510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION

DECISION SUMMARY

A. 510(k) Number:

k113349

B. Purpose for Submission:

New device

C. Measurand:

CH50, total complement activity

D. Type of Test:

Quantitative, turbidometric

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Human CH50 reagent pack for use on the SPA $_{PLUS}$, Human CH50 calibrator set for use on the SPA $_{PLUS}$, Human CH50 controls for use on the SPA $_{PLUS}$

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, Device and Calibrator

   Class I, Quality Control Material

3. Product codes:

   DAE, Complement 9, Antigen, Antisera, Control

JIX, Calibrator, Multi-Analyte Mixture

JJY, Multi-analyte controls, All kinds (Assayed and Unassayed)

4. Panel:

Immunology (82)

Chemistry (75)

H. Intended Use:

1. Intended use(s):

Human CH50 reagent pack for use on the SPA $_{PLUS}$:
These reagents are intended for the quantification of total classical complement activity (CH50) in human serum on the Binding Site SPA $_{PLUS}$ analyser. Measurement of complement activity aids in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components. The test results are to be used in conjunction with clinical findings and other laboratory tests.

Human CH50 calibrator set for use on the SPA $_{PLUS:}$
The Human CH50 calibrator set is intended for use on the SPA $_{PLUS}$ in conjunction with the Binding Site Human CH50 reagent pack (product code: NK095.S) for the determination of total complement activity.

Human CH50 controls for use on the SPA $_{PLUS:}$
The Human CH50 controls are intended for use in conjunction with the Binding Site Human CH50 reagent pack (product code: NK095.S).

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

The Binding Site SPA_{plus}™

I. Device Description:

The device consists of the following:

CH50 reagent pack includes: CH50 Liposome reagent R1(Liquid reagent containing

G6PDH); CH50 Substrate R2 (lyophilized, containing anti-DNP antibody (goat), 24 mmol/L G6P and 9 mmol/L NAD); CH50 Substrate diluent R2a (Liquid reagent containing 10 mmol/L maleate buffer pH 5.0).

The CH50 calibrator set includes: Six levels, lyophilized.

The CH50 controls includes 3 level controls (low, high and elevated), 4x0.45 mL for each level, lyophilized.

J. Substantial Equivalence Information:

1. Predicate device name(s) and K number(s):

   WAKO Autokit CH50, WAKO CH50 Calibrator, WAKO Complement Control; k954145

2. Comparison with predicate:

Similarities		
Item	Binding Site Human CH50 reagent pack	WAKO Autokit CH50
Intended Use:	Measurement of total complement activity	Same
Sample matrix	Serum	Same
Detection Method	Turbidimetric, absorbance	Same
Reagents	CH50 liposome reagent, substrate and substrate diluent	Same
CH50 anti-DNP antibody substrate mammalian source	Goat	Same

Differences		
Item	Binding Site Human CH50 reagent pack	WAKO Autokit CH50
Instruments	SPA $_{PLUS}$ analyser	WAKO-30R analyzer
Measuring range	12-95 U/mL	10-60 U/mL
Expected values	41.68-95.06 U/mL	23-46 U/mL

Similarities		
Item	Binding Site Human CH50 calibrator set and controls	WAKO CH50 Calibrators and Complement Controls
Reagents	Lyophilized	Same

Differences		
Item	Binding Site Human CH50 calibrator set and controls	WAKO CH50 Calibrators and Complement Controls
Traceability	Value assigned using Internal Reference which is compared to the predicate Internal Reference	Internal reference value assignment and determination per Mayer et al in Complement and complement fixation 1967 2nd ed. Springfield, Charles C. Thomas text book
Calibrator stability after reconstitution	Store on ice or 2-8°C and use within 10 hours	Store on ice and use within a day
Control stability after reconstitution	Store on ice or 2-8°C and use within 10 hours	Store on ice and use within 8 hours

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

CLSI EP-5A2: Evaluation of Precision Performance of Clinical Chemistry; Approved Guideline – Second Edition

CLSI EP-6A: Evaluation of Linearity of Quantitative Measurements; Approved Guideline

CLSI EP-17A: Determinations of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

Liposomes encapsulating glucose-6-phosphate dehydrogenase (G6PDH) are used to mimic an invading microorganism. On 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 the

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:

The intra-assay and inter-assay precision was determined by testing four serum samples over 21 days with two runs per day on three different reagent lots. Results are summarized below.

Sample	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	78.73	1.67	2.1	3.04	3.90	3.26	4.1	4.76	6.0
Serum 2	55.22	0.66	1.2	0.65	1.2	1.86	3.4	2.08	3.8
Serum 3	46.05	0.84	1.8	1.03	2.2	1.00	2.2	1.66	3.6
Serum 4	25.34	0.34	1.4	0.39	1.5	0.99	3.9	1.12	4.4

b. Linearity/assay reportable range:

Linearity across the assay range (12.68 – 95.47 U/mL) was confirmed by testing a serum pool with high range concentrations up to 95.9 U/mL. The samples were serially diluted 11 times with buffer (1:10) down to the lower measuring range (12.41 U/mL). All testing were performed three times. The regression plot equations where y is the measured level of CH50 concentration and x the theoretical concentration is as follows: y=0.996x – 2.709 (U/mL). r=0.994

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

Value assigned to the new device internal reference standard was compared to the predicate internal reference (IR) standard which was determined according to methods used by Mayer et al as described in Complement and complement fixation text book published in 1967 2nd ed. Springfield by Charles C. Thomas). The IR standard is used to assign values to the 6 calibrators and 3 controls.

Stability:
Stability studies demonstrated the following claims:

- Unopened reagent pack, calibrators and controls: 7 months at 2-8°C
- On-board reagent pack: 30 days at 2-8°C
- Reconstituted calibrator and controls: 10 hours stored on ice or at 2-8°C

d. Detection limit:

The detection limits were determined by testing 60 replicates of a blank sample, the lowest calibrator, and a sample with value close to the blank sample. The limit of blank claim for this assay is 0.272 U/mL as determined by testing 60 replicates of a blank sample. The limit of detection represents the lowest measurable analyte level that can be distinguished from zero and has been estimated at 1.654 U/mL. The limit of quantitation is defined as the lowest amount of analyte that can be quantitatively determined and has been estimated as 11/98 U/mL for this assay.

e. Analytical specificity:

Interference by endogenous and other substances:
No significant assay interference was demonstrated by 1500 formazine turbidity units (FTU) of chyle, 200 mg/L bilirubin, or 5g/L hemoglobin and 0.5 g/L ascorbic acid using CH50 sample at 36.26 U/mL.

The package insert states that "turbidimetric assays are not suitable for measurement of highly lipemic 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:

The cut-off of 41.68 U/mL for the Binding Site Human CH50 assay was established from 120 serum samples from normal healthy adult blood donors. The CH50 results ranged from 36.295 to 98.210 U/mL. A non-parametric distribution of CH50 results was seen that gave a 95 percentile reference lower limit of 41.68 U/mL.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study between Human CH50 reagent pack, calibrator and controls on a SPA_{PLUS}™ analyzer and predicate device was performed using 110 samples (29 normal, 62 known suppressed CH50 samples and 19 moderate level mixed samples to obtain results between 14-59 U/mL (5 samples were between 14-21 U/mL and 14 samples were between 32-59 U/mL). A total of 62 samples with results "<13.88 U/mL*" results were

excluded from the regression analysis. The study demonstrated the following Passing-Bablok fit: y = 1.15x -4.29 (U/mL) with a correlation coefficient (r) of 0.961. (*<13.88 U/mL is the lowest point of the calibration curve of calibrator used.)

Percent agreements between CH50 Reagent pack, calibrator and controls on a SPA_{PLUS}™ analyzer and predicate in identifying CH50 deficient samples were calculated and summarized in the table below.

		WAKO Autokit CH50		
		Normal	CH50 deficient	Total
Binding Site Human CH50	Normal	42	0	42
	CH50 deficient	1	67	68
	Total	43	67	110

Positive percent agreement: 97.7% (42/43) (95% CI: 93.2-102.2%)

Negative percent agreement: 100% (67/67) (95% CI: 100-100%)

Overall percent agreement: 99.1% (109/110) (95% CI: 97.3-100.9%)

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A clinical study evaluated 91 samples on this kit : 29 normal and 62 CH50 deficient clinical samples. Of the 62 CH50 deficient samples, 33 were from autoimmune disease patients (19 SLE, 10 RA, 3 Sjögren's Syndrome, 1 Raynaud's syndrome), 8 from kidney disease patients (3 nephrotic syndrome, 3 nephritis, 1 nephropathy, 1 proteinuria), 21 other pathological disorders (1 neutropenia, 1 myelodysplasia, 1 bi-clonal gammopathy, 1 urticaria, 1 face swelling, 2 stroke, 14 undetermined). The 62 CH50 deficient clinical samples all had CH50 levels below 41.6 U/mL.

N=91		Patient Group		
		CH50 Deficient Autoimmune (33), kidney (8) and other pathological disorders (21)	CH50 Sufficient Healthy controls	Total
Binding Site Human CH50	<41.6 U/mL	62	0	62
	>41.6 U/mL	0	29	29
	Total	62	29	91

Sensitivity: 100% (62/62) (95% CI: 100-100%)
Specificity: 100% (29/29) (95% CI: 100-100%)

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

Not applicable.

4. Clinical cut-off**:**

Same as assay cut-off.

5. Expected values/Reference range:

Adult reference range:
Adult reference range was assessed using a total of 120 serum samples from healthy adult UK blood donors. A non-parametric distribution of CH50 results was seen that gave a 95 percentile reference interval of 41.68-95.06 U/mL with a mean of 68.37 U/mL and a median of 68.70 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.