DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

ROCHE DIAGNOSTICS OPERATIONS (RDO)
BARBARA MCWHORTER
REGULATORY AFFAIRS PRINCIPAL
9115 HAGUE ROAD
SUITE 114
INDIANAPOLIS IN 46250

October 19, 2016

Re: K162593
Trade/Device Name: HDL-Cholesterol Gen.4
Regulation Number: 21 CFR 862.1475
Regulation Name: Lipoprotein test system
Regulatory Class: I, meets the limitation of exemption 21 CFR §862.9(c)(4)
Product Code: LBS
Dated: September 14, 2016
Received: September 16, 2016

Dear Ms. McWhorter:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
k162593

Device Name
HDL-Cholesterol Gen.4

Indications for Use *(Describe)*
In vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in human serum and plasma on Roche/Hitachi cobas c systems.

A lipoprotein test system is a device intended to measure lipoprotein in serum and plasma. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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HDL-Cholesterol Gen.4
510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

In accordance with 21 CFR 807.87, Roche Diagnostics hereby submits official notification as required by Section 510(k) of the Federal Food, Drug and Cosmetics Act of our intention to market the device described in this Premarket Notification 510(k).

The purpose of this Traditional 510(k) Premarket Notification is to obtain FDA review and clearance for the HDL-Cholesterol Gen.4 reagent.

Submitter Name	Roche Diagnostics
Address	9115 Hague Road P.O. Box 50416 Indianapolis, IN 46250-0457
Contact	Barbara McWhorter Phone: (317) 521-2336 FAX: (317) 521-2324 Email: barbara.mcwhorter@roche.com
Date Prepared	September 13, 2016
Proprietary Name	HDL-Cholesterol Gen.4
Common Name	HDL-Cholesterol
Classification Name	Lipoprotein test system
Product Codes, Regulation Numbers	LBS, 21 CFR § 862.1475, Class I, meets the limitations to the exemptions 21 CFR§ 862.9(c)(4)
Predicate Devices	Ultra N-geneous HDL Cholesterol Reagent, k021316
Establishment Registration	For the HDL-Cholesterol Gen.4, the establishment registration number for Roche Diagnostics GmbH in Mannheim, Germany is 9610126. The establishment registration number for Roche Diagnostics in the United States is 1823260.

1. DEVICE DESCRIPTION

The HDL-Cholesterol Gen.4 is a homogeneous enzymatic colorimetric test. Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of CHER and CHOD towards non-HDL lipoproteins is blocked. Finally only HDL-particles can react with CHER and CHOD. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD.

2. INDICATIONS FOR USE

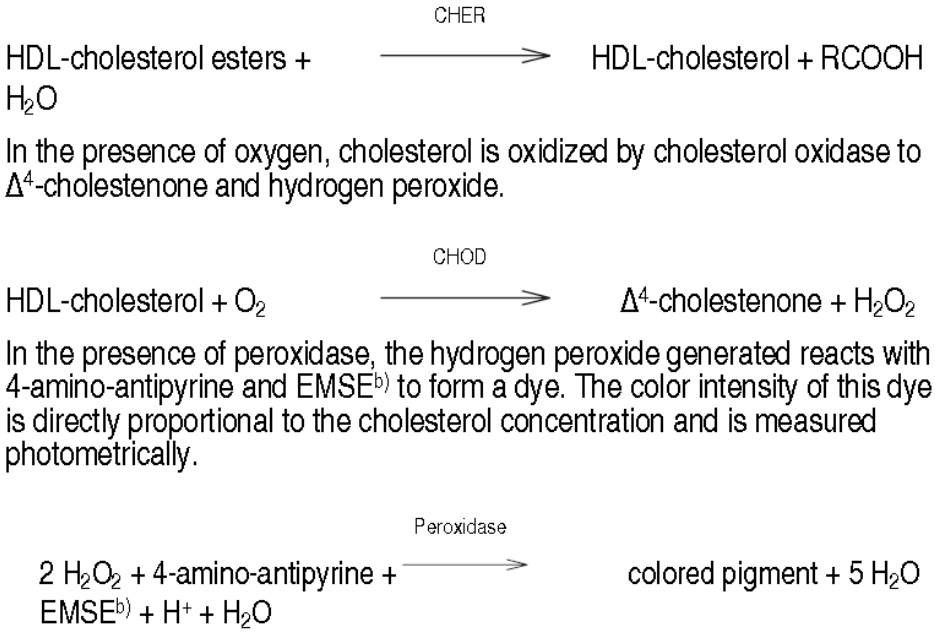
In vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in human serum and plasma on Roche/Hitachi cobas c systems.

A lipoprotein test system is a device intended to measure lipoprotein in serum and plasma. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases.

3. TECHNOLOGICAL CHARACTERISTICS

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by CHER. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSE to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Figure 1: Test Principle



The following table compares the HDL-Cholesterol Gen.4 with its predicate device, Ultra N-geneous HDL Cholesterol Reagent (k021316).

Table 1: Assay Comparison

Feature	Predicate Device Ultra N-geneous HDL Cholesterol Reagent, (k021316)	Candidate Device HDL-Cholesterol Gen.4
Intended Use	For the quantitative measurement of high-density lipoprotein cholesterol (HDL-C) concentration in human serum or plasma.	In vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in human serum and plasma on Roche/Hitachi cobas c systems.

Feature	Predicate Device Ultra N-geneous HDL Cholesterol Reagent, (k021316)	Candidate Device HDL-Cholesterol Gen.4
Test Principle	The method is in a two reagent format and depends on the properties of a unique detergent, as illustrated. This method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent. In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding a colorless product. The second reagent consists of a detergent capable of solubilizing HDL specifically, cholesterol esterase (CE) and chromogenic coupler to develop color for the quantitative determination of HDL-C. This may be referred to as the Accelerator Selective Detergent methodology.	Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by CHER. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSE to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.
Reagent Composition	R1: Cholesterol oxidase, (Fr: E. Coli),Peroxidase, (Fr: Horseradish), N,N-bis(4-sulphobutyl)-mtoluidine-disodium(DSBmT), Accelerator, Preservative, Ascorbic Oxidase,(Fr: Curcubita sp.) R2: Buffer, Cholesterol esterase (Fr:Pseudomonas sp.), 4-Aminoantipyrine (4-AAP), Detergent ,Preservative	R1: TAPSO buffer: 62.1 mmol/L, pH 7.77; polyanion: 1.25 g/L; EMSEb) : 1.08 mmol/L; ascorbate oxidase (cucurbita): ≥ 50 µkat/L; peroxidase (horseradish): ≥ 166.7 µkat/L; detergent; BSA: 2.0 g/L; preservative R2: Bis-Tris buffer: 20.1 mmol/L, pH 6.70; cholesterol esterase (microorganism): ≥ 7.5 µkat/L; cholesterol oxidase (recombinant E. coli): ≥ 7.17 µkat/L; cholesterol oxidase (microorganism): ≥ 76.7 µkat/L; peroxidase (horseradish): ≥ 333 µkat/L; 4-amino-antipyrine: 1.48 mmol/L; BSA: 3.0 g/L; detergents; preservative
Sample Type/Matrix	Serum Plasma: EDTA or lithium or sodium heparin.	Serum. Plasma: Li-heparin, K2- and K3-EDTA plasma
Calibrator	Sekisui Diagnostics Ultra N-geneous® HDL Cholesterol Calibrator or the HDL Ultra Cholesterol Calibrator	S1: H2O S2: C.f.a.s. Lipids

Feature	Predicate Device Ultra N-geneous HDL Cholesterol Reagent, (k021316)	Candidate Device HDL-Cholesterol Gen.4
Controls	The National Cholesterol Education Program (NCEP) Lipid Standardization Panel (LSP) recommends two levels of controls, one in the normal range (40-65 mg/dL) and one near the concentrations for decision making (<40 mg/dL). An acceptable range of HDL cholesterol values should be established by each laboratory. If control values are not within the expected range, confirm that procedures were performed correctly and follow normal troubleshooting measures.	PreciControl ClinChem Multi 1 PreciControl ClinChem Multi 2
Reagent Stability	Reagent 1 is stable open on the analyzer for 4 weeks at 2-8°C. Reagent 2 is stable open on the analyzer for 4 weeks at 2-8°C. DO NOT FREEZE	Shelf life at 2-8 °C: See expiration date on cobas c pack label. On-board in use and refrigerated on the analyzer: 12 weeks
Measuring Range	2.5 mg/dL to 200 mg/dL	3.09-150 mg/dL

4. NON-CLINICAL PERFORMANCE EVALUATION

The following performance data were provided in support of the substantial equivalence determination:

Precision according to CLSI EP5-A3

Detection Limit: LoB, LoD and LoQ according to CLSI EP17-A2

Linearity according to CLSI EP6-A

Endogenous Interferences - H, L and I Indices

Endogenous Interferences - Triglycerides

Exogenous Interferences - Drugs

Method Comparison to Predicate

Matrix Comparison - Anticoagulants

4.1. Precision

4.1.1. Repeatability and Intermediate Precision

Precision was evaluated per a protocol with modifications from the study design recommended in CLSI Guideline EP5-A3. The testing was run for 21 days; 4 individual replicates were tested in one run per day. Two replicates were tested at the beginning of the run, followed by 10 patient samples and then two replicates were tested at the end of the run. Five human serum pools and two control samples were tested on one cobas c 501, using 3 lots of reagent. Mean, Repeatability (within run precision) and Intermediate precision (within-lab precision) %CV and SD values were calculated.

4.1.2. Results and Conclusions

Table 2: Repeatability Summary

Specimen	Mean (mg/dL)	SD (mg/dL)	CV (%)
PreciControl ClinChem Multi 1	28.0	0.20	0.7
PreciControl ClinChem Multi 2	68.1	0.44	0.6
Human Serum 1	9.48	0.17	1.8

Specimen	Mean (mg/dL)	SD (mg/dL)	CV (%)
Human Serum 2	40.5	0.26	0.7
Human Serum 3	59.4	0.32	0.5
Human Serum 4	79.4	0.51	0.6
Human Serum 5	141	0.83	0.6

Table 3: Intermediate Precision Summary

Specimen	Mean (mg/dL)	SD (mg/dL)	CV (%)
PreciControl ClinChem Multi 1	28.4	0.30	1.1
PreciControl ClinChem Multi 2	66.4	0.9	1.4
Human Serum 1	9.48	0.20	2.2
Human Serum 2	40.7	0.33	0.8
Human Serum 3	59.4	0.40	0.7
Human Serum 4	79.4	0.65	0.8
Human Serum 5	141	1.07	0.8

4.2. Analytical Sensitivity

4.2.1. Limit of Blank (LoB)

LoB of the HDL-Cholesterol Gen.4 on the cobas c 501 analyzer was determined according to CLSI EP17-A2. Limit of Blank determines the highest observed measurement values for samples free of analyte. The Limit of Blank was determined as the 95th percentile of measurements of blank samples.

One analyte free sample was measured on three lots of reagent in 10-fold determinations in 6 runs, distributed over 3 days, on one analyzer. In total, 60 measurements were obtained per reagent lot. Data analysis was based on determination of the 95th percentile of the 60 measured values.

LoB Observed: 0.00 mg/dL

LoB Claim: 3.09 mg/dL

4.2.2. Limit of Detection (LoD)

LoD of the HDL-Cholesterol Gen.4 on the cobas c 501 analyzer was determined according to CLSI EP17-A2. The LoD determines the lower limit for samples with analyte. The LoD is the concentration at which there is a 95% probability that a sample contains analyte.

For determination of LoD, five samples with low-analyte concentration (approximately up to 4 times the LoB) were measured with three lots of reagent in two-fold determination in 6 runs, distributed over 3 days, on one analyzer. In total 60 measurements were obtained per lot of reagent.

LoD was determined using the following equation:

$$LoD = LoB + 1.653 \times SD_{tot}$$

Where:

$$SD_{total} = \text{Square root } [0.2 \times ((SD_{sample\,1})^2 + (SD_{sample\,2})^2 + (SD_{sample\,2})^2 + (SD_{sample\,4})^2 + (SD_{sample\,5})^2)].$$

LoD Observed: 0.50 mg/dL

LoD Claim: 3.09 mg/dL

4.2.3. Limit of Quantitation (LoQ)

The LoQ of the HDL-Cholesterol Gen.4 was determined on the cobas c 501 analyzer according to CLSI Guideline EP17-A2.

The LoQ is derived from a plot (mean concentration versus %CV) with the goal of %CV less than or equal to 20% which was met. A low level sample set was prepared by diluting 5 human serum samples with an analyte free diluent (0.9% NaCl). The low level sample set was tested in 5 replicates per sample on 5 days, one run per day on one analyzer.

LoQ Observed: 2.89 mg/dL

LoQ Claim: 3.09 mg/dL

4.3. Linearity/Assay Reportable Range

4.3.1. Regression Analysis

The linearity study was conducted to demonstrate that measurements across the claimed measuring range for each parameter are linear. The study was performed according to CLSI guideline EP6-A.

Dilution series were prepared using the human sample pools (1 serum pool and 1 plasma pool) with HDL-C concentrations above the upper end of the measuring range. Dilutions were made using 0.9% NaCl. The dilution series contain 11 concentrations for serum and 15 concentrations

for plasma. Sample dilution levels were measured in triplicate and data analysis was done separately for each sample type.

Linear regression analysis was done according to EP6-A.

In a first step, a linearity check was performed with first order (linear) regression and then with higher order models (quadratic and cubic). A linearity check was performed with a first order (linear) regression for plasma and additionally a 2nd order regression for serum. The linear regression was weighted $1/Conc^2$.

The nonlinearity of the assay did not deviate by more than 10% during testing.

Table 4: Linearity Results

	Slope	Intercept	Correlation Coefficient (r2)	Repeatability	Claimed Measuring Range
Serum	1.020	-0.399	0.9992	1.5%	3.09 to 150 mg/dL
Plasma	1.022	-0.173	0.9929	0.8%	3.09 to 150 mg/dL

4.4. Endogenous Interferences

4.4.1. L, H, and I Indices

The effect on quantitation of analyte in the presence of endogenous interfering substances was determined at two HDL-Cholesterol concentrations (approximately 33 and 65 mg/dL) and a dilution set of the added interfering substances. Interfering substances evaluated include:

Hemolysis up to an H index of 1200

Lipemia up to an L index of 2000

Icterus/Conjugated and Unconjugated Bilirubin up to an I index of 60

High concentrated stock solutions of the interference substances were prepared in a suitable solvent. Two human serum pools were spiked with the defined HDL-Cholesterol concentrations and divided into two aliquots. The potential interfering substance was added to one aliquot, while the other aliquot was mixed with the same amount of solvent without the interfering substance. A

dilution series was prepared with 11 dilution steps for each interferent by mixing the 2 aliquots. Three aliquots per level were tested in 1 run on 1 instrument and 1 lot.

The aliquot containing the interfering substance had the same HDL-Cholesterol concentrations as the aliquots containing no interfering substance. When diluting those two aliquots the HDL-Cholesterol concentration remained constant while the concentration of interferent varied. Thus the effect of increasing concentrations of interferent can be determined.

Mean of the measured results were compared to the expected result (aliquot with no interfering substance) and the recovery was determined (paired difference testing). Interference was defined as bias greater than 10%.

This procedure was repeated for each of the interfering substances.

Table 5: H, L, I Indices/Serum Indices Interference

Interferent	Claim
Hemolysis	No Interference up to 1200 H index
Lipemia	No Interference up to 2000 L index
Unconjugated Bilirubin	No Interference up to 60 I index
Conjugated Bilirubin	No Interference up to 60 I index

4.4.2. Triglycerides Interference

The effect on quantitation of analyte in the presence of endogenous interfering substances was determined at two HDL-Cholesterol concentrations (approximately 33 and 65 mg/dL) and a dilution set of the added interfering substance.

High concentrated stock solution of triglycerides was prepared in a suitable solvent. Two human serum pools were spiked at the defined HDL-Cholesterol concentrations and divided into two aliquots. The potential interfering substance was added to one aliquot, while the other aliquot was mixed with the same amount of solvent without the interfering substance. A dilution series was prepared with 11 dilution steps for each interferent by mixing the 2 aliquots. Three aliquots per level were tested in 1 run on 1 instrument and 1 lot.

The parts containing the interfering substance had the same HDL-Cholesterol concentrations as the aliquots containing no interfering substance. When diluting those two aliquots the HDL-

Cholesterol concentration remained constant while the concentration of triglycerides varied. Thus the effect of increasing concentrations of interferent can be determined.

Mean of the measured results were compared to the expected result (aliquot with no interfering substance) and the recovery was determined (paired difference testing).

Interference was defined as bias greater than 10%.

Table 6 Triglycerides Interference

Interferent	Claim
Triglycerides	No Interference up to 1200 mg/dLTriglycerides

4.5. Exogenous Interferences – Drugs

Two human serum sample pools spiked with approximately 30 and 60 mg/dL HDL-Cholesterol concentrations were divided into two aliquots. One aliquot of each concentration were used as the reference sample for HDL-Cholesterol concentration and were not spiked with the drugs but the solvent for the drug.

The other aliquots, with either the high or low HDL-Cholesterol concentration, were spiked with the respective amount of drug. The HDL-Cholesterol concentration of the spiked aliquots were tested with 3 replicates in one run, 1 reagent lot and one instrument. The defined drug compounds were spiked into samples with concentrations according to EP7-A2 or higher concentrations. The drugs included:

Table 7: Potentially Interfering Drugs and Test Concentrations Results

Potential Interferent	Concentration mg/L
Acetylcystein	553
Ampicillin-Na	1000
Ascorbic acid	500
Cyclosporine	5
Cefoxitin	2500
Heparin	5000 U

Potential Interferent	Concentration mg/L
Intralipid	10000
Levodopa	4
Methyldopa +1.5	4
Metronidazole	200
Phenylbutazone	400
Doxycyclin	50
Acetylsalicylic Acid	1000
Rifampicin	60
Acetaminophen	200
Ibuprofen	500
Theophyllin	100
Bezafibrate	120
Simvastatin	16

4.6. Method Comparison to Predicate

One hundred eleven routine laboratory serum samples were used in the method comparison testing. No patient information was obtained.

Four samples were spiked with high human serum HDL-Cholesterol. One sample was diluted with 0.9% NaCl.

The samples were tested in singlicate using the HDL Ultra Cholesterol reagent from Sekisui on Roche/Hitachi 917 analyzer and the HDL-Cholesterol Gen.4 reagent on cobas c 501 analyzer.

The data was evaluated using Passing Bablok Regression analysis.

Regression analysis results:

$y = 0.956x - 0.949$, $r = 0.995$

Bias at medical decision points:

-6.7 % at 40.2 mg/dL

-6.0 % at 59.9 mg/dL

4.7. Matrix Comparison - Anticoagulants

Each pair of serum and plasma of a single donor were spiked with HDL-Cholesterol. The 38 paired sample tubes are tested in singlicate.

A method comparison was executed by using the serum data as the reference. Only samples within the measuring range were used.

The following method comparisons were completed:

Gel separation tubes

K2-EDTA plasma vs serum

K3-EDTA plasma vs serum

Li-Heparin plasma vs serum

Table 8: Matrix Comparison Results

Anticoagulant	Linear Regression	Range Tested
Serum vs. Serum Gel Separation	y = 0.99x - 0.33, r = 0.999	3.48 to 145 mg/dL
Serum vs. Li-heparin	y = 0.99x - 0.32, r = 1.000	3.48 to 147 mg/dL
Serum vs. K2-EDTA	y = 0.98x - 0.70, r = 0.999	3.87 to 144 mg/dL
Serum vs. K3-EDTA	y = 0.95x - 0.08, r = 0.999	3.87 to 145 mg/dL

5. CLINICAL PERFORMANCE EVALUATION

Not Applicable

6. ADDITIONAL INFORMATION

6.1. Other Devices Required But Not Provided

- The Calibrator f.a.s. Lipids, k011658
- The PreciControl ClinChem Multi 1 and 2, k102016
- Diluent NaCl 9%

There have been no changes to these items marketed with the new HDL-Cholesterol Gen.4.

7. CONCLUSIONS

The submitted information in this premarket notification supports a substantial equivalence decision.