510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k122965

B. Purpose for Submission:

New Device

C. Measurand:

Human ceruloplasmin

D. Type of Test:

Turbidimetry, Quantitative

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Human Ceruloplasmin Kit for use on SPA_{PLUS}

G. Regulatory Information:

1. Regulation section:

   21 CFR§866.5210 – Ceruloplasmin Immunological Test System

2. Classification:

   Class II

3. Product code:

   DDB, Ceruloplasmin, antigen, antiserum, control

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   The Human Ceruloplasmin Kit for use on SPA_{PLUS} is intended for the quantitative *in vitro* measurement of human ceruloplasmin in serum using the SPA_{PLUS} turbidimetric analyzer. The measurement of ceruloplasmin levels in serum is an aid in the diagnosis of copper metabolism disorders. This test 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):

   Prescription use only

4. Special instrument requirements:

   SPA_{PLUS} analyzer

I. Device Description:

The kit contains the following materials:

- Human ceruloplasmin antiserum (1 x 50 testes), liquid form with preservatives
- Calibrator sets 1-6 (12 vials), lyophilized
- Controls (High and Low), lyophilized
- Reaction Buffer (1 x 50 tests)

J. Substantial Equivalence Information:

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

   N Antisera to Human Ceruloplasmin and Hemopexin (k053074)

2. Comparison with predicate:

Similarities and Differences		
Item	Device Human Ceruloplasmin Kit for Use on SPA_{PLUS}	Predicate N Antisera to Human Ceruloplasmin and Hemopexin
Intended use	Quantification of ceruloplasmin.	Quantification of ceruloplasmin and hemopexin
Indication for use	Measurement of ceruloplasmin levels in serum is an aid in the diagnosis of copper metabolism disorders.	Same
Analyte	Ceruloplasmin	Ceruloplasmin and hemopexin
Sample type	Serum	Serum and heparinized plasma
Method	Turbidimetry	Nephelometry
Instrument	SPA_{PLUS}	BN system
Antibody	Sheep anti-human ceruloplasmin	Rabbit anti-human ceruloplasmin
Measuring range	0.03 – 0.82 g/L (at 1/10 standard dilution) 0.06 – 1.64 g/L (at 1/20 dilution)	0.07 – 2.20 g/L
Stability	Open vial: 2 – 8°C for 2 months On-board: 30 days	Open vial: 2 – 8°C for 4 weeks On-board: A minimum of 3 days at 8 hours/day
Reference range	0.2 – 0.6 g/L (adult)	Same

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; Approved Guideline".
CLSI EP7-A2 "Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition"
CLSI EP17-A "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline"
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 photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test samples. 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 precision was evaluated based on CLSI EP05-A2 by testing three pooled samples (0.047, 0.214 and 0.809 g/L) with using 1/10 standard dilution according to the instruction. One high analyte level sample was run at a 1/20 sample dilution with concentration of 0.750 g/L. Each sample was assayed in duplicate with 2 runs per day for 21 days (n=84) using three reagent lots and three instruments. The results are summarized in the following table.

Mean Conc. (g/L)	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Lot**		**Total**	
	SD (g/L)	CV %	SD (g/L)	CV %	SD (g/L)	CV %	SD (g/L)	CV %	SD (g/L)	CV %
0.809	0.013	1.6	0.012	1.5	0.042	5.2	0.028	3.4	0.046	5.6
0.214	0.002	1.1	0.004	1.6	0.019	8.9	0.021	10.0	0.020	9.1
0.047	0.001	2.1	0.002	4.3	0.005	10.6	0.003	6.3	0.006	11.6
0.750	0.009	1.1	0.025	3.4	0.055	7.4	0.059	7.8	0.061	8.2

b. Linearity/assay reportable range:

Linearity: The linearity across the assay range was conducted based on CLSI EP06-A. A dilution series was prepared by diluting the high serum pool (1.066 g/L) in the low serum pool (0.024 g/L) to generate 11 dilutions with concentration ranging from 1.066 g/L to 0.024 g/L. Each dilution was tested in triplicate. The observed values (y) were graphed against the expected values (x), and the regression analysis gives the following equation:

$y=1.067x - 0.009$, $R^2= 0.9943$

The assay was shown to give a linear response over the claimed measuring range, 0.03 – 0.82 g/L (at standard 1/10 sample dilution).

Antigen excess (hook effect): The susceptibility of the assay to antigen excess was investigated. The results demonstrated that the assay is not susceptible to antigen excess up to a concentration of 2 g/L.

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

Traceability: There is no international reference material for the analyte. The calibration of the assay is traceable to the Siemens N Protein Standard (predicate).

The table below summarizes the target values for calibrators and controls:

	Target Value (g/L)
Calibrator	
Calibrator 1	0.03
Calibrator 2	0.06
Calibrator 3	0.12
Calibrator 4	0.25
Calibrator 5	0.50
Calibrator 6	1.00
Controls	
High Control	0.65
Low Control	0.20

Stability:

Closed vial stability: The real time stability of human ceruloplasmin kit was performed using three batches of kits stored under the recommended temperature at 2 – 8°C. Data were collected at point 0, 3, 6, and 13 months. The results support stability of the kits under the recommended storage of 2 – 8°C for up to 12 months.

Open vial stability: The study was done to evaluate the reagent (the ceruloplasmin antiserum and reaction buffer) stability after first opening. Three batches of reagents were stored at 2 – 8°C after first opening. Data were collected at 1, 2, and 3 months. The results support that the reagents are stable once opened for up to 2 months when stored at 2 – 8°C.

On-board stability: On-board stability of human ceruloplasmin kit was done by placing the kit in the reagent carousel of the SPA_{PLUS}. The reagent carousel was covered and cooled to 8 – 12°C. A calibration curve was generated on Day 0 and validated. The test data were collected at Day 0, Day 7, Day 14, Day 21, Day 28 and Day 35. The result supports that the reagents are stable up to 30 days on-board the SPA_{PLUS}.

Calibrator and control stability: Stability of calibrator set and controls was evaluated. The results supports that the calibrator set is stable up to 7 days and controls are stable up to 30 days once reconstituted and opened and stored at 2 – 8°C.

Calibration curve stability: There is no stability claim for the calibration curve. The controls should be run in all assay performed. Should a control measurement be out of the acceptance range (within ±15% of the concentration(s) stated) when assayed with a stored curve, the assay must be recalibrated and validated.

d. Detection limit:

The study was performed in accordance with CLSI EP17-A. The limit of blank (LoB) for this assay was determined by testing instrument diluent. The limit of detection (LoD) was determined by testing serum sample with low analyte concentration at concentration of 0.018 g/L. Sixty (60) replicates of each sample were run, and the mean and standard deviation for each of the samples was calculated. LoD = LoB + 1.645 x SDs where SDs is the standard deviation of the replicate samples. To determine LoQ, 40 replicates of the lowest calibrator fluid with an assigned concentration 0.03 g/L were assayed to calculate the mean, SD and %CV. The claimed LoB, LoD and LoQ are summarized in the following table:

LoB	LoD	LoQ
0.014 g/L	0.017 g/L	0.03 g/L

e. Analytical specificity:

Endogenous interference: Interference by endogenous interference was performed according to CLSI EP7-A. Two base pools of patient serum at 0.193 g/L (close to the lower end of the reference range) and 0.452 g/L (close to the top of the reference range) were spiked with endogenous substances, hemoglobin, bilirubin and chye. The negative samples were prepared by spiking the same volume of commercially obtained blank reagents. The resulting samples were tested in triplicate and the mean values were used to calculate % interference. No significant interference was noted for sample containing hemoglobin at 4.88 g/L, bilirubin at 200 mg/L, and chye at 1550 formazine turbidity units (FTU). Rheumatoid Factor (RF) interference was not evaluated.

f. Assay cut-off:

The reference range for ceruloplasmin concentration is 0.2 – 0.6 g/L according to the literature. To validate the reference range, 89 serum samples from normal healthy people were tested and results support the cited reference range.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare human ceruloplasmin kit on SPA_{PLUS} (y) and the predicate device on Siemens BNII system (x). Total 102 samples were tested. Samples included 48 normal, 50 clinical, and 4 spiked samples which were spiked with purified ceruloplasmin in order to fully span the measuring range for both proposed and predicate devices. The following regression analysis (Passing/Bablok) was obtained:

$$y = 1.08x - 0.02$$

Slope (95% CI): 1.02 – 1.14
Intercept (95% CI): -0.03 – 0.00

b. Matrix comparison:

Not applicable, assay use serum sample only

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):

Not applicable

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

The reference range for adult is 0.2 – 0.6 g/L according to the literature. It is recommended that each laboratory establishes its own reference range.

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.