Mercodia Oxidized LDL ELISA

Features

- Measuring circulating levels of Oxidized Low Density Lipoprotein
- Monoclonal
- Results within 3.5 h
- Breakable strips

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Summary

The oxidative conversion of low density lipoproteins(LDL) to oxidized low density lipoproteins (oxidized LDL) is now considered to be an essential step in the atherogenic process. Experimental studies have demonstrated that oxidized LDL is directly involved in the initiation and acceleration of the atherosclerotic lesion, from the early development of the macrophage- derived foam cells and the fatty streak to the later development of the stenosed artery and the rupture of the plaque[1-7].

Oxidized LDL should be viewed as a unique plaque-specific lipoprotein, the most atherogenic lipoprotein yet described. Oxidized LDL is a "killer" molecule-a life-threatening molecule, found in monocyte-derived macrophages in atherosclerotic lesions, but not in normal arteries.

Patients with coronary artery disease have significantly elevated plasma levels of oxidized LDL, according to studies of Holvoet *et al.*[8]. They found plasma oxidized LDL concentrations 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 a more recent study, Holvoet´s group[9] demonstrated the usefulness of circulating oxidized LDL levels in identifying patients with coronary artery disease.

In the publication of Holvoet[8], plasma oxidized LDL levels were measured by a competitive ELISA utilizing a specific murine monoclonal antibody, mAb-4E6. It should be noted that the Mercodia Oxidized LDL ELISA assay uses the same specific murine monoclonal antibody. However, the Mercodia assay is a "sandwich" ELISA, in which the wells of the microtiter plates are coated with the capture antibody, mAb-4E6. Comparison studies have shown a correlation of 87% between the two assays.

Test principle

Mercodia 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, that removes non-reactive plasma components, a peroxidase conjugated anti-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 labelled 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 that is read spectrophotometrically at 450 nm.

References

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Reagents

• Standards 0; 2; 4; 9 and 33 mU/l*	6 vials
• Assay buffer	1 vial
• Anti-apoB conjugate stock solution	1 vial
• Conjugate buffer	1 vial
• Anti-Oxidized LDL coated microtitration strips	12x8 wells breakable strips
• Peroxidase substrate	1 vial
• Stop solution	1 vial
• Wash concentrate	1 bottle
• Sample buffer	2 bottles
• Oxidized LDL Controls L and H Conc. indicated at the vial label	2 vials

**For each lot the exact value is stated on each vial.*

Test procedure

- 25 µl standard/sample
- 100 µl assay buffer

  2 h incubation, room temperature on a shaker

- Wash 6 times
- Add 100 µl conjugate

  1 h incubation, room temperature on a shaker

- Wash 6 times
- Add 200 µl substrate

  15 min incubation, room temperature, no shaking

- Add 50 µl stop solution, shake for 5 sec. to ensure mixing
- Measure at 450 nm

Order no:

10-1143-01 1x96 kit

Test characteristics

Recovery

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

Precision

The following coefficients of variation have been obtained, each sample assayed in 3-8 replicates on 20 different occasions.

Sample	Obtained value mU/l	Coefficient of variation: Within assay %	Between assay %	Total assay %
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

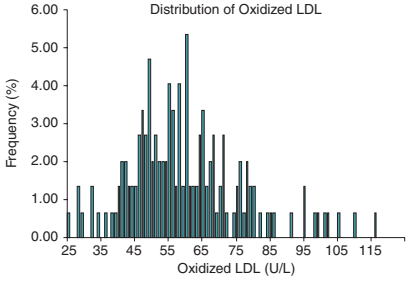
Mean obtained / expected values is 1.03 range 0.94 - 1.13.

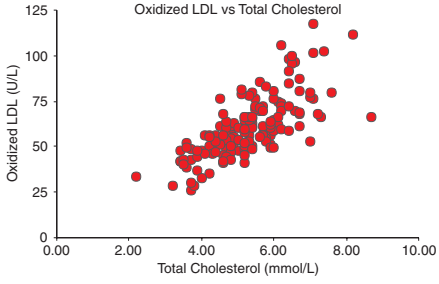
Sensitivity

Detection limit is < 1 mU/l, defined as three standard deviations above the 0-standard.

Expected values

Good practice dictates that each laboratory establishes its own expected range of values. The following results were obtained from 149 ambulatory, randomly selected individuals in the Stockholm area, Sweden.





Performance limitations

Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

Samples

EDTA plasma.

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