibrators and Controls: The calibrator is made from fresh frozen serum. A Master Calibrator was used to control calibration between batches. The Master Calibrator value was transferred from the FDA cleared standards (Wako Autokit CH50, K954145).

A single calibrator and three (low, high and elevated) levels of controls are provided in the kit as shown in the table below.

Optilite® CH50 Controls	Target (U/mL)	Range (U/mL)
--------------------------------	---------------	--------------

Low	20.0	18.0 – 22.0
High	40.0	36.0 – 44.0
Elevated	67.0	60.30 – 73.70
Optilite® CH50 Calibrator	50.0	45.0 – 55.0

ii) *Stability:*

The Optilite CH50 Liposome Reagent is identical to the reagent used in the predicate device (SPA_{PLUS} CH50 Reagent Pack). The open vial stability of the reagent was previously demonstrated in K113349 (SPA_{PLUS} CH50 Reagent Pack).

Stability studies demonstrated the following claims:

- Real-Shelf life (CH50 Kit): The study demonstrates that the kits are stable for at least 6.5 months when stored at 2-8°C. This study is on-going.
- On-board stability on Optilite Analyzer (CH50 Kit): 30 days at 2-8°C
- Reconstituted stability (controls and calibrators): 8 hrs stored at 2-8°C.

The sponsor recommends not freezing the kits.

d. *Detection limit:*

The analytical sensitivity was determined in accordance with CLSI EP17-A. The Limit of Blank (LoB) was determined using a pool consisting of five analyte-depleted serum samples with a concentration approximately 10 times lower than that of the LoQ sample. The LoB sample was tested 60 times in one day, using one batch of reagent with one analyzer and the mean and SD was calculated.

The Limit of Detection (LoD) was calculated using the LoB value and the precision of the new assay for the five (5) samples that were tested for the LoQ study [$LoD = LoB + (1.645 \times \text{combined SD of LoQ})$].

The LoQ study was performed by analyzing five different samples with a concentration within $\pm 10\%$ of the lowest calibrator (12.5 U/mL). These samples were tested 12 times over five days in one instrument to give 60 replicate results. The data were used to demonstrate that the total error (TE) is acceptable (pre-determined acceptance criteria, $<10\%$), at the bottom of the measuring range of the assay.

The results are summarized as:

$$LoB = 8.54 \text{ U/mL}$$

LoD = 9.13 U/mL
 LoQ = 12.50 U/mL

e. *Analytical specificity:*

i) *Endogenous and exogenous Interference:*

Interferences were assessed using three base serum samples containing three clinically relevant concentrations of CH50 [68 U/mL(normal range/negative), 41.68 U/mL (cut-off) and 20 U/mL (complement deficient sample)] supplemented with various amounts of interfering agents following CLSI EP7-A2 guidance. Control samples were made by using same base sera spiked with saline instead of the interfering agents. Thirty replicates of each serum base pool was run in order to calculate mean and SD. The data demonstrated that assays were not adversely affected by high levels (i.e. the values are within $\pm 10\%$ of acceptance criteria) of the following substances tested up to the concentrations listed in the table below:

Interferent	Conc. of interferent	Percent Interference (%)		
		Normal 68 U/mL	Cut-off 41.68 U/mL	Deficient 20 U/mL
Hemoglobin	5 g/mL	-1.30	2.18	3.11
Bilirubin	200 mg/mL	0.92	-3.15	-7.62
Intralipid	2.0 g/L	-9.64	-8.55	-10.29**
Intralipid	500 mg/mL	-4.46	-3.48	-10.12**
Intralipid	250 mg/mL	-3.21	-1.56	3.44
Triglyceride	1.0 g/mL	-5.73	-0.96	1.41
Theophylline	222 μ mol/L	-2.07	-1.63	-4.56
Vancomycin	69 μ mol/L	0.14	1.51	3.89
Amoxicillin	206 μ mol/L	-0.12	3.00	9.63
Chloramphenicol	245 μ mol/L	-3.12	3.30	-0.53
Fluconazole	155 μ mol/L	-2.30	1.20	-4.33
Caffeine	308 μ mol/L	-1.78	-5.25	9.13
Cimetidine	79.2 μ mol/L	0.39	-1.33	-0.64
Ascorbic acid	0.5 g/mL	-0.68	-0.35	0.83
Ibuprofen	2.425 g/mL	-4.80	1.11	-9.98
Acetaminophen	1324 μ mol/L	1.11	0.04	-1.83
Acetylsalicylic acid	3.63 mmol/L	0.91	1.20	2.44
Phenytoin	198 μ mol/L	0.47	0.07	3.13
Digoxin	7.8 nmol/L	-1.12	2.30	0.51
Penicillin	75 mg/L	1.08	-0.01	8.58
Cefuroxime	1416 μ mol/L	0.59	1.95	7.7
Cefotaxime	673 μ mol/L	1.62	2.44	8.94

** The package insert states that “turbidimetric assays are not suitable for measurement

of highly lipaemic or hemolyzed samples, or samples containing high levels of circulating immune complexes due to the unpredictable degree of non-specific scatter these sample types might generate. Unexpected results should be confirmed using alternative assay method.”

f. Assay cut-off

Not applicable

2. Comparison Studies

a. Method comparison with predicate device:

A method comparison study between the Optilite CH50 reagent for use on the Optilite analyzer and the predicate assay was performed using 345 samples include: 27 systemic lupus erythematosus (SLE); 28 Rheumatoid Arthritis (RA); 5 Sjögren’s syndrome; 74 suspected complement deficiency samples; 10 asthma; 2 neurological disease; 11 inflammatory bowel disease (5 ulcerative colitis; 6 Crohn’s disease); 4 diabetes; 12 thyroid disease (8 hypothyroid and 4 hyperthyroid); 15 cancer samples; 4 liver cirrhosis; 20 hepatitis C; 15 liver disease; 7 autoimmune samples; 58 normal samples; and 10 spiked samples (to cover the very top of the measuring range and the area around the cut-off). The clinical samples were obtained by a large reference laboratory in Paris, France. The remaining 43 clinical samples assay (3 anti-GBM, 13 kidney disease samples with no further clinical information available and 27 lupus nephritis samples) had an admission diagnosis relevant to the intended use and were sourced by Binding Site from the United Kingdom. Samples, ranged between 14–97 U/mL. The sponsor’s pre-determined acceptance criteria were slope = 1.0 ± 0.1 and R ≥ 0.975. The regression analysis was performed and the results are summarized below:

Regression analysis	U/mL	Slope (95% CI)	Intercept (95% CI)
Passing-Bablok	$y = 1.09x - 3.37$	1.05 – 1.14	- 5.30 – 1.77
Deming (weighted)	$y = 1.04x - 1.55$	0.99 – 1.09	- 3.47 – 0.36
Linear	$y = 1.07x - 2.74$	1.03 – 1.12	- 4.82 – 0.66

The agreement between the CH50 Reagent pack on an Optilite analyzer and the predicate in identifying CH50 deficient samples (i.e. samples below the cut-off) is summarized in the table below :

Predicate Assay

		CH50 Deficient	Not Deficient	Total
Test Assay	CH50 Deficient	263	7	270
	Not Deficient	2	73	75
	Total	265	80	345

Positive percent agreement: 99.25% (263/265) (95% CI: 97.30 – 99.91%)

Negative percent agreement: 91.25% (73/80) (95% CI: 82.80 – 96.41%)

Overall percent agreement: 97.30% (336/345) (95% CI: 95.04 – 98.70 %)

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical sensitivity and specificity:

Not applicable

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

The table below shows the disease conditions that fall under CH50 deficiency and CH50 sufficiency with a total of 287 samples (healthy subjects and contrived samples were removed from the 345 samples described above). The same data from the method comparison are intended to demonstrate substantial equivalence to the cleared predicate, rather than establish clinical truth.

Diagnostic Groups	Number of Samples	CH 50 Deficient	CH50 Not Deficient
SLE (without nephritis)	27	21 (78%)	6 (22%)
RA	28	21 (75%)	7 (25%)
5 Sjögren's syndrome	5	5 (100%)	0
Kidney disease (Total 43 samples)	3 anti-GBM	3 (100%)	0
	13 kidney disease without clinical information	13 (100%)	0
	27 lupus nephritis	25 (93%)	2 (7%)
Differential diagnosis samples (Total 39 samples)	10 asthma	10 (100%)	0
	2 neurological disease	2 (100%)	0
	5 ulcerative colitis	5 (100%)	0
	6 Crohn's disease	6 (100%)	0
	4 diabetes	4 (100%)	0
	8 hypothyroid	7 (88%)	1 (12%)

	4 hyperthyroid	4 (100%)	0
Other Clinical Samples (Total 61 samples)	15 Cancer	15 (100%)	0
	4 Liver cirrhosis	4 (100%)	0
	20 hepatitis C	20 (100%)	0
	15 liver disease	13 (87%)	2 (13%)
	7 Small vessel vasculitis (4 PR3, 3 MPO)	7 (100%)	0
Suspected complement deficiency samples	74	71 (96%)	3 (4%)

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Reference intervals (values) for CH50 were validated and transferred from predicate SPAPLUS (K113349) to Optilite CH50 using 50 donor samples (UK and US) collected from various individual of different demographics by following CLSI C28-A3 guidance: *Defining, Establishing and Verifying Reference Intervals in a Clinical Laboratory*). The results demonstrate that 98% (49 out of 50) of the samples agreed with predicate reference interval values. The 95% reference interval of Optilite CH50 kit is 41.68-95.06 U/mL with a mean of 60.99 U/mL and a median of 60.50 U/mL.

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.