510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k132711

B. Purpose for Submission:

New device

C. Measurand:

HDL Cholesterol assay

D. Type of Test:

Quantitative, colorimetric assay

E. Applicant:

Carolina Liquid Chemistries Corp.

F. Proprietary and Established Names:

Carolina Liquid Chemistries HDL Cholesterol Reagent

G. Regulatory Information:

1. Regulation section:

CFR 21 section 862.1475 - Lipoprotein test system

2. Classification:

Class I, meets limitations per exemption 21 CFR 862.9(c)(4)

3. Product code:

LBS

4. Panel:

Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indication(s) for use.

2. Indication(s) for use:

   For the quantitative determination of high-density lipoprotein cholesterol (HDL-C) in serum using the Carolina Liquid Chemistries CLC720® Clinical Chemistry Analyzer. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Carolina Liquid Chemistries CLC720® Clinical Chemistry Analyzer (Cleared in k112377)

I. Device Description:

The Carolina Liquid Chemistries HDL Cholesterol assay is a homogeneous method for directly measuring serum HDL-C levels without the need for any off-line pretreatment or centrifugation steps. The method is in a two-reagent format. The first reagent contains a polyanion and a chromagen. The second reagent contains standard cholesterol enzymes along with a special detergent that has selective affinity for HDL versus other lipoproteins (LDL, VLDL, Chylomicrons).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Olympus HDL Cholesterol Reagent

2. Predicate 510(k) number(s):

   k040692

3. Comparison with predicate:

Reagent Similarities		
Item	Candidate Device: Carolina Liquid Chemistries HDL Cholesterol Reagent (k132711)	Predicate Device: Olympus HDL Cholesterol Reagent (k040692)
Intended Use	System reagent for the quantitative determination of HDL-Cholesterol. For in vitro diagnostic use	Same
Measurand Analyte	HDL-Cholesterol	Same
Test Principle	Detergent solubilization of HDL to selectively measure HDL cholesterol using an enzymatic method	Same
Reagent Composition	Reactive ingredients: Cholesterol esterase, Cholesterol oxidase, Peroxidase, DSBmT (N,N-bis(4-sulphobutyl)-m-toluidine-disodium salt), 4-aminoantipyrine	Same
Reagent Storage and Stability	Unopened reagent is stable until the expiration date stated on the vial label when stored at 2-8°C; opened reagents are stable for 30 days when stored in the refrigerator compartment of the analyzer	Same

Reagent Differences		
Item	Candidate Device: Carolina Liquid Chemistries HDL Cholesterol Reagent (k132711)	Predicate Device: Olympus HDL Cholesterol Reagent (k040692)
Sample Type	Serum	Serum and Plasma

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

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition.

CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.

CLSI EP9-A2, Method Comparison and Bias Estimation Using Patient Samples, 2002

L. Test Principle:

The method is in a two-reagent format. The first reagent contains a polyanion and a chromagen. The second reagent contains standard cholesterol enzymes along with a special detergent that has selective affinity for HDL verses the other lipoproteins (LDL, VLDL, Chylomicrons).

The reaction can be described as follows:

$$\text{LDL, VLDL, Chylomicrons} \xrightarrow[\text{Polyanion}]{\text{Detergent}} \text{Stable Complexes}$$

$$\text{HDL} \xrightarrow{\text{Detergent}} \text{HDL Disrupted}$$

$$\text{HDL Cholesterol} \xrightarrow[\text{Cholesterol Oxidase}]{\text{Cholesterol Esterase}} \text{Cholest-4-en-3-one}$$

$$\text{H2O2 + DSBmT + 4-AA} \xrightarrow[\text{Peroxidase}]{} \text{Color Development}$$

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision Studies were conducted utilizing the Carolina Liquid Chemistries HDL Cholesterol Reagent on the Carolina Liquid Chemistries CLC 720 Chemistry Analyzer following the recommendations in the CLSI guideline EP5-A2. A total of three serum-based control materials were analyzed 40 times for the within-run precision, and were analyzed in triplicate two times per day for 20 non-consecutive days for the total precision analysis. A single reagent lot and instrument was used for the analysis. The results are summarized below:

Precision						
	WITHIN RUN			**TOTAL PRECISION**		
	Level 1	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**
N	40	40	40	120	120	120
MEAN (mg/dL)	65.3	45.0	26.9	65.3	45.0	26.9
SD (mg/dL)	1.04	0.92	0.66	1.60	1.32	1.13
CV (%)	1.6	2.0	2.5	2.4	2.9	4.2

The sponsor performed the following additional precision studies on the Carolina Liquid Chemistries CLC 720 Chemistry Analyzer following recommendations in the CLSI guideline EP5-A2. Two clinical sample pools were analyzed 20 times for the within-run precision, and were analyzed two times per day, for 30 non-consecutive days, for the total precision determination. A single reagent lot (different lot from above described study) and instrument (same as above study) was used for the analysis. The results are summarized below:

Precision					
	WITHIN RUN			**TOTAL PRECISION**	
	Level 1	**Level 2**		**Level 1**	**Level 2**
N	20	20		60	60
MEAN (mg/dL)	43.2	83.6		41.2	81.4
SD (mg/dL)	0.796	0.582		1.80	2.01
CV	1.8	0.7		4.4	2.5

b. Linearity/assay reportable range:

Linearity studies were carried out using six commercially available HDL Linearity

standards. All samples were tested five times. The sample range tested was 8.6 mg/dL to 165.2 mg/dL.

Range tested	Intercept	Slope	r^2
8.6 to 165.2 mg/dL	0.83	1.002	1.00

The sponsor demonstrated acceptable linearity from 8.6 to 165.2 mg/dL.

The claimed measuring range of the device is from 9 to 150 mg/dL.

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

The sponsor recommends the use of a commercially available calibrator (the JAS Diagnostics HDL Calibrator traceable to NIST SRM 911b) which was previously cleared in k021671.

Stability testing was performed by the manufacturer. The reagents are stable for 12 months when stored between 2-8 °C.

On board stability was performed on the CLC 720 Chemistry Analyzer demonstrated that the reagents are stable for 30 days when stored on board the CLC 720 Chemistry Analyzer when stored at 2-8 °C.

The reagent system has not been tested or certified by the Cholesterol Reference Method Laboratory Network (CRMLN). The labeling contains language that the device has not been certified by the CRMLN.

d. Detection limit:

A limit of detection study was conducted based on procedures described in CLSI EP17-A. The limit of the blank (LoB) of 0.16 mg/dL was calculated from data using a pooled serum sample diluted with 0.85 % sodium chloride assayed 8 times and repeated for a total of ten individually calibrated runs to produce 80 results. The LoD of 0.53 mg/dL was calculated as the LoB plus the one-tailed 95% confidence interval of the test results. The LoQ of 6.48 mg/dL was determined with a performance goal of $\leq$20% CV and is lower than the lower limit of the measuring range which is set at 9 mg/dL.

e. Analytical specificity:

To determine the level of interference from substances normally present in human serum, the Carolina Liquid Chemistries HDL Cholesterol Reagent was tested with low level HDL serum samples containing about 32-38 mg/dL HDL-C and high level HDL serum samples containing about 62-72 mg/dL HDL-C. Both levels were spiked with various concentrations of potentially interfering substances following the recommendations in the

CLSI guideline EP7-A2 on the CLC 720 Chemistry Analyzer. A total of four or six levels were tested for interferents and all samples were tested in six runs with eight replicates. The following substances normally present in serum produced less than 10% deviation when tested at levels equal to the following concentrations:

Interference Substance	Concentration
Bilirubin (conjugated and unconjugated)	20 mg/dL
Hemolysis	200 mg/dL
Lipemia	800 mg/dL
Ascorbic Acid	30 mg/dL
γ-Globulin	5 g/dL

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were conducted following the recommendations in the CLSI EP9-A2 guideline.

80 serum samples were assayed in parallel by singlicate runs on both the Carolina Liquid Chemistries HDL Cholesterol Reagent on the Carolina Liquid Chemistries CLC 720 Chemistry Analyzer and a predicate method. The results were analyzed by using Deming regression. The range tested was 11.2 mg/dL to 150.2 mg/dL. Altered samples were included in the study (16%).

The comparison by Deming regression resulted in a slope of 0.958 (95%CI = 0.939 to 0.977), an intercept of 1.06 (95%CI = -0.32 to 2.45), correlation coefficient = 0.9961, and a standard error of 2.43.

b. Matrix comparison:

Not applicable

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**:**

Not applicable

5. Expected values/Reference range:

The expected values for serum HDL cholesterol are as follows:

Low: <40 mg/dL
High: ≥60 mg/dL

* National Cholesterol Education Program. Third report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. NIH Pub No. 02-5215. Bethesda, MD: National Heart, Lung, and Blood Institute (2002).

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.