LDL-CHOLESTEROL

Selective Detergent Technique

INTENDED USE:

This reagent kit is intended for ***"in vitro"*** quantitative determination of **Low Density Lipoprotein CholesteroL (LDL-C)** in serum & plasma.

CLINICAL SIGNIFICANCE:

The LDL particles are lipoproteins that transport cholesterol to the cells. Often called "bad cholesterol" because high levels are risk factor for coronary heart disease and are associated with obesity, diabetes and nephrosis
Clinical diagnosis should not be made on a single test result; it should integrate with clinical and other laboratory data.

PRINCIPLE:

When a sample is mixed with Reagent 1, the protecting (masking) reagent binds to LDL and protects LDL from enzyme reactions. Cholesterol esterase (CHE) and cholesterol oxidase (CO) react with non-LDL lipoproteins [chylomicrons (CM), very low density Lipoprotein (VLDL) and HDLJ. Hydrogen peroxide produced by the enzyme reactions with non-LDL cholesterol is decomposed by catalase in Reagent 1. When Reagent 2 is added, the protecting (masking) reagent is removed from LDL and catalase is inactivated By sodium azide (NaN). In this second process, CHE and CO reactonly with LDL-C. Hydrogen peroxide produced by the enzyme reactions with LDL-C yields a color complex upon oxidative condensation with N-(2-hydroxy-3-sulfopropyl)-3.5-dimethoxyaniline (HDAOS) and 4-aminoantipyrine (4AA) in the presence of peroxidase (POD). By measuring the absorbance of the blue color complex produced, at approximately 546 nm, the LDL-C concentration in the sample can be calculated when compared with the absorbance of the LDL-C Calibrator.

REAGENT COMPOSITION:

Reagent 1: Buffer Reagent
Reagent 2: Substrate Reagent
LDL-C Calibrator: Concentration Printed on the Vial.

SAMPLE:

Serum or heparinized plasma, free of hemolysis, removed from the blood clot as soon as possible.
Anticoagulants containing citrate should not be used.

WORKING REAGENT PREPARATION & STABILITY:

Reagent 1 & Reagent 2 are ready to use and are stable up to the expiry date stated on the label.
LDL-C Calibrator: Dissolve with distilled water (Qty. Printed on the vial). Cap and mix gently to dissolve contents. Reconstituted calibrator is stable for 7 days at 2°C to 8°C or 21 days at -20°C.

GENERAL SYSTEM PARAMETERS:

Reaction type	Endpoint
Wave length	546 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank/Zero Setting	Reagent
Reagent Volume	1 ml
Sample Volume	10 µl
Incubation Time	10 Min.
Calibrator Concentration	Printed on vial
Linearity	250 mg/dl

ASSAY PROCEDURE:

	Blank	Calibrator	Sample
Reagent 1	750 µl	750 µl	750 µl
Calibrator		10 µl	
Sample			10 µl

Mix and incubate for 5 Minutes at 37°C and then add

Reagent 2	250 µl	250 µl	250 µl

Mix and read the optical density (A) after 10 minutes incubation at 37°C.

CALCULATION:

$$\text{LDL-C. (Mg/dl)} = \frac{\text{OD of Sample}}{\text{OD of Standard}} \text{ X Conc. of Calibrator}$$

LINEARITY:

Reagent is Linear up to 250 mg/dl.
Dilute the sample appropriately and re-assay if LDL-C Concentration exceeds 250 mg/dl.

REFERENCE NORMAL VALUE:

Desirable value: <130 mg/dl
Increased risk for coronary heart disease: 130-159 mg/dl
High risk for coronary heart disease: >160 mg/dl

It is recommended that each laboratory should assign its own normal range.

QUALTY CONTROL:

For accuracy it is necessary to run known controls with every assay.

LIMITATION & PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.

BIBLIOGRAPHY:

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