

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

A. 510(k) Number:

k072523

B. Purpose for Submission:

New device

C. Measurand:

Low Density Lipoprotein Cholesterol

D. Type of Test:

Quantitative colorimetric Trinder reaction

E. Applicant:

General Atomics, Diazyme Laboratories Division

F. Proprietary and Established Names:

Proprietary Name – Diazyme LDL-Cholesterol Reagent
Established Name – LDL Cholesterol

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1475: Lipoprotein Test System
21 CFR 862.1660: Quality control material (assayed and unassayed)

2. Classification:

Class I (meets limitations of exemptions 21 CFR 862.9 (c) (4)) - Diazyme LDL-Cholesterol Reagent
Class I – Diazyme LDL Controls

3. Product code:

Diazyme LDL-Cholesterol – LBR
Diazyme LDL- Cholesterol Control - JJX

4. Panel:

75 - Chemistry

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The Diazyme LDL-Cholesterol reagent is intended for the in vitro quantitative determination of Low Density Lipoprotein Cholesterol in human serum or plasma. The reagents can assist in the diagnosis and treatment of patients at risk of developing coronary heart disease. Elevated LDL Cholesterol is the primary target of cholesterol-lowering therapy

Calibrator – For calibration of the Diazyme LDL-Cholesterol Reagent Assay in serum or plasma. For In Vitro Diagnostic Use

Controls – To monitor the performance of the Diazyme LDL-Cholesterol Reagent. For In Vitro Diagnostic Use

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

All testing was performed on the Roche Diagnostics Hitachi 917.

I. Device Description:

The Diazyme LDL-Cholesterol kit contains two reagents Reagent 1 4X70ml bottles and Reagent 2 2X50ml bottles and 2X1ml calibrator materials. In addition, control materials consisting of three lyophilized human serum based materials are sold separately. All donor pools for the serum pool used to make the control and calibrator materials have been tested by FDA licensed methods and found non reactive for hepatitis B surface antigen, hepatitis C and HIV I and II. The assay is based upon a

modified polyvinyl sulfonic acid and polyethylene-glycol-methyl ether coupled precipitation to LDL, VLDL and chylomicrons making them inaccessible to cholesterol oxidase and cholesterol esterase. HDL then reacts with cholesterol oxidase and cholesterol esterase. Addition of special detergents in the R2 reagent release LDL which reacts with H₂O₂ which is then quantified by the Trinder Reaction.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Ultra N-Geneous LDL Cholesterol Reagent and Calibrator
Bio-Lipid Lipid Controls

2. Predicate K number(s):

k021316, k951749

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indication for Use	The Diazyme LDL-Cholesterol reagent is intended for the in vitro quantitative determination of Low Density Lipoprotein Cholesterol in human serum or plasma. The reagents can assist in the diagnosis and treatment of patients at risk of developing coronary heart disease. Elevated LDL Cholesterol is the primary target of Cholesterol-lowering therapy	For the quantitative determination of low density lipoprotein cholesterol (LDL-C) in human serum or plasma
Type of Test	Quantitative	Quantitative
Specimen Type	Serum or Plasma	Serum or Plasma
Calibrator	Lyophilized	Lyophilized
Control	3 levels, lyophilized	3 levels, lyophilized

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

The following standards have been used to support this submission:

EP05-A2 – Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

EP06-A - Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

EP09-A2 – Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline -Second Edition

EP21-A - Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline

L. Test Principle:

Colorimetric Trinder reaction (Cholesterol Oxidase and Cholesterol Esterase)

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision of the Diazyme HDL- Cholesterol Reagent was evaluated using the CLSI EP5-A guideline. In the study three levels of serum samples were tested with 2 runs per day in duplicate over 20 days using the Roche Diagnostics Hitachi 917. An additional precision study was performed on the Hitachi 917 using one level of serum containing about 70 mg/dL of LDL-C tested in two runs per day in duplicate for 5 working days. The results are presented in the table below.

Within Run Precision

	Level 1	Level 2	Level 3	Level 4
N	20	80	80	80
Mean	71.19	97.14	147.37	211.47
SD	0.50	1.00	1.19	1.38
CV%	0.70	1.0	0.8	0.7

Total Precision

	Level 1	Level 2	Level 3	Level 4
N	20	80	80	80
Mean	71.19	97.14	147.37	211.47

	Level 1	Level 2	Level 3	Level 4
SD	1.15	1.55	2.23	2.98
CV%	1.6	1.6	1.5	1.4

b. Linearity/assay reportable range:

Linearity determination was based upon NCCLS (CLSI) EP6-A. 16 samples were prepared by diluting a serum sample containing 250 mg/dL of LDL-C. Each level was measured in triplicate on the Hitachi 917. The resulting regression equation was: $Y=0.9848X-1.89$, $r^2=0.9989$. The sponsor determined an acceptable % error was $\leq 10\%$. The reportable range for the assay is 2 – 250 mg/dL

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

This device has not been certified or tested by the Cholesterol Reference Method Laboratory Network (CRLMN). A statement reflecting this is included in the labeling.

The NIST SRM 1915b material was tested with the Diazyme LDL-Cholesterol reagent and found to have a deviation from the NIST target value of 2.8% for level I (116.39 mg/dL) and 0.95% for level II (154.05 mg/dL).

Values are assigned to the control materials by repeat testing on the Hitachi 917 using the Diazyme LDL-cholesterol reagent and calibrator. The assigned ranges are lot specific. The following ranges were obtained for a tested lot: Level I 142-214 mg/dL; Level II 94-140 mg/dL and Level III 26-38 mg/dL.

Reagent Stability: Reagents from two lots were stored in incubators at 37°C, 25°C and 4°C. Reagents were tested at 0 day, 2 days, 6 days, 7 days and 10 days. Accelerated study results show that the reagent is stable for up to 12 months when stored at 2-8°C. Real time studies are being conducted and adjustments to shelf life will be made as needed. Stability on board the Hitachi 917 was studied by storing the two reagents (R1 and R2) in the 2-8°C storage chamber of the Hitachi 917. Results indicate the reagents are suitable for on board storage for up to 60 days.

Calibrator Stability: Calibrators from two lots were stored in incubators at 37°C, 25°C and 4°C. The calibrators were removed from storage a predetermined times and tested with the Diazyme LDL-Cholesterol reagent. The accuracy of the Calibrators was determined by testing LDL-C samples of know concentrations determined by the predicate kit. The shelf life of the calibrators was determined to be 12 months at 2-8°C. Real time studies are being conducted and adjustments to shelf life will be made as needed.

Control Stability: Controls from two lots were stored in incubators at 37°C,

25°C and 4°C. The controls were removed from storage at predetermined times and tested in duplicate with the Diazyme LDL-Cholesterol reagent. The accuracy of the controls was determined by testing LDL-C samples of known concentrations determined by the predicate kit. The shelf life of the calibrators was determined to be 12 months at 2-8°C. Open vial stability for controls was determined to be one month. Real time studies are being conducted and adjustments to shelf life will be made as needed.

d. Detection limit:

The Limit of Blank (LOB) and Limit of Quantitation (LOQ) for the Diazyme LDL-Cholesterol assay were determined based upon the CLSI EP17-A Protocol. The LOB was determined by testing zero calibrator 12 times on the Hitachi 917. The LOB obtained was 1.64 mg/dL (the mean + 3SD). The Limit of Quantitation of the Diazyme LDL-Cholesterol Reagent was determined by diluting a serum sample into 4 other samples. The 5 samples were tested 5 times over 3 days for a total of 125 replicates. The value estimated for LOQ where a 20% CV was obtained was 2.0 mg/dL.

e. Analytical specificity:

To determine the level of interference from substance normally present in human serum, the Diazyme LDL-Cholesterol Assay was tested with normal serum samples containing about 79 mg/dL LDL-C spiked with various concentrations of interfering substances following CLSI guideline EP7-A. The following substance normally present in serum produced less than 10% deviation when tested at levels equal to the following concentrations:

Interference Substance	Concentration
Ascorbic Acid	10mM/L
Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Hemoglobin	1000 mg/dL
Triglycerides	1000 mg/dL

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A total of 82 samples were compared with the Equal LDL Ultra Cholesterol and calibrator on the Hitachi 917. Samples were tested based upon

recommendations in NCCLS (CLSI) EP-9A2 guidance. The following regression equation was obtained: $Y = 0.9776X + 17.291$, $r^2 = 0.9821$.

b. Matrix comparison:

29 samples were compared between serum and EDTA and citrate plasma on the Hitachi 917. Samples were compared across the measuring range; some samples were spiked or diluted to cover the entire range.

Serum vs EDTA plasma $Y = 1.0642x + 1.6446$, $r^2 = 0.9510$, $N=29$

Serum vs Citrate plasma $Y = 1.0573x + 4.7913$, $r^2 = 0.9768$, $N=29$

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:

<100 mg/dL Optimal

100-129 mg/dL Near optimal/Borderline

130-150 mg/dL Borderline High

160-189 mg/dL High

≥ 190 mg/dL Very High

* Third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and treatment of High Blood Cholesterol in Adults (NCEP ATPIII)

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.