

Intended Use

This reagent is intended for *in vitro* quantitative determination of LDL Cholesterol in serum or plasma.

- Direct determination of LDL Cholesterol
- Selective Solubilisation Method
- Linear up to 700 mg/dL
- Ready to use liquid stable reagents

Clinical Significance

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, low density lipoprotein cholesterol (LDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong positive relationship between LDL-C concentration and the incidence of CHD was reported. LDL Cholesterol acts as a key factor in the pathogenesis of atherosclerosis and coronary artery disease.

Principle

This assay method uses a surfactant for selectively solubilizing LDL alone in the cholesterol assay system that employs cholesterol esterase and cholesterol oxidase. It passes the ester cholesterol and free cholesterol contained in the LDL to the cholesterol reaction system to determine LDL cholesterol. The enzyme reactions to other, non-LDL lipoproteins (HDL, VLDL, chylomicrons) are inhibited by the surfactant and by the sugar compounds. These lipoproteins are therefore not passed to the cholesterol reaction system and consequently remain in the reaction liquid as lipoproteins. Based on this principle it is thus possible to directly determine LDL-cholesterol on its own.

Kit Components

Reagent/ Component	Product code 11415003	Product code 11415004	Description
LDL –C Direct R1	2 x 30 mL	2 x 45 mL	HSDA - 1mmol/L Good's buffer pH 6.3
LDL –C Direct R2	2 x 10 mL	1 x 30 mL	Cholesterol esterase 2.0 U/mL Cholesterol oxidase 1.0 mmol/L 4-Aminoantipyrin 2.5mmol/L Good's buffer pH 6.3
LDL –C Direct Calibrator	1 x 3 mL	1 x 3 mL	Calibrator Concentration as mentioned on the vial label

Risk & safety

Material safety data sheets (MSDS) will be provided on request

Reagent Preparation

Reagent1 & Reagent 2 are ready to use.

Calibrator : Reconstitute with 3 mL of distilled water. Let it stand for 2 hrs at room temperature. Dissolve the content of the vial by swirling gently to avoid the formation of foam.

Reagent Storage and Stability

The sealed reagents are stable up to the expiry date stated on the label, when stored at 2 - 8°C, protected from light. Do not freeze

Stability : Reconstituted calibrator is stable only for 7 days at 2- 8°C.

Open Vial Stability

Once opened, the reagent is stable up to four weeks at 2- 8°C if contamination is avoided.

Onboard Calibration Stability

Onboard Calibration stability is 20 days

Reagent Deterioration

Turbidity or precipitation on in any kit component indicates deterioration and the component must be discarded. Values outside the recommended acceptable range for the Agappe Qualicheck Norm & Path control may also be an indication of reagent instability and associated results are invalid. Sample should be retested using fresh vial of reagent.

Precaution

To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.

Avoid direct exposure of reagent to light.

Do not blow into the reagent bottles.

This reagent is only for IVD use and follow the normal precaution required for handling all laboratory reagents

Waste Management

Reagents must be disposed off in accordance with local regulations

Sample

Fresh serum /Plasma (free of haemolysis)

Interferences

No interference for	:	
Bilirubin up to	:	20 mg/dL
Ascorbic acid up to	:	50 mg/dL
Haemoglobin up to	:	500 mg/dL
Triglyceride up to	:	3000 mg/dL

*(when triglyceride in a sample exceeds 1000 mg/dL, dilute the sample 1+9 with saline, repeat the assay and multiply result by 10)

Materials provided

LDL Direct Reagent R1,R2 & Calibrator

Materials required but not provided

- Pipettes & Tips
- Test Tubes & racks
- Timer
- Incubator
- Analyzer

Test Parameters

	Fully Auto Analyser	Semi Auto Analyser
Mode of reaction	End Point (Differential)	End Point
Slope of Reaction	Increasing	Increasing
Wavelength I	600 nm	578 nm (578 – 610)
Wavelength II	700 nm	630 nm (630 – 700)
Temperature	37°C	37°C
Calibrator concentration	As on the vial label	As on the vial label
Linearity	700 mg/dL	700 mg/dL
Incubation time	5 +5 min	5 +5 min
Blank	Reagent	Reagent
Sample Volume	3 µL	5 µL
Reagent 1 Volume	270 µL	450 µL
Reagent 2 Volume	90 µL	150 µL
Cuvette	1 cm light path	1 cm light path

Application parameters for various instrument are available. Please contact customer support department for specific information.

Unit Conversion

Traditional Unit	SI Unit	Conversion from Traditional to SI
mg/dL	mmol/L	x 0.026

Calibration

Agappe LDL- C Calibrator is recommended for calibration of this assay.

Reconstitute with 3 mL of distilled water. Let it stand for 2 hrs at room temperature. Dissolve the content of the vial by swirling gently to avoid the formation of foam. Reconstituted calibrator is stable only for 7 days at 2- 8°C.

SYMBOLS USED ON THE LABELS

IVD IN VITRO DIAGNOSTIC USE  SEE PACKAGE INSERT FOR PROCEDURE  LOT LOT NUMBER  MANUFACTURER'S ADDRESS  MANUFACTURING DATE  EXPIRY DATE  TEMPERATURE LIMIT

**AGAPPE DIAGNOSTICS LTD.**

'Agappe Hills', Dist. Ernakulam, Kerala, India-683 562.
Tel. +91 484 2867 000 | Customer Support No.: 1800 425 7151(Toll Free)
customersupport@agappe.in | www.agappe.com

REV. NO.: ADL/IFU/LDLC/LIQ/R02

 ISO 9001 : 2015
EN ISO 13485: 2016

Procedure Notes

Laboratory Procedure for Fully Auto Analyzer			
	Blank	Calibrator	Sample/control
Reagent 1	270 µL	270 µL	270 µL
Calibrator	-	3 µL	-
Sample / control		-	3 µL
Mix & incubate for 5 min at 37°C. Measure the absorbance (OD1) at 600 nm/ 700 nm.			
Reagent 2	90 µL	90 µL	90 µL
Mix and incubate for 5 min at 37°C. Measure the absorbance (OD2) at 600 nm/ 700 nm.			

Calculation

$$\text{LDL-C Concentration (mg/dL)} = \frac{(\text{OD2}-\text{OD1}) \text{ Sample}}{(\text{OD2}-\text{OD1}) \text{ Calibrator}} \times \text{Calibrator Concentration}$$

Laboratory Procedure for Semi Auto Analyzer			
	Blank	Calibrator	Sample/control
Reagent 1	450 µL	450 µL	450 µL
Calibrator	-	5 µL	-
Sample / control	-	-	5 µL
Mix & incubate for 5 min at 37°C.			
Reagent 2	150 µL	150 µL	150 µL
Mix and incubate for 5 min at 37°C. Measure the absorbance of calibrator & sample against reagent blank at 578 & 630 nm.			

Calculation

$$\text{LDL-C Concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Conc.}$$

Quality control

It is recommended to use Agappe Qualicheck Norm & Path (11601001) to verify the performance of the assay. Each laboratory has to establish its own internal quality control scheme and procedure for corrective action, if control do not recover within the acceptable range.

Reference Range

It is recommended that each laboratory establish its own reference values.

The following value may be used as guide line.

- Desirable < 130 mg/dL
- Borderline High Risk for CHD 130-159 mg/dL
- High Risk for CHD >160 mg/dL

Results obtained for patient samples are to be correlated with clinical findings of the patient for interpretation and diagnosis.

Performance

1. Linearity

This reagent is linear upto 700 mg/dL

If the concentration is greater than linearity (700 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor

2. Comparison

A comparison study has been performed between Agappe Reagent and another internationally available reagent yielded a correlation coefficient of r²=0.9922 and a regression equation of y =0.9592x.

3. Precision

Intra Run		
	Control Level 1	Control Level 2
n	20	20
Mean (mg/dL)	108.7	50.5
SD	2.11	1.19
CV(%)	1.94	2.36

Inter Run		
	Control Level 1	Control Level 2
n	20	20
Mean (mg/dL)	109.66	51.08
SD	4.07	2.00
CV(%)	3.71	3.92

Accuracy (mg/dL)		
Control	Expected Value	Measured Value
Control Level 1	130 ± 26	131.4
Control Level 2	56 ± 12.2	55.1
Qualicheck Norm	63 ± 9.0	62.8
Qualicheck Path	115 ± 18.5	118.3

4. Sensitivity

Lower detection Limit is 1.0 mg/dL

Bibliography

1. Crouse, J.R., *et al.*; Studies of Low Density Lipoprotein molecular weight in human being with coronary artery disease. J.Lipid Res 26:5666 (1985)

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IVD IN VITRO DIAGNOSTIC USE SEE PACKAGE INSERT FOR PROCEDURE LOT LOT NUMBER MANUFACTURER'S ADDRESS MANUFACTURING DATE EXPIRY DATE TEMPERATURE LIMIT