510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K161508

B. Purpose for Submission:

New Device

C. Measurand:

Human ceruloplasmin

D. Type of Test:

Turbidimetry, Quantitative

E. Applicant:

Beckman Coulter Ireland Inc.

F. Proprietary and Established Names:

Ceruloplasmin

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):

   System reagent for the quantitative determination of Ceruloplasmin (CER) in human serum and plasma on Beckman Coulter AU analyzers as an aid in the diagnosis of copper metabolism disorders.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For Prescription Use only

4. Special instrument requirements:

   For use on the Beckman Coulter AU analyzers: AU400/AU400^{e}/AU480, AU640/AU640^{e}/AU680 (K961274), AU2700 (K003721), AU5400 (K011720), AU5800 (K112412)

I. Device Description:

The Ceruloplasmin reagent kit consists of:

- R1: Rabbit anti-human Ceruloplasmin antiserum
- R2: Solution of polymers in Tris buffer (pH 7.4–7.6) with preservatives

The calibrator is a Beckman Coulter Serum Protein Multi-calibrator 2 (K992086) that is sold separately.

J. Substantial Equivalence Information:

1. Predicate device name:

   Siemens N Antisera to Human Ceruloplasmin

2. Predicate 510(k) number:

   K053074

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	System reagent for the quantitative determination of Ceruloplasmin (CER) in human serum and plasma on Beckman Coulter AU analyzers as an aid in the diagnosis of copper metabolism disorders.	In-vitro diagnostic reagents for the quantitative determination of ceruloplasmin and hemopexin in human serum and heparinized plasma by means of immunonephelopetry on the BN II and BN Prospec® system.
Assay Format	Quantitative	Same
Sample Type	Serum and plasma (sodium-heparin and lithium-heparin)	Serum and heparinized plasma
Antibody	Rabbit anti-human ceruloplasmin antiserum	Same
Solid Phase	Polystyrene microwells	Same
Traceability	Serum Protein Multi-calibrator is traceable to IFCC CRM470	N Protein Standard is traceable to ERM470
Expected Values	200–600 mg/L	Same
Reagent	Liquid, Ready to use	Same
Reagent Storage	2–8°C for one year	Same

Differences		
Item	**Device**	**Predicate**
Test Principle	Turbidimetry	Nephelometry
Analyte	Ceruloplasmin	Ceruloplasmin and hemopexin
Instrument(s)	Beckman Coulter AU analyzers	BNII, BN 100, and BN Prospec analyzers
Linearity Range	60–2000 mg/L	70–2200 mg/L
On-board Stability	90 days	3 days
Calibration Interval	14 days	Not specified

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

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
- CLSI EP6-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 EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient

Samples; Approved Guideline, Third Edition.
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition
- CLSI C28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Addition

L. Test Principle:

Ceruloplasmin in the specimen reacts with rabbit anti-human ceruloplasmin antibody to form immune complexes in solution. The formed insoluble complexes scatter light in proportion to their size, shape and concentration. The amount of transmitted light is indirectly proportional to the concentration of ceruloplasmin in the specimen. Ceruloplasmin concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

M. Performance Characteristics:

1. Analytical performance: The results presented below were within the sponsor's pre-determined acceptance criteria for each study.

   a. Precision/Reproducibility:

   Precision: The precision of the ceruloplasmin assay was evaluated on six pooled human serum samples containing various concentrations of ceruloplasmin. Each sample was run in duplicate, two runs per day, for 20 days (n = 80) by multiple operators using one lot of reagent on one AU5800 analyzer. Data were analyzed for within run, between run, between day and total precision. The results are summarized in the table below.

Sample	Mean (mg/L)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	95.99	1.04	1.1	1.81	1.9	6.09	6.3	6.44	6.7
2	148.36	1.69	1.1	1.94	1.3	3.18	2.1	4.09	2.8
3	254.17	2.30	0.9	2.77	1.1	4.40	1.7	5.69	2.2
4	596.43	5.46	0.9	4.87	0.8	8.68	1.5	11.36	1.9
5	915.61	6.78	0.7	3.45	0.4	12.35	1.3	14.51	1.6
6	1791.94	9.52	0.5	11.35	0.6	20.43	1.1	25.23	1.4

   Lot-to-lot Reproducibility: To evaluate lot-to-lot reproducibility, three samples with ceruloplasmin concentration at low, medium and high levels were tested. Each sample was tested in replicates of five per run, one run a day for five days on one AU5800 analyzer with three difference lots of reagent (N = 75 per sample). Mean and %CV for each sample were calculated. The results are summarized in the table below:

Sample	Mean (mg/L)	Within-Day		Between-Day		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Low	93.56	1.12	1.2	1.60	1.7	0.60	0.6	2.05	2.2
Medium	920.90	4.46	0.5	7.73	0.8	4.05	0.4	9.80	1.1
High	1786.64	10.29	0.6	33.50	1.9	0.00	0.0	35.07	2.0

b. Linearity/assay reportable range:

Linearity: The linearity across the assay's analytical measuring grange was evaluated by a study according to CLSI EP6-A. Eleven serially diluted samples with ceruloplasmin concentrations ranging from 17.37 to 2197.33 mg/L were prepared by mixing high positive serum samples with analyte depleted serum. Each dilution was tested in quadruplicate on the AU5800 analyzer using one lot of reagent. The weighted linear regression analysis resulted in the following equation:

Range (mg/L)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient	% Bias
17.37–2039.70	1.03 (1.02–1.04)	5.49 (1.02–9.96)	1.00	−1.82–6.36

The data support the linearity of the claimed measuring range of the Ceruloplasmin: 60–2000 mg/L.

Auto-Dilution: A study was performed to evaluate whether the analyzer dilutes the sample correctly using pre-dilution settings when a sample has a concentration above the assay range. Deionized water or saline were tested as diluents. A sample with a concentration within the range of 2000–3000 mg/L was prepared by spiking with Level 5 of Serum Protein multi-calibrator (ODR3023) or an in-house serum pool. The sample was tested using the 1:5 automatic dilution setting on one AU5800 analyzer and the diluted sample was run in replicates of 20 using one lot of reagent. The same sample prepared manually with a 1:5 fold dilution was used as the reference. The % bias for the results obtained with the auto-dilution compared to the results obtained with the manual dilution was 2.3% when deionized water was used as a diluent, and 5.8% when saline was used as a diluent.

Hook Effect: Hook effect was evaluated by using a spiked human serum sample with ceruloplasmin concentration of 3470 mg/L. The sample was diluted to multiple levels and the diluted samples were measured in triplicate using one lot of reagent on one AU5800 analyzer. No hook effect was observed for the sample with concentration up to 3470 mg/L.

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

Traceability: The calibration material is traceable to IFCC International Reference Preparation CRM470 (RPPHS).

Value assignment: The calibrator used for the Ceruloplasmin is Serum Protein Multi-calibrator 2 which was cleared under K992086.

Stability:

Kit stability (unopened): A real-time stability study was performed using three lots of reagents stored under the recommended temperature at 2–8°C. The data were collected at different time points up to 19 months. The results support stability of the kits under the recommended storage of 2–8°C for 18 months.

On-board (In-use) stability: On-board stability was tested using one lot of reagent and one lot of calibrator. The reagent was calibrated and placed in the refrigerated compartment (2-8°C) of the analyzer. The data were collected at different time points up to 90 days. The results support that the on-board reagent stability is up to 90 days and the calibration stability is up to 14 days.

d. Detection limit:

Limit of Blank (LoB) was determined by assaying four blank samples (5% BSA in 0.9% saline) with three replicates per sample over five days with three different reagent lots . A total of 60 data points per lot were generated. LoB for each lot was calculated separately as the 95th percentile value. The LoB for three lots was determined to be 5.56 mg/L, 5.72 mg/L and 6.29 mg/L. The claimed LoB value is 6.29 mg/L.

The Limit of Detection (LoD) was determined by assaying four human serum pools that were diluted with 0.9% Saline/BSA to achieve low analyte values. Each sample was tested in triplicate across five days with three different reagent lots. The LoD value was calculated as the LoB + 1.645 x SD of the replicates for the low level samples. The LoD for three lots was determined to be 12.15 mg/L, 11.56 mg/L and 11.84 mg/L. The claimed LoD is 12.15 mg/L.

The Limit of Quantitation (LoQ) was determined based on the analysis of total imprecision of the LoD study with a total error goal of 20%. The LoQ was determined using seven pooled human serum samples that were diluted to have analyte concentrations between 2.00 to 40.00 mg/L. Each sample was measured in triplicate over the course of five days with three different reagent lots. The LoQ for three lots was determined to be 22.55 mg/L, 22.09 mg/L and 20.83 mg/L. The claimed LoQ is 22.55 mg/L.

e. Analytical specificity:

Endogenous Interference: Interferences were assessed following CLSI EP7-A2 by testing two human serum pools with low concentration (150–200 mg/L) and intermediate concentration (500–600 mg/L) of ceruloplasmin. Each sample was spiked with the interfering substances and tested in replicates of four on one AU5800 analyzer using one lot of reagent. The sample without spiking the interfering

substances was used as reference. The % recovery of the results from the sample with the interference substance compared to the reference was calculated and no interference was detected in the samples up to the concentrations listed in the table below:

Interfering Substances	Final Test Concentration	% Recovery
Bilirubin (unconjugated)	40 mg/dL	97.78–100.27 %
Hemoglobin	500 mg/dL	98.86–101.24 %
Triglycerides (Intralipid)	1000 mg/dL	100.60–101.98 %
Rheumatic Factor	76 IU/mL	92.00–102.00 %

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A total of 120 serum samples including 103 samples from individual donors and 17 contrived samples were tested with the Ceruloplasmin and the predicate. The contrived samples were used to cover the analytical measuring range of both devices and were prepared by spiking purified human ceruloplasmin into normal human serum samples. The results are summarized in the table below:

N	Range*(mg/L)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
120	9.0–1880.0	1.06 (1.04–1.08)	−26.10 (−31.95– −20.36)	0.99

b. Matrix comparison:

A study was performed to demonstrate that heparinized plasma (drawn in lithium-heparin and sodium-heparin plasma separation tubes) yields values comparable to serum when tested with the device. A total of 110 or 111 samples with concentrations spanning the assay range were assayed on one AU5800 analyzer using one lot of reagent. Deming regression analysis was performed using the data from different plasma preparations compared to the data from serum samples. The results are summarized in the tables below:

	N	Range* (mg/L)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
Li-heparin Plasma vs. Serum	110	72.60–1745.47	0.97 (0.96–0.98)	3.64 (0.13–7.15)	1.00
Na-heparin Plasma vs. Serum	111		0.99 (9.99–1.00)	−4.44 (7.95–−0.93)	1.00

* values from the serum sample

3. Clinical studies:

   a. Clinical Sensitivity and Clinical 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 range:

   The reference range of the serum ceruloplamin in the normal healthy adults is 200–600 mg/L according to Baudner and Dati (1996)*. To validate this reference range, 20 serum samples from healthy adults were tested in duplicate using two lots of reagents on one representative AU5800 analyzer. One sample tested with a mean value of 199.25 mg/L and the rest of samples with a ceruloplamin level within the reference range of the assay. The sponsor provided the following statement in the package insert: "Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values".

   * Baudner, S. and Dati, F. (1996). Standardization of the measurement of 14 proteins in human serum based on the new IFCC/BCR/CAP international reference material CRM 470. J. Lab Med 20:145-152

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.