DRG® Oxidized LDL (EIA-3430)

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Revised 4 Jan. 2007

INTENDED USE

The DRG® Oxidized LDL ELISA kit is intended to be used for the *in vitro* quantitative measurement of oxidized low density lipoproteins (oxidized LDL) in human blood serum or plasma. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases. In the United States, this kit is intended for Research Use Only.

SUMMARY AND EXPLANATION OF THE TEST

The oxidative conversion of low density lipoproteins (LDL) to oxidized low density lipoproteins (oxidized LDL) is now considered to be a key event in the biological process that initiates and accelerates the development of the early atherosclerotic lesion, the fatty streak [1–5].

Experimental studies have shown that native LDL becomes atherogenic when it is converted to oxidized LDL, and that oxidized LDL is more atherogenic than native LDL[1–5]. Oxidized LDL is found in monocyte-derived macrophages in atherosclerotic lesions, but not in normal arteries [6]. The uptake of LDL into macrophages does not occur by way of the classic Brown/Goldstein LDL receptor [7]. Numerous studies [1–5,8] have established that LDL, the major carrier of blood cholesterol, must first be converted to oxidized LDL so that it can be recognized by “scavenger” or “oxidized LDL” receptors on monocyte-derived macrophages. The binding of oxidized LDL to macrophages is a necessary step by which oxidized LDL induces cholesterol accumulation in macrophages, thus transforming the macrophages into lipid-laden foam cells [8].

Holvoet and his colleagues [9] were the first to clearly demonstrate that patients with coronary artery disease had significantly elevated plasma levels of oxidized LDL, and that these circulating levels of oxidized LDL were very similar in patients with stable coronary artery disease and in patients with acute coronary syndromes. They found plasma oxidized LDL results to be significantly higher in patients with stable angina, unstable angina and acute myocardial infarction when compared to age-matched, presumably healthy, control subjects.

In the publication of Holvoet [9,10], plasma oxidized LDL levels were measured by a competitive ELISA utilizing a specific murine monoclonal antibody, mAb-4E6. It should be noted that the DRG® Oxidized LDL ELISA kit uses the same specific murine monoclonal antibody, mAb-4E6, that Holvoet [9,10] used in his assays. However, the DRG® assay kit is a capture ELISA (also known as a “sandwich” ELISA), in which the wells of the microtiter plates are coated with the capture antibody, mAb-4E6.

Several noteworthy studies have been reported by clinical researchers who have used the DRG® Oxidized LDL ELISA kits. Hulthe and Fagerberg [11] demonstrated the relationship between subclinical atherosclerosis and circulating oxidized LDL levels by showing that oxidized LDL levels were related to intima-media thickness and plaque occurrence in the carotid and femoral arteries. Sigurdardottir, Fagerberg and Hulthe [12] found elevated levels of oxidized LDL in patients with metabolic syndrome. In addition, they found that elevated oxidized LDL levels in metabolic syndrome patients were associated with small LDL-particle size. Kopprasch et al [13] found elevated levels of circulating oxidized LDL in subjects with impaired glucose tolerance (IGT). And Duntas, Mantzou, and Koutras [14] found significantly elevated plasma oxidized LDL levels in untreated patients with overt hypothyroidism.

At the American Heart Association Scientific Sessions 2002, Johnston et al [15] reported that plasma levels of oxidized LDL were substantially higher in patients with unstable coronary artery disease compared to healthy controls. Most

important, there was no significant difference between the cholesterol levels of the unstable coronary artery disease patients and the healthy controls.

PRINCIPLE OF THE PROCEDURE

DRG® Oxidized LDL ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. During incubation oxidized LDL in the sample reacts with anti- oxidized LDL antibodies bound to microtitration well. After washing, which removes non-reactive plasma components, a peroxidase conjugated anti-human apolipoprotein B antibody recognizes the oxidized LDL bound to the solid phase. After a second incubation and a simple washing step that removes unbound enzyme labeled antibody, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint then read spectrophotometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use. Not for internal or external use in humans or animals. In the United States, this kit is intended for Research Use Only.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All patient samples should be handled as capable of transmitting infections.

Warning! This kit contains reagents that may be infectious!
This kit contains reagents manufactured from human blood components. The source of material have been tested by immunoassay for hepatitis B surface antigen, antibodies for hepatitis C virus and for antibodies for HIV virus and found to be negative. Nevertheless, all recommended pre-cautions for the handling of blood derivates should be observed. Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/national guidelines on laboratory safety procedures.

MATERIAL REQUIRED BUT NOT PROVIDED

- 25 µl micropipette with disposable tips
- 50 µl, 100 µl, 200 µl and 1000 µl repeating pipettes
- Beakers and cylinders for reagent preparation
- Redistilled water
- Test tubes with caps, 3.5 ml
- Microplate reader with 450 nm filter
- Plate shaker (The recommended velocity is 700–900 cycles per minute, orbital movement)
- Wash device for microplates
- "Vortex"-mixer

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REAGENTS

Each Oxidized LDL ELISA kit contains reagents for 96 wells, sufficient for 40 samples, two Controls and one calibration curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical Lot numbers.
The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is +2–8 °C.

- **Coated Plate** 1 plate, 8-well strips 96 wells Ready for use
  (mouse monoclonal anti-oxidized LDL)
  For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.
- **Calibrators** 5 vials 1000 µl Lyophilized
  Concentrations stated on vial label (human oxidized LDL).
  Add 1000 µl redistilled water per vial
- **Calibrator 0** 1 vial 1000 µl Ready for use
  Colour coded yellow
- **Controls (L), (H)** 2 vials 1000 µl Pre-diluted, lyophilized
  Concentrations stated on vial label
  Add 1000 µl redistilled water per vial.
- **Enzyme Conjugate 11X** 1 vial 1.2 ml Preparation see below

(Peroxidase conjugated mouse monoclonal anti-apoB (6 µg/ml))
Note! Light sensitive,

- **Enzyme Conjugate Buffer** 1 vial 12 ml Ready for use
  Colour coded blue
- **Assay Buffer** 1 vial 12 ml Ready for use
  Colour coded red
- **Sample Buffer 4X** 1 bottle 50 ml
  Colour coded yellow
  Dilute with 150 ml redistilled water to make Sample Buffer.
  Note! Precipitate may occur when stored at +2–8 °C Allow Sample Buffer 4X to reach room temperature.
  Shake or vortex until precipitate has dissolved.
- **Wash Buffer 21X** 1 bottle 40 ml
  Dilute with 800 ml redistilled water to make Wash Buffer
- **Substrate TMB** 1vial 22 ml Ready for use
  Colourless solution. *Note! Light sensitive!*
- **Stop Solution** 1 vial 7 ml Ready for use
  0.5 M H_2SO_4

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Preparation of Enzyme Conjugate Solution

Dilute the Enzyme Conjugate 11X (1.2 ml) by adding the Enzyme Conjugate Buffer (12 ml).
If less than 12 strips are used, prepare the needed volume according to the table below. Mix gently.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µl	7.0 ml
6 strips	500 µl	5.0 ml
4 strips	350 µl	3.5 ml.

Stability of reconstituted and opened reagents

Calibrators	1 week at 2–8 °C, 4 weeks at –20 °C
Controls (L), (H)	1 week at 2–8 °C
Wash Buffer	1 month at 2–8 °C
Sample Buffer	1 month at 2–8 °C
Coated Plate, unused strips	2 months at 2–8 °C, reseal the bag with adhesive tape
Enzyme Conjugate Solution	1 month at 2–8 °C

SPECIMEN COLLECTION AND HANDLING

The recommended use of specimen in the DRG® Oxidized LDL ELISA is fresh EDTA-plasma.
Heparin-plasma and serum may also be used.

Plasma

Collect blood by venipuncture into tubes containing EDTA or heparin as anticoagulant, and separate the plasma fraction. Samples can be stored at –80 °C for at least six months. Avoid repeated freezing and thawing.

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Samples can be stored at –80 °C for at least six months. Avoid repeated freezing and thawing.

DILUTION OF SAMPLES

Samples must be diluted the same day as the assay performance. Prepare two tubes for each patient sample. Each sample has to be diluted in two steps to a final dilution of 6561 times as follows:

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1	Patient sample	25 µl	
	Sample Buffer	2000 µl	
	Cap tubes, invert three times and Vortex-mix*		Dilution 1/ 81
2	1/81 dilution of sample	25 µl	
	Sample Buffer	2000 µl	
	Cap tubes, invert three times and Vortex-mix*		Dilution 1/ 6561

* It is IMPORTANT to ensure that each dilution step is properly mixed before the next step.
As a result of this procedure the samples will be diluted 1/6561.
Dilution 1/6561 is stable for 1 day. Dilution 1/81 is stable up to 4 days stored at 2–8 °C.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Prepare a standard curve for each assay run.

1. Prepare sufficient Coated Plate wells to accommodate Calibrators, Controls and samples in duplicate
2. Pipette 25 µl of each Calibrator, Control and diluted sample into appropriate wells.
3. Add 100 µl Assay Buffer to each well.
4. Incubate on a plate shaker for 2 hours at room temperature (18–25 °C).
5. Wash 6 times with automatic washer or: Aspirate the reaction volume and fill each well completely with 350 µl Wash Buffer. Discard liquid completely. Repeat 5 times. After final wash, invert and tap the plate firmly against absorbent paper.
6. Add 100 µl Enzyme Conjugate Solution to each well.
7. Incubate on a plate shaker for 1 hour at room temperature (18–25 °C).
8. Wash as described above.
9. Add 200 µl Substrate TMB.
10. Incubate for 15 minutes at room temperature, no shaking.
11. Add 50 µl Stop Solution. Place plate on the shaker for 15 seconds to ensure mixing.
12. Read optical density at 450 nm and calculate results. Read within 30 minutes.

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INTERNAL QUALITY CONTROL

Commercial controls* and/or internal plasma/serum pools with low, intermediate and high oxidized LDL concentrations should routinely be assayed as unknowns, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number; reconstitution dates of kit components; OD values for the blank, Calibrators and Controls.

* Commercial controls (e.g. Ox-LDL Control) are available at request. Please contact DRG International, Inc.

CALCULATION OF RESULTS

Computerized calculation

The concentration of oxidized LDL is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator O, versus the concentration using cubic spline regression. Multiply the concentration of the unknown samples with the dilution factor (e.g. × 6561).

Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the Oxidized LDL concentration on a lin-log paper and construct a calibration curve.
2. Read the concentration of the Controls and unknown samples from the calibration curve. • Multiply the concentration of the Controls and the unknown samples with the dilution factor (e.g.× 6561).

Example of results

Wells	Identity	A450 nm	Mean conc. mU/l	× 6561 U/l
1A–B	Calibrator 0 mU/l	0.072		
1C–D	Calibrator 1.8 mU/l	0.185		
1 E–F	Calibrator 3.6 mU/l	0.369		
1 G–H	Calibrator 6.7 mU/l	0.664		
2A–B	Calibrator 14 mU/l	1.405		
2C–D	Calibrator 28 mU/l	2.469		
2E–F	Control (H)	1.234	12.2	80.04
2G–H	Control (L)	0.513	5.2	34.12

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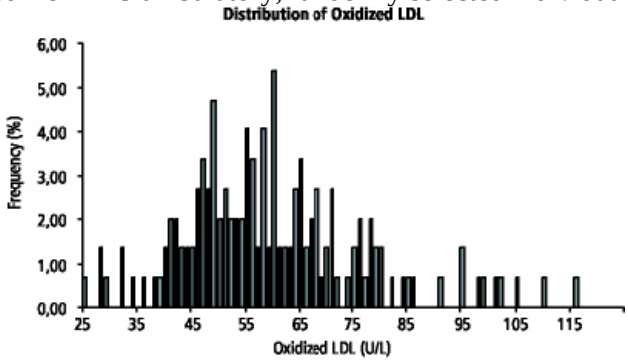
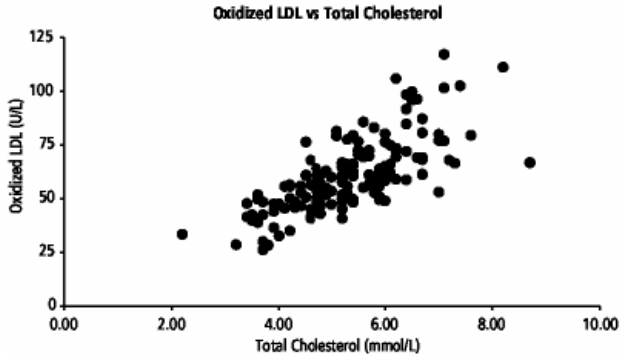
LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.
Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own.
The following results were obtained from 149 ambulatory, randomly selected individuals in the Stockholm area, Sweden.





The following results were obtained from 149 ambulatory, randomly selected individuals in the Stockholm area, Sweden

	Mean	Median	Range
OxLDL (U/l)*	61	59	26–117
Chol/HDL ratio**	4.10	3.90	1.68–7.89

* Arbitrary units. See CALIBRATION.
** Measured data Cholesterol (mmol/l) and HDL (mmol/l).

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Distribution of Oxidized LDL and Cholesterol/HDL ratio

Oxidized LDL U/l	Cholesterol/HDL Ratio	Patient/Total (%)	
	Quartile 1		
Q1 (26–49)	1.68–3.13	22/149	(14.8)
Q2 (50–59)	1.68–3.13	10/149	(6.7)
Q3 (60–69)	1.68–3.13	5/149	(3,4)
Q4 (70–117)	1.68–3.13	0/149	(0.0)
	Quartile 2		
Q1 (26–49)	3 .2 1–3 .86	7/149	(4.7)
Q2 (50–59)	3 .2 1–3 .86	17/149	(11.4)
Q3 (60–69)	3 .2 1–3 .86	10/149	(6.7)
Q4 (70–117)	3 .2 1–3 .86	3/149	(2.0)
	Quartile 3		
Q1 (26–49)	3.87–4.79	7/149	(4.7)
Q2 (50–59)	3.87–4.79	11/149	(7.4)
Q3 (60–69)	3.87–4.79	11/149	(7.4)
Q4 (70–117)	3.87–4.79	9/149	(6.0)
	Quartile 4		
Q1 (26–49)	4.80–7.89	1/149	(0.7)
Q2 (50–59)	4.80–7.89	4/149	(2.7)
Q3 (60–69)	4.80–7.89	7/149	(4.7)
Q4 (70–117)	4.80–7.89	25/149	(16.8)

The following results were obtained from 147 ambulatory, randomly selected individuals in the Stockholm area, Sweden.

	Mean	Median	Range
OxLDL (U/l)*	61	59	26–117
LDL/HDL ratio**	2.51	2.36	0. 55–5. 56

* Arbitrary units. See CALIBRATION.
** Measured data LDL (mmol/l) and HDL (mmol/l)

Distribution of Oxidized LDL and LDL/HDL ratio

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Oxidized LDL U/l	LDL/HDL Ratio	Patient/Total (%)	
	Quartile 1		
Q1 (26–49)	0.55–1.79	19/147	(13.0)
Q2 (50–59)	0.55–1.79	12/147	(8.2)
Q3 (60–69)	0.55–1.79	6/147	(4.1)
Q4(70–117)	0.55–1.79	0/147	(0.0)
	Quartile 2		
Q1 (26–49)	1.79–2.33	10/147	(6.8)
Q2 (50–59)	1.79–2.33	13/147	(8.8)
Q3 (60–69)	1.79–2.33	10/147	(6.8)
Q4(70–117)	1.79–2.33	3/147	(2.0)
	Quartile 3		
Q1 (26–49)	2.36–3.08	8/147	(5.4)
Q2 (50–59)	2.36–3.08	11/147	(7.5)
Q3 (60–69)	2.36–3.08	10/147	(6.8)
Q4(70–117)	2.36–3.08	8/147	(5.4)
	Quartile 4		
Q1 (26–49)	3.09–5.56	0/147	(0.0)
Q2 (50–59)	3.09–5.56	5/147	(3.4)
Q3 (60–69)	3.09–5.56	8/147	(5.4)
Q4(70–117)	3.09–5.56	24/147	(16.3)

PERFORMANCE CHARACTERISTICS

Detection Limit

The detection Limit is < 1 mU/l calculated as three standard deviations above the zero Calibrator.

Recovery

Recovery upon addition is 85–107% (mean value is 95%)

Precision

Precision was calculated from three samples assayed in 3–8 replicates on 20 different occasions.

Sample	Obtained value mU/l	Coefficient of variation %		
		within	between	total
1	8.5	5.5	6.2	8.3
2	19	7.3	4.0	8.3
3	32	6.2	4.0	7.4

Dilutions

Sample/	Dilution	Obtained value mU/l	Obtained/ Expected
		(Assay 1/ Assay 2)	
Sample 1	1:3321		
	1:6642	19.9/18.3	
	1:13284	9.4/9.5	0.94/1.04
Sample 2	1:3321	–	
	1:6642	20.6/20.4	
	1:13284	10.6/9.8	1.02/0.97
Sample 3	1:3321	29.1/32.0	
	1:6642	15.6/15.5	1.07/0.97
	1:13284	7.7/8.0	1.05/1.00
Sample 4	1:3321	21.6/20.2	
	1:6642	10.4/10.4	0.97/1.03
	1:13284	5.9/5.7	1.08/1.12
Sample 5	1:3321	15.9/1 5.7	
	1:6642	8.1/8,0	1.02/1.02
	1:13284	4.0/4.4	1.02/1.13

Mean Obtained/Expected value is 1.03, range 0.94–1.13.

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Calibration

No international reference is at date available. The DRG® Oxidized LDL ELISA is calibrated in relative arbitrary units against an in house reference preparation.

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by DRG® may affect the results, in which event DRG® disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. DRG® and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.