s LDL-EX "SEIKEN"

Reagent kit for the quantitative determination of small, dense LDL-cholesterol

REF 562616

INTENDED USE

The s LDL-EX "SEIKEN" kit is for the quantitative determination of small, dense (sd) LDL cholesterol (-C) in human serum or plasma. The device is intended to be used in clinical laboratories on routine clinical chemistry analyzers capable of accommodating two reagent assays. The measurement of sd LDL-C, when used in conjunction with other biochemical markers and coronary risk factors, is useful in the prediction of coronary artery disease/coronary heart disease (CAD/CHD) risk and the assessment of CAD/CHD severity in individuals with intermediate CAD/CHD risk.

SUMMARY AND EXPLANATION OF THE TEST

LDL-C is considered a critical risk factor for developing CHD and cardiovascular disease (CVD). The qualitative features of the LDL particles also play an important role in the development of CHD, particularly in view of the predominance of sd LDL particles. sd LDL particles have been suggested to be highly atherogenic due to their higher penetration into the arterial wall, their lower binding affinity for the LDL receptor, their prolonged plasma half-life and their lower resistance to oxidative stress compared to that of large buoyant LDL(L LDL) [1-4].

A recent report has confirmed that a predominance of sd LDL-C is a strong and independent predictor of CAD/CHD [5]. Another study demonstrated that the LDL size is markedly smaller, and that small, dense LDL-cholesterol levels are significantly higher, in CAD/CHD patients than in controls; there also is a clear relationship between sd LDL levels and the severity of CAD/CHD [6, 7].

To date, ultracentrifugation and electrophoresis-based methods are used for the measurement of sd LDL-C but these methods are both laborious and time-consuming [8]. The s LDL-EX "SEIKEN" test is a direct method for the quantitative determination of sd LDL-C, using automated chemistry analyzers capable of accommodating two-reagent assays. The test is completed within 10 minutes.

CHEMICAL PRINCIPLE OF THE PROCEDURE

The assay 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 (CHE) and then oxidized by the cholesterol oxidase (CO). Produced hydrogen peroxides are finally decomposed to water and oxygen by the catalase.

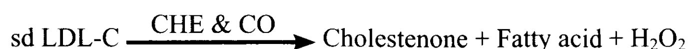
In the second step, another surfactant in Reagent-2 releases cholesterol only from sd LDL particles and cholesterol released from sd LDL is then subject to the enzymatic reactions. As catalase in the reaction mixture is inhibited by sodium azide in Reagent-2, hydrogen peroxides, produced from the reaction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

1st step

$$\text{chylomicrons, VLDL, IDL, L LDL and HDL} \xrightarrow{\text{CHE \& CO}} \text{Cholestenone} + \text{Fatty acid} + H_2O_2$$

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

2nd step



$$\text{sd LDL-C} \xrightarrow{\text{CHE \& CO}} \text{Cholestenone} + \text{Fatty acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4-aminoantipyrine} + \text{TOOS*} \xrightarrow{\text{POD}} \text{Purple-red color} + 4H_2O$$

* N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

MATERIALS PROVIDED

Reagent Kits

- **R1 Reagent-1 (18mL x 1 vial)**

Component	Concentration
Good's buffer	pH 7.0
Cholesterol esterase (CHE) (microorganism)	1600 U/L
Cholesterol oxidase (CO) (microorganism)	600 U/L
Sphingomyelinase (microorganism)	2700 U/L
Catalase (microorganism)	1200 KU/L
N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS)	2.0 mmol/L
Bovine Serum Albumin	1.0 w/v%

- **R2 Reagent-2 (6mL x 1 vial)**

Component	Concentration
Good's buffer	pH 7.0
Peroxidase (POD) (horseradish)	5000 U/L
4-aminoantipyrine	4.0 mmol/L
Sodium azide	0.05 w/v%

MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrator:

  Randox sLDL Calibrator **(Randox: Cat. No. CH5050)**

- Controls:

  Randox sLDL Control Level 1 **(Randox: LE 5013)**
  Randox sLDL Control Level 2 **(Randox: LE 5014)**
  Randox sLDL Control Level 3 **(Randox: LE 5015)**

WARNINGS AND PRECAUTIONS

CAUTION: This product requires the handling of human specimens. It is recommended that all human sourced materials are considered potentially infectious. Standard Precautions (Directive 2000/54/EC of the European Parliament and of the council of 18 September 2000) should be followed in handling, storing and disposing of all specimens and all items contaminated with blood and biosafety practices should be used for materials that contain, or are suspected of containing, infectious agents.

1) For in vitro diagnostic use.
2) Read all instructions carefully before starting the test.
3) Store the reagents at appropriate conditions and do not use reagents after the expiration date on the label.
4) Bottles supplied with the kit should be handled carefully and disposed of properly after use. Do not use the bottles for other purposes.
5) Reagents with different lot numbers should not be mixed.
6) All patient specimens used in this test should be considered potentially infectious and as such be treated with universal precautions.
7) Avoid direct skin contact. In the event that reagents come into contact with the skin, eyes or mouth, flush with plenty of water. Seek medical advice if any symptoms are observed or if it is considered necessary.

Application parameters for Hitachi analyzers

Hitachi 717		Hitachi 911		Hitachi 917	
Temperature:	37℃	Temperature:	37℃	Temperature:	37℃
ASSAY CODE	2POINT	ASSAY CODE	2POINT END-10	ASSAY CODE	2POINT END-10
ASSAY POINT	24-50	ASSAY POINT	15-31	ASSAY POINT	14-34
SAMPLE VOLUME	6 (μL)	SAMPLE VOLUME	6 (μL)	SAMPLE VOLUME	3.6 (μL)
R1 VOLUME	300 (μL)	R1 VOLUME	300 (μL)	R1 VOLUME	180 (μL)
R2 VOLUME	100 (μL)	R2 VOLUME	100 (μL)	R2 VOLUME	60 (μL)
WAVELENGTH	700 / 600	WAVELENGTH	700 / 600	WAVELENGTH	700 / 600
CALIB. METHOD	LINEAR-0-0	CALIB. METHOD	LINEAR-2-2	CALIB. METHOD	LINEAR-2-2
UNITS	mg/dL	UNITS	mg/dL	UNITS	mg/dL

Refer to each analyzer's Operation Manual for detailed instrument procedures.

Calibration

1. The designated calibrator should be used for calibration, and saline or water should be used for the zero calibrator (blank solution).
2. The assigned value to the calibrator is lot-dependent. Please refer to the package insert of calibrator for the specific value assigned to the lot in use.
3. Each laboratory should determine the calibration frequency, as this would depend on the analyzer in use, as well as the types and number of other assays being run. It is recommended that a new calibration curve be drawn at least once every two weeks, or when a new lot of reagent is used.
4. Calibration range: 0.0-100 mg/dL.
5. For a detailed description of how to calibrate an assay, refer to the package insert of calibrator.

Quality Control

The control testing intervals and assay limits should be established at each laboratory in accordance with State and local regulations, and laboratory policies. If the reported values do not fall within the labeled limits, check if the procedures were correct, and follow normal troubleshooting measures.

Results

1) Refer to the instrument-specific operations manual for information on results calculations.
2) Results are reported in mg/dL. To convert to mmol/L, multiply the results in mg/dL by 0.0259. (mg/dL x 0.0259 = mmol/L)

Limitations

1) The assessment of CHD risk should include the patient's history, clinical information, and other clinical laboratory test results in addition the results from this assay.
2) Samples must be stored frozen at – 80 ℃ or below if they are stored over 3 days. Samples stored at temperature higher than – 80 ℃ may result in falsely low sd LDL-C measurements.

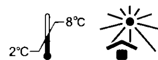
EXPECTED VALUES

The data shown below has been obtained by the assay using sd-LDL-C "SEIKEN" (for Europe, polyanion/ divalent cation precipitation method) .

Reference Intervals

A reference interval study was performed in accordance with Clinical Laboratory Standard Institute (CLSI) protocol C28-A2. 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 gender, according to the following four parameters: (1) males 21 – 44 years of age and (2) males 45 – 75 years, and (3) women 21 – 54 years of age (presumed pre-menopausal/peri-menopausal) and (4) women 55 – 75 years (presumed post-menopausal). The inclusion criteria for the reference populations were ambulatory status and presumptively healthy, HDL-C ≥ 40 mg/dL, LDL-C < 160 mg/dL, TG < 200 mg/dL, Fasting glucose < 126 mg/dL.

8) Laboratories should follow their instrument manufacturer's recommendations for sterilization procedures after use. Reagent-2 contains 0.05 w/v% sodium azide. As sodium azide may react with lead and/or copper piping to form explosive metal azides, it should be disposed of by flushing with copious amounts of water.



STORAGE AND HANDLING

All unopened reagents are stable until the expiration date printed on the label when stored at 2 to 8 ℃ and protected from light.
The s LDL-EX "SEIKEN" kit may be stored on board the instrument for a maximum of 30 days. After 30 days, the reagent kit must be discarded.

Preparation of reagents and stability of working solutions

Reagent	Preparation	Stability of working solution
Reagent-1	Ready for use	Once opened, the reagent is stable for 30 days when stored at 2 to 8 ℃.
Reagent-2	Ready for use	

SPECIMEN COLLECTION AND HANDLING

Specimen Types

1) Human serum and plasma (EDTA-2K, EDTA-3K, Heparin-Lithium) shall be used for the assay.
2) For specific information on interference, refer to the section on PERFORMANCE CHARACTERISTICS.

Specimen Collection and Handling Conditions

1) Fasting and non-fasting samples can be used. However, due to the circadian rhythm of sd LDL-C serum concentrations, it is recommended that specimens be collected in the morning [9].
2) When collecting serum specimens, the blood tubes should be centrifuged after the blood has clotted thoroughly.
3) When serum collection tube contains coagulation accelerators, draw the blood volume as instructed by the manufacturer. If the blood volume drawn is too small, erroneous test results may be produced.
4) Refrigerate the samples after the separation.
5) Blood samples should be kept in a refrigerator if centrifugation does not occur immediately after the blood collection.
6) Dilute samples with saline manually two- or three-fold before applying to the measurement of sd LDL-C when their total LDL-C levels are over 300 mg/dL as a guide. Correct the results for the dilution factor.

Specimen Storage and Shipping

1) It is recommended that samples be stored at refrigerated temperatures (2 to 8 ℃) after the separation process, and may remain refrigerated for up to 3 days. For longer storage, samples should be stored frozen at – 80 ℃ or below.
2) If samples need to be shipped, they should be shipped on dry ice.
3) It is recommended that samples be avoided to be subject to more than three freeze/thaw cycles.

ASSAY PROCEDURE

For a detailed description of how to perform an assay, refer to the instrument-specific operations manual.
The assay should be conducted according to the specific application parameters for the automated chemistry analyzer in use. Below is a general example of the assay procedure for an automated analyzer. Please contact the manufacturer for specific application parameters for the automated chemistry analyzer in use.

1. Incubate 6.0 μL of sample with 300 μL of Reagent-1 at 37 ℃ for 5 minutes.
2. Add 100 μL of Reagent-2.
3. Read the absorbance change at 600 nm (or difference between 700 nm and 600 nm) for 5 minutes after the addition of Reagent-2.
4. Calculate sd LDL-C value with the measured absorbance change by interpolation from a calibration curve prepared with Randox sLDL Calibrator.

Age differences associated with the sd LDL-C level were significant in both genders ($p<0.0001$). No significant difference was observed in the sd LDL-C level between males and females ($p=0.925$). 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 of males and 21 – 54 yrs of females (n=246)	9.5 to 42.5 mg/dL
Older group	45 – 75 yrs of males and 55 – 75 yrs of females (n= 214)	10.7 to 48.7 mg/dL

Clinical Cut-Off Value for CAD/CHD Risk Assessment

The clinical cut-off value for sd LDL-C was established by an additional analysis performed on the data obtained from 623 subjects, including normolipidemic and dyslipidemic cases who were not CAD/CHD or diabetic patients. From this analysis, the 75th percentile value was selected as the cut-off level for CAD/CHD risk assessment.

Cut-off value for CAD/CHD: 35mg/dL

CLINICAL STUDY RESULTS

The data shown below has been obtained by the assay using sd-LDL-C "SEIKEN" (for Europe, polyanion/ divalent cation precipitation method).

Angiography Studies (case-control)

One clinical study was conducted in the US with patients whose coronary artery disease was diagnosed angiographically. The study involved 57 subjects without coronary artery stenosis (non-CAD group) and 96 subjects with coronary artery stenosis equal to or greater than 50% in at least one vessel (CAD group). The mean sd LDL-C concentration was significantly higher in CAD subjects than in non-CAD subjects ($p=0.0400$). Performance characteristics for assessing CAD severity were compared among sd LDL-C and other lipid tests. The sensitivity (32.3%) of sd LDL-C was equal to that of LDL-C (32.3%), and better than that of total cholesterol (27.1%) or triglyceride (27.1%). The specificity of sd LDL-C (87.7%) was the highest of all lipid parameters assessed. Accuracy of sd LDL-C (52.9%) for assessing CAD risk was similarly high as that of HDL-C (57.5%). It was also found that some CAD patients were solely identified by sd LDL-C, but not by other lipid parameters. All these results demonstrate the clinical utility of the sd LDL-C. This was also proved in another clinical study conducted in Germany. Multinomial regression analysis from that study demonstrated that only age, gender, and sd LDL-C were independently associated with CAD severity, which was judged by angiography as progression greater than 50% of coronary atherosclerosis, but other well-known risk factors and lipid parameters, including LDL-C, were not independent risk factors.

Framingham Offspring Study

The relationship between sd LDL-C concentrations and other risk factors associated with the development of CHD were epidemiologically determined by longitudinal analysis of the subjects from the Framingham Offspring Study. By means of Cox's proportional hazard model, the hazard ratio of sd LDL-C for the development of CHD was obtained from 2,639 subjects who are the offspring and their spouses of the original Framingham Heart Study cohort. The subjects participated in examination Cycle 6, and then underwent follow-up observation at Cycle 8 (approximately eight years later). It was shown that the sd LDL-C level was significantly associated with the development of CHD events. The hazard ratio for the development of CHD by sd LDL-C remained highly significant, even after taking the effects from other risk factors into consideration (Hazard ratio=1.44, $p=0.0244$).

Hazard ratio for the development of CHD events predicted by sd LDL-C.

covariates	n**	Hazard ratio	p-value
none	2,639	1.9606	<0.0001*
Sex, Age, Smoke	2,636	1.5343	0.0080*
Sex, Age, Smoke, Sbp, Htn, Diab	2,632	1.4410	0.0244*

The Cox's proportional hazard model analysis was performed using the log-transformed sd LDL-C concentrations.

* : $p<0.05$

** : Subjects who were on medication were excluded from the analysis.

Sbp: Systolic blood pressure, Htn: Hypertension treated, Diab: Diabetes mellitus (type I and II)

Conclusion

The sd LDL-C assay is a useful diagnostic marker for predicting the CHD risk and the severity of CAD for clinical management of patients with intermediate CHD risk.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

When distilled water was measured as the test sample, the absorbance at 600 nm (or difference between 700 nm and 600 nm) was below 0.025. When a control serum containing 100 mg/dL sd LDL-cholesterol was measured, the subtracted absorbance change between 600 nm and 700 nm was always in the range of 0.170 – 0.230.

2. Specificity

Recoveries from control sera were all in the range plus or minus 3 mg/dL for the sd LDL-C level below 30 mg/dL and plus or minus 10% for the sd LDL-C level equal or over 30 mg/dL against target values.

3. Precision

Within–run precision was assessed in 10-replicated measurements and CVs were always below 3%.

4. Assay range

The study was performed by diluting a high sd LDL-C spiked serum sample with a saline. Comparison of the observed concentrations with the theoretical concentrations showed bias within plus or minus 3 mg/dL for the sd LDL-C level below 30 mg/dL and plus or minus 10% for the sd LDL-C level equal or over 30 mg/dL. The linearity range of the assay is 4.0 – 100 mg/dL.

	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9	Level 10	Level 11
Theoretical value (mg/dL)	0.0	8.7	19.0	29.4	39.8	50.2	60.5	70.9	81.3	91.7	102.0
Mean (mg/dL)	-0.1	9.8	19.4	28.7	38.8	48.5	59.0	70.2	81.1	91.6	105.0
%Recovery	-	113.0%	102.1%	97.4%	97.5%	96.7%	97.4%	99.0%	99.8%	99.9%	102.9%
Bias(mg/dL)	-0.1	1.1	0.4	-0.7	-1.0	-1.7	-1.5	-0.7	-0.2	-0.1	3.0
Mean Recovery	100.6%										

5. Method comparison

The s LDL-EX "SEIKEN" assay (y) was compared to ultracentrifugation method and s LDL-C "SEIKEN" (precipitation method) (x) using 60 and 180 human serum samples respectively. The linear regression analysis showed: $r = 0.954$, $y = 0.990x - 3.4$, and $r = 0.913$, $y = 0.943x + 2.0$, respectively.

6. Interferences

Ascorbic acid (up to 50 mg/dL), hemoglobin (up to 500 mg/dL), bilirubin (up to 30 mg/dL) did not interfere with the assay (target within + / – 10% recovery).

Potentially Interfering Compound	Concentration	% Recovery
Ascorbic acid	50 mg/dL	99.3 to 100.3%
Hemoglobin	500 mg/dL	100.0 to 109.8%
Bilirubin (conjugated)	30 mg/dL	97.4 to 100.0%
Bilirubin (unconjugated)	30 mg/dL	99.7 to 100.7%

Triglycerides (up to 1000 mg/dL) did not interfere with the assay.

References

1) Griffin BA, et al. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk, Atherosclerosis, 106, 241 (1994).
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3) Berneis KK, Krauss RM., Metabolic origins and clinical significance of LDL heterogeneity, J Lipid Res., 43, 1363 (2002).
4) Austin MA, et al. Low-density lipoprotein subclass patterns and risk of myocardial infarction, JAMA, 260(13), 1917 (1988).
5) St-Pierre AC, et al. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec cardiovascular study, Aterioscler Thromb Vasc Biol., 25, 553 (2005).

6) Hirano T, et al. Clinical significance of small dense low-density lipoprotein cholesterol levels determined by the simple precipitation method, Arterioscler Thromb Vasc Biol., 24, 558 (2004).

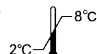
7) S. Koba, et al. Significance of small dense low-density lipoprotein-cholesterol concentrations in relation to the severity of coronary heart diseases, Atherosclerosis, 189, 206 (2006).

8) Hirano T, et al. Measurement of small dense low-density lipoprotein particles, J Atheroscler Thromb., 12(2), 67 (2005).

9) Ogita K, et al. Circadian rhythm of serum concentration of small dense low-density lipoprotein cholesterol, Clinica Chimica Acta, 376, 96 (2007).

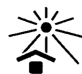
SYMBOL GLOSSARY

In vitro diagnostic medical device



Temperature limitation

Catalogue number



Keep away from sunlight

Contents of kit

Manufacturer

Batch code

Authorized representative in the European Community

Use by

CE marked product

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