

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

A. 510(k) Number:

k072395

B. Purpose for Submission:

New device

C. Measurand:

High Density Lipoprotein Cholesterol

D. Type of Test:

Quantitative, colorimetric Trinder reaction

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Proprietary Name – Diazyme HDL-Cholesterol Reagent

Established Name – HDL 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 HDL-Cholesterol Reagent

Class I – Diazyme HDL Controls

3. Product code:

LBS - Diazyme HDL-Cholesterol
JJX - Diazyme HDL- Cholesterol Control

4. Panel:

75 – Clinical Chemistry

H. Intended Use:

1. Intended use(s):

See Indications for use below.

2. Indication(s) for use:

The Diazyme HDL-Cholesterol reagent is intended for the in vitro quantitative determination of High 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.

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

Control – To monitor the performance of the Diazyme HDL-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 HDL-Cholesterol kit contains two reagents: Reagent 1 4X70ml bottles and Reagent 2 2X50ml bottles and 2X1ml calibrator materials. The control materials consist of three lyophilized human serum based materials which 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. These enzymes react with HDL via a Trinder reaction.

This device has not been certified or tested by the Cholesterol Reference Method Laboratory Network.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Ultra N-Geneous HDL 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 HDL-Cholesterol reagent is intended for the in vitro quantitative determination of High 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.	For the quantitative determination of high density lipoprotein cholesterol (HDL-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. Testing was performed on the Roche Diagnostics Hitachi 917. The results are presented in the table below.

Within Run Precision

	Level 1	Level 2	Level 3
N	80	80	80
Mean	29	53.07	90.56
SD	0.3	0.41	0.84
CV%	1.0	0.8	0.9

Total Precision

	Level 1	Level 2	Level 3
N	80	80	80
Mean	29	53.07	90.56
SD	0.65	1.36	2.02
CV%	2.3	2.6	2.2

Additional precision studies were performed at or near the clinical significant ranges of HDL. These precision studies of the Diazyme HDL- Cholesterol Reagent were 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 5 days. Testing was performed on the Roche Diagnostics Hitachi 917.

Within Run Precision

	Level 1	Level 2	Level 3
N	20	20	20
Mean	21.63	44.28	159.59
SD	0.18	0.30	1.77
CV%	0.9	0.7	1.10

Total Precision

	Level 1	Level 2	Level 3
N	20	20	20
Mean	21.63	44.28	159.59
SD	0.61	0.79	5.90
CV%	2.8	1.8	3.7

b. *Linearity/assay reportable range:*

Linearity determination was based upon NCCLS (CLSI) EP6-A and was performed on the Roche Diagnostics Hitachi 917. Each level was measured in triplicate. Samples were serially diluted from a level of 184.18 to 0 mg/dL. The linearity for this method is 1.06 – 184.8 mg/dL, $Y=0.998x+0.7275$, $r^2=0.9999$. The sponsor determined an acceptable % error was $\leq 10\%$. Results of the study support the claimed linearity range.

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

This device has not been certified or tested by the Cholesterol Reference Method Laboratory Network. The NIST SRM 1915b material was tested with the Diazyme HDL-Cholesterol reagent and found to have a deviation from the NIST target value of 1.9% for level I (48.7 mg/dL) and -3.4% for level II (51.9 mg/dL).

Values are assigned to the control materials by repeat testing on the Hitachi 917 using the Diazyme HDL-cholesterol reagent and calibrator. The assigned ranges which are lot specific are as follows: Level I 23-35 mg/dL; Level II 44-66 mg/dL and Level III 74-110 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, 9 days and 12 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 30 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 at predetermined times and tested with the Diazyme HDL-Cholesterol reagent. The accuracy of the Calibrators was determined by testing HDL-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. 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 HDL-Cholesterol reagent. The accuracy of the controls was determined by testing HDL-C samples of known concentrations determined by the predicate kit. The shelf life of the control materials 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:

To demonstrate the limit of blank of Diazyme HDL-Cholesterol, HDL zero calibrator was tested in 12 replicates on the Hitachi 917. The Limit of blank is defined as the mean + 3SD. N = 12, Mean = 0.37, SD = 0.27 LOD = 1.06. To determine the limit of quantitation (LOQ), a serum sample was diluted into 5 other concentrations and assayed over 3 days with 2 runs per day for a total of 150 replicates on the Hitachi 917. The five concentrations were 0.30, 0.75, 1.50, 3.00 and 5.00 mg/dL. In the study, the estimated LOQ where the inter-assay CV is 20% was 1.39 mg/dL.

e. Analytical specificity:

To determine the level of interference from substance normally present in human serum, the Diazyme HDL-Cholesterol Assay was tested with normal serum samples containing about 34 mg/dL HDL-C spiked with various concentrations of interference substances following CLSI guideline EP7-A and was performed on the Roche Diagnostics Hitachi 917. 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
Bilirubin	40 mg/dL
Bilirubin Conjugated	30 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 84 samples (68 non-altered, 16 altered) were compared with the Equal HDL Ultra Cholesterol and calibrator on the Hitachi 917. $Y = 1.0484x - 4.6906$, $r^2 = 0.9871$. Samples ranged from 7.17 to 181.84 mg/dL were tested based upon recommendations in NCCLS (CLSI) EP-9A2 guidance.

b. *Matrix comparison:*

Samples were compared between serum and EDTA, citrate and heparin plasma on the Hitachi 917. Samples were compared across the measuring range, from 14.03 to 184.4 mg/dL some samples were spiked or diluted to cover the entire range.

Serum vs EDTA plasma $Y = 1.0264X + 1.1334$, $R^2 = 0.9959$

Serum vs Citrate plasma $Y = 1.0661X + 2.0933$, $R^2 = 0.996$

Serum vs Heparin plasma $Y = 1.05X + 0.3388$, $R^2 = 0.994$

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:

Less than 40 mg/dL – A major risk factor for heart disease

60 mg/dL and above – Considered protective against heart disease

* 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.