

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

A. 510(k) Number:

k161679

B. Purpose for Submission:

New device

C. Measurand:

Small, dense (sd) LDL cholesterol (-C)

D. Type of Test:

Quantitative colorimetric assay

E. Applicant:

Denka Seiken Co., Ltd.

F. Proprietary and Established Names:

s LDL-EX“SEIKEN”

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
PYP Low-density lipoprotein (LDL) cholesterol sub-fraction test	Class I, meets the limitation to the exemption 21 CFR §862.9(c)(4)	21 CFR 862.1475 Lipoprotein test system	Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The s LDL-EX"SEIKEN" test is for the quantitative determination of small, dense (sd) LDL cholesterol (-C) in human serum or plasma. The s LDL-EX"SEIKEN" test is used in conjunction with other lipid measurements and clinical evaluations to aid in the risk management of lipoprotein disorders associated with cardiovascular disease.

3. Special conditions for use statement(s):

For *in vitro* diagnostic use only; for prescription use only

4. Special instrument requirements:

The performance of the assay was evaluated on the Roche Diagnostics Hitachi 917 analyzer.

I. Device Description:

The assay consists of two reagents: Reagent 1 and Reagent 2.

The content of the reagent is the following:

- R1 Reagent-1
Good's buffer pH 7
Cholesterol esterase (CHE) (microorganism) <3500U/L
Cholesterol oxidase (CO) (microorganism) <2000 U/L
Sphingomyelinase (microorganism) <7000 U/L
Catalase (microorganism) <2500 KU/L
N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS) <5.0 mmol/L
- R2 Reagent-2
Good's buffer pH 7
Peroxidase (POD) (horseradish) <12000 U/L
4-aminoantipyrine <10.0 mmol/L
Sodium azide 0.05w/v%

Material required but not provided with the reagents:

- Calibrator:
Lipid Calibrator D (Denka Seiken)
- Controls:
Lipid Control I (Denka Seiken)
Lipid Control II (Denka Seiken)

J. Substantial Equivalence Information:

1. Predicate device name(s):

NMR Lipoprofile Test

2. Predicate 510(k) number(s):
k111516

3. Comparison with predicate:

Similarities		
Item	Device s LDL-EX“SEIKEN” (k161679)	Predicate NMR LipoProfile (k111516)
Intended Use	For the quantitative determination of LDL cholesterol in human serum or plasma to be used in conjunction with other lipid measurements and clinical evaluations to manage lipoprotein disorders associated with cardiovascular disease.	Same.

Differences		
Item	Device s LDL-EX“SEIKEN” (k161679)	Predicate NMR LipoProfile (k111516)
Reported analyte	Small dense LDL cholesterol (sd LDL-C) sub-fraction	LDL-particle number
Methodology	Colorimetric Assay	NMR Spectrometer

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

CLSI EP5-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Third Edition

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

CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition

CLSI EP9-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Third Edition

CLSI EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline –Second Edition

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Products; Approved Guideline

CLSI EP28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition

L. Test Principle:

The test consists of two steps and is based on the technique to use well-characterized surfactants and enzymes that selectively react with certain groups of lipoproteins.

In the first step, non-sd LDL lipoproteins, that is, chylomicrons, VLDL, IDL, L-LDL and HDL are decomposed by a surfactant and sphingomyelinase in Reagent-1 that is reactive to those non-sd LDL lipoproteins. The cholesterol released from such non-sd LDL lipoproteins is then degraded to water and oxygen by the action of enzymes. Cholesterol ester is hydrolyzed by the cholesterol esterase and then oxidized by the cholesterol oxidase. Produced hydrogen peroxides are finally decomposed to water and oxygen by the catalase.

In the second step, another surfactant in Reagent-2 releases cholesterol only from sd LDL particles and cholesterol released from sd LDL is then subject to the enzymatic reactions. As catalase in the reaction mixture is inhibited by sodium azide in Reagent-2, hydrogen peroxides, produced from the reaction with the cholesterol esterase and cholesterol oxidase, develop a purple-red color with the coupler in the presence of peroxidase.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision testing was performed at a total of three sites (one internal and two external sites) according to the Clinical and Laboratory Standards Institute (CLSI) EP05-A3 guideline. Samples were assayed twice a day, two replicates per run, for 20 days, for a total of 80 results per sample. Total precision (N=80) was calculated and presented in the tables below.

The study included five samples, 2-level control set, and three serum-based pools. The three serum based pools used in the sites were different, but were representative of low, intermediate, and high sd LDL-C concentrations. Each site employed one lot of assay reagents, and one instrument.

Site 1

	Lipid Control I	Lipid Control II	Human Serum L	Human Serum M	Human Serum H
n	80	80	80	80	80
Mean (mg/dL)	17.03	56.89	7.80	43.85	74.55
SD (mg/dL)	0.39	0.92	0.16	0.58	1.05
Total precision	2.3%	1.6%	2.1%	1.3%	1.4%

Site 2

n	Lipid Control I	Lipid Control II	Human Serum L	Human Serum M	Human Serum H
	80	80	80	80	80
Mean (mg/dL)	20.98	54.05	7.90	44.53	75.85
SD (mg/dL)	0.45	1.02	0.25	0.81	1.34
Total precision	2.1%	1.9%	3.2%	1.8%	1.8%

Site 3

n	Lipid Control I	Lipid Control II	Human Serum L	Human Serum M	Human Serum H
	80	80	80	80	80
Mean (mg/dL)	18.05	55.39	7.78	44.96	75.82
SD (mg/dL)	0.74	1.33	0.34	0.86	1.67
Total precision	4.1%	2.4%	4.3%	1.9%	2.2%

b. Linearity/assay reportable range:

The linearity study was performed according to the CLSI guideline EP6-A. The study included 13 evenly distributed concentrations from 0 to 127 mg/dL prepared from a high and low serum pool. Regression analyses were used, as described in the guideline, to check for nonlinearity. The third-order model fit the data better than the linear model. However, the test results within the claimed measuring range did not deviate from linearity by more than 11%. The linear regression analysis is summarized below:

$$y = 1.0099x + 1.0039.$$

The sponsor claims that the measuring range of the candidate device is 4.0 - 100 mg/dL. Test results below 4.0 mg/dL are reported as <4 mg/dL and test results above 100 mg/dL are reported as >100 mg/dL.

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

The method is traceable to an internal standard prepared from pooled human serum and value assigned using an internal standard measurement method.

d. Detection limit:

For the limit of blank (LoB), saline samples were assayed in replicates of 15 over 5 days (5 runs) using 3 lots of reagents on one analyzer for a total of 75 replicates per reagent lot. The classical approach (nonparametric option) described in the CLSI EP17-A2 guideline was followed. The claim was based on the reagent lot with the highest estimated LoB.

For the limit of detection (LoD) and the limit of quantitation (LoQ) the sponsor

assayed multiple low level serum samples (6 for the LoD study and 10 for the LoQ study) in duplicate over 5 days using 3 lots of reagents and one analyzer for a total n of 60 per lot for the LoD studies and a total n of 10 per sample per reagent lot for the LoQ study. The LoD was estimated following the classical approach (parametric option) described in EP17-A2. The LoQ was estimated using the precision profile approach described in the guideline and was defined as the concentration point where the imprecision is no greater than 10 %CV. The LoD and LoQ claims are based on the reagent lot with the highest estimate. The LoB was 0.20 mg/dL, the LoD was 0.38 mg/dL, and the LoQ was 1.14 mg/dL.

e. Analytical specificity:

The studies were designed following the recommendations in the CLSI guideline EP7-A2. Three human serum samples that spanned the dynamic range were used for each compound. The samples were divided into two series of aliquots: one aliquot was spiked to represent the interfering sample, and another aliquot remained neat to represent the control sample. All samples were assayed in triplicate and results were averaged. The results between the neat and spiked samples were all within $\pm 10\%$. The sponsor claims that the following compounds at the concentrations listed did not interfere with the performance of the test.

The test results are summarized below:

Hemoglobin: No significant interference up to 1,000 mg/dL

Bilirubin: No significant interference up to 60 mg/dL for both conjugated and unconjugated bilirubin

Chyle: No significant interference up to 1420 FTU

Sodium L-ascorbate: No significant interference up to 100 mg/dL

Intralipid to assess turbidity: No significant interference up to 10% Intralipid (approximately 2,200 mg/dL triglycerides)

Uric acid: No significant interference up to 15 mg/dL

Triglyceride: No significant interference up to 1,500 mg/dL

Drugs: No interference was found at 3 fold higher than therapeutic levels using Pravastatin, Pitavastatin, Atorvastatin, Rosuvastatin, Simvastatin, Fluvastatin, Ezetimibe, Fenofibrate, Gamma-Oryzanol, Bezafibrate, Probucol, Tocophenol Nicotinate, and Riboflavin Tetrabutryate.

f. Assay cut-off:

See Clinical Cutoff in 3 c below.

2. Comparison studies:

a. Method comparison:

Not applicable

b. Matrix comparison:

Multiple matched serum and plasma samples were drawn from 48 subjects. The tube types were: serum (reference, “plain”), serum separator tubes (SST), K2 EDTA plasma tubes, and lithium heparin plasma tubes. Samples from each tube type were assayed in duplicate, but for the analyses (least squares linear regression statistics), the x-axis was the mean of the duplicate testing of the plain tube, and the y-axis was the first result from the other four (4) tubes. The plain tube was considered the reference condition. The concentration of the samples ranged from 10 to 90 mg/dL sd LDL-C. The results are summarized below:

	Serum (SST)	Plasma (K2 EDTA)	Plasma (Lithium Heparin)
Correlation Coefficient	1.00	1.00	1.00
Slope	1.00	0.96	0.99
Intercept	+0.1	-0.1	-0.4

3. Clinical studies:

a. Clinical Sensitivity:

See 3 c below.

b. Clinical specificity:

See 3 c below.

c. Other clinical supportive data (when a. and b. are not applicable):

The sd LDL-C cutoff value of 50.0 mg/dL was validated using subjects without prevalent CHD (defined as self-reported myocardial infarction, silent MI (diagnosed by electrocardiographic changes), validated MI or revascularization procedure) from the ARIC study Visit 4 participants.

For the clinical validation, a total of 10,290 subjects were tested. The subjects ranged from 52 to 75 years of age at the time of ARIC study Visit 4 (1996-1998), 56% females and 44 % males and were recruited from four U.S. communities (Washington County MD; Forsyth County, NC; Jackson, MS; and Minneapolis, MN) and were followed for a maximum of 16 years. The primary endpoint used in the clinical validation study was a composite of total CHD events, comprising of 1) hospitalized MI, 2) fatal CHD or 3) cardiac procedure.

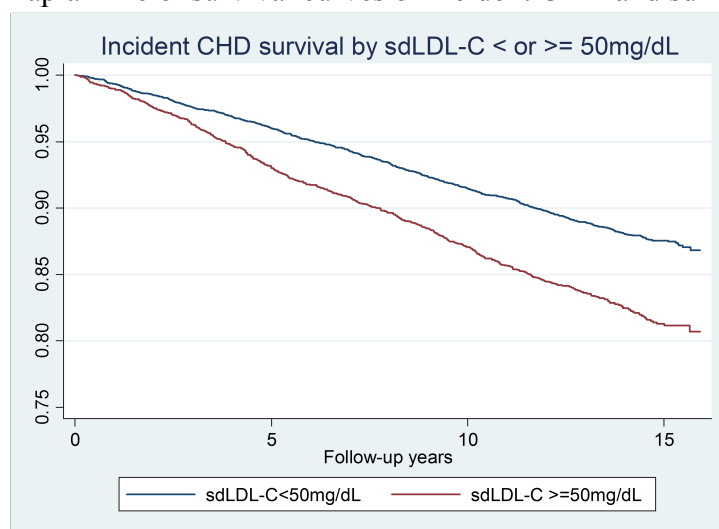
Associations between sd LDL-C and incident CHD was determined using Cox proportional hazards modeling, in both minimally adjusted and fully adjusted models. The basic model (Model 1) was adjusted for age, sex, and race as potential confounders. Model 2: was adjusted for the Model 1 variables + ever smoker, BMI, hypertension (systolic blood pressure and/or hypertension medications), HDL, log(triglycerides), lipid-lowering medication, diabetes, and log(high sensitivity C-reactive protein). Triglycerides and high sensitivity C-reactive protein were log-transformed to account for their non-Gaussian distributions.

The following analyses were provided:

16-year Absolute Risk for incident CHD by sd LDL-C cut-off (50mg/dL)

sd LDL-C < 50 mg/dL Event/population % (95%CI)	sd LDL-C ≥ 50 mg/dL Event/population % (95%CI)	P-value
825/7123 11.58% (10.82-12.34%)	569/3167 17.97% (16.60-19.33%)	<0.0001

Kaplan-Meier survival curves of incident CHD and sd LDL-C < or ≥ 50.0 mg/dL



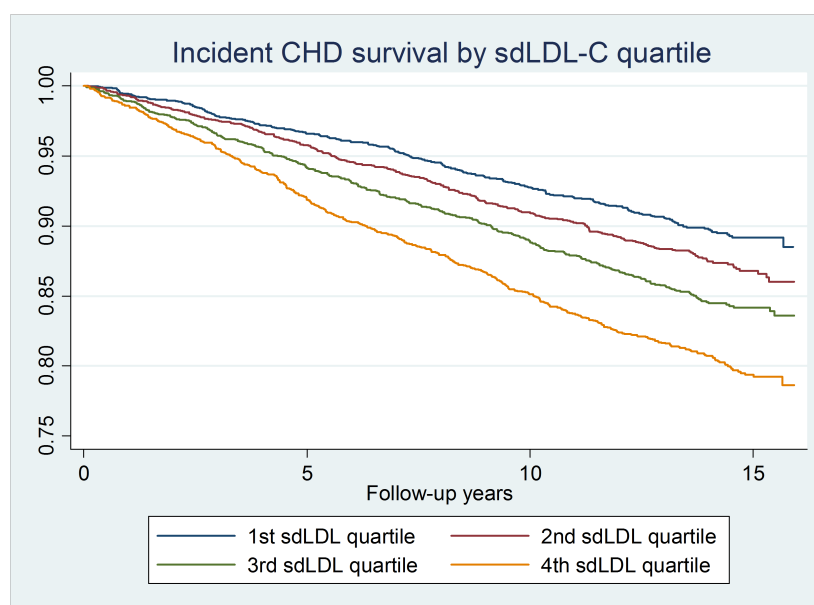
Over the follow-up years (16 years), proportional hazards regression analyses were used to investigate the association of incident CHD with baseline levels of sd LDL-C in medians, using sd LDL-C <50.0 mg/dL as the reference group. The HRs are presented by the aforementioned Models 1 and 2.

Hazard ratio (95% CI) for incident CHD by sd LDL-C cutoff (50.0 mg/dL)

	sd LDL-C <50.0 mg/dL	sd LDL-C ≥50.0 mg/dL
Events/Population	825/7123	569/3167
Model 1	Reference	1.55 (1.39 - 1.73) (p<0.0001)
Model 2	Reference	1.26 (1.10 - 1.43) (p=0.0006)

As supplemental information, the Kaplan-Meier survival curves of CHD risk for sd LDL-C quartiles, adjusted by age, race, and sex, are shown in Figure 2, and hazard ratios (95% CI) for incident CHD by sd LDL-C quartiles are shown in Table 3.

Kaplan-Meier survival curves of risk of CHD by sd LDL-C quartiles.



Hazard ratios (95% CI) for incident CHD by sd LDL-C quartiles (p-values (Pr > chiSq) for linear hypothesis testing results of sd LDL-C quartiles).

	sd LDL-C, Quartile				p-trend
	1 (≤ 27.8 mg/dL)	2 (27.8 mg/dL – <39.5 mg/dL)	3 (39.5 mg/dL – <54.6 mg/dL)	4 (≥54.6 mg/dL)	
Model 1	ref	1.20 (1.01-1.42)	1.43 (1.22 - 1.68)	1.95 (1.67 - 2.28)	<0.0001
Model 2	ref	1.10 (0.92 - 1.31)	1.19 (0.99 - 1.43)	1.47 (1.21 - 1.80)	0.0006

Additional studies:

- The sponsor provided a sensitivity analysis to estimate the impact on the interpretation of the test result because of the change in the universal definition of myocardial infarction (which is a component of the composite endpoint used) that occurred during the study period. The sponsor estimated potentially missed myocardial infarctions by reevaluating the data on symptoms, cardiac biomarkers levels and electrocardiographic evidence used to determine MIs in all ARIC study participants for the entire period where the new definition of MI was not utilized. The results of the sensitivity analysis showed that there was no significant impact on the association of sd LDL-C with risk of incident CHD since there was no change in estimated hazard ratios for incident CHD in the 50 mg/dL cut point analysis. The sponsor also showed that the hazard ratios for incident CHD were similar to the hazard ratios of revascularization procedures and CHD-related death (and these endpoints are included in the incident CHD endpoint).
- Data was provided supporting the stability of the frozen specimen used in the clinical validation study.

The labeling includes the following information as “Limitations”:

- The assessment of coronary heart disease (CHD) risk should include the patient’s history, clinical information, and other clinical laboratory test results in addition the results from this assay.
- The s LDL-EX”SEIKEN” test is not a replacement for LDL-C measurement. It should not be used in risk assessment calculators.

The labeling also includes the following information as “Limitations of the Clinical Study”:

- During the clinical study, the universal definition of myocardial infarction was updated. This change impacted the number of myocardial events routinely identified. As a result, the clinical information provided in support of this device may not reflect the total number of myocardial infarctions that the study participants experienced.

4. Clinical cut-off:

The sd LDL-C cutoff value of 50.0 mg/dL was established using samples from the Multi-Ethnic Study of Atherosclerosis (MESA) and was based on the 75th percentile value of sd LDL-C for normolipidemic and dislipidemic subjects who showed no sign of coronary heart disease or diabetes mellitus at baseline (n=3,938).

5. Expected values/Reference range:

A reference interval study was performed in accordance with the CLSI guideline EP28-A3c. Eligible subjects were enrolled at two US regions and consented to a single blood draw after an overnight fast. Subjects were partitioned by age and sex, according to the following four parameters: (1) males 21 – 44 years of age and (2) males 45 – 75 years, and (3) females 21 – 54 years of age (presumed pre-menopausal/peri-menopausal) and (4) females 55 – 75 years (presumed post-menopausal). The inclusion criteria for the reference populations described as ambulatory status and presumptively healthy, HDL-C $\geq$ 40 mg/dL, LDL-C < 160 mg/dL, TG < 200 mg/dL, fasting glucose < 126 mg/dL. Age differences associated with the sd LDL-C level were significant in both sexes (p=0.0030 in males and p<0.0001 in females). No significant difference was observed in the sd LDL-C level between males and females (p=0.7564).

According to the CLSI guideline, the normal range was defined as the 2.5th percentile value to the 97.5th percentile value, as described below.

Group	Subjects of the study	Reference Intervals
Younger group	21 – 44 yrs for males and 21 – 54 yrs for females (n=240)	12.7 to 48.3 mg/dL
Older group	45 – 75 yrs for males and 55 – 75 yrs for females (n= 202)	12.6 to 51.7 mg/dL

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.