HDL-CHOLESTEROL (D) WITH CALIBRATOR

2 x 40 mL ,2 x 60 mL
51414003,52013001

Intended Use

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

- Direct determination of HDL Cholesterol – Highly specific to HDL determination
- Selective Inhibition Method
- Linear up to 150 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, high density lipoprotein cholesterol (HDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong negative relationship between HDL-C concentration and the incidence of CHD was reported.

Principle

The reaction between cholesterol other than HDL and the enzyme for cholesterol assay is suppressed by the electrostatic interaction between polyanions & cationic substances. Hydrogen peroxide is formed by the free cholesterol in HDL by cholesterol oxidase. Oxidative condensation of EMSE and 4-AA is caused by hydrogen peroxide in the presence of peroxidase, and the absorbance of the resulting red-purple Quinone is measured to obtain the cholesterol value in HDL

$$\text{Other lipoproteins than HDL} \xrightarrow[\text{Cationic substances}]{\text{Polyanions}} \text{Suppress reaction with enzyme}$$

$$\text{HDL (cholesterol esters)} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{HDL (free cholesterol)} + \text{Free fatty acids}$$

$$\text{HDL (free cholesterol)} + O_2 + H^+ \xrightarrow{\text{cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{EMSE} + H_3 + O \xrightarrow{\text{Peroxidase}} \text{Violet quinone} + 5\,H_2O$$

Kit Components

Reagent/ Component	Product Code		Description
	51414003	52013001	
HDL–C Direct R1	2 x 30 mL	2 x 45 mL	N—ethyl-N-(3-methylphenyl)-N'succinylethyenediame (EMSE)
HDL–C Direct R2	2 x 10 mL	2 x 30 mL	Cholesterol Oxidase 4-Aminoantipyrin (4-AA)
HDL–C Direct Calibratior	1 x 2 mL	1 x 2 mL	Calibrator Concentration as mentioned on the vial label

Risk & Safety

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

Reagent Preparation

The Reagent1 & Reagent 2 are ready to use.

Calibrator: Reconstitute with 2 mL of distilled water. Let it stand for 30 minutes 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 4 weeks at 2- 8°C if contamination is avoided.

Onboard Calibration Stability

On board Calibration stability is 20 days.

Reagent Deterioration

Turbidity or precipitation 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.

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 forhandling 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	40 mg/dL
Ascorbic acid up to	50 mg/dL
Haemoglobin up to	500 mg/dL
Triglyceride up to	1000 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

HDL- D 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 x dilution factor	As on the vial label x dilution factor
Linearity	150 mg/dL	150 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

SYMBOLS USED ON THE LABELS

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

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ADS/V.02/060114

CE ISO 9001 : 2008
ISO 13485 : 2003

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Calibration

Agappe HDL Calibrator is recommended for calibration this assay.Reconstitute with 2 mL of distilled water. Let it stand for 30 minutes 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^{0}C.

Procedure notes

Laboratory Procedure for Fully Auto Analyzer			
	Blank	Calibrator	Sample/control
Reagent	270 µL	270 µL	270 µL
Calibrator	-	3 µL	-
Sample / control	-	-	3 µL
Mix & incubate for 5 min at 37^{0}C.			
Reagent	90 µL	90 µL	90 µL
Mix and incubate for 5 min at 37^{0}C and read absorbance of calibrator &sample against reagent blank at 578 & 630 nm.			

Calculation

$$\text{HDL-C Concentration (mg/dL)} = \frac{\text{(OD2 – OD1) sample}}{\text{(OD2 – OD1) Calibrator}} \times \text{standard conc.}$$

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^{0}C.			
Reagent	150 µL	150 µL	150 µL
Mix and incubate for 5 min at 37°C and read absorbance of calibrator & sample against reagent blank at 578 & 630 nm.			

Calculation

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

Quality Control

It is recommended to use Agappe Qualicheck Norm & Path (51601001) 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.

Male : 35 - 80 mg/dL

Female : 42 - 88 mg/dL

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

Performance

1. **Linearity**

This reagent is linear up to 150 mg/dL

If the concentration is greater than linearity (150 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 r^2 = 0.9848 and a regression equation of y =0.9854x.

3. **Precision**

Intra Run		
	Control Level 1	Control Level 2
n	20	20
Mean (mg/dL)	67.1	27.1
SD	0.87	0.85
CV(%)	1.30	3.14

Inter Run		
	Control Level 1	Control Level 2
n	20	20
Mean (mg/dL)	66.48	27.16
SD	1.21	0.55
CV(%)	1.82	2.04

Accuracy (mg/dL)		
Control	Expected Value	Measured Value
Control Level 1	65 ± 9.8	69.7
Control Level 2	24 ± 6.6	26.6
Qualicheck Norm	30 ± 4.5	32
Qualicheck Path	85 ± 15.5	83.9

4. **Sensitivity**

Lower detection Limit is 1 mg/dL

Bibliography

1. Williams, P., *et al.;* High density lipoprotein and coronary risk factor, Lancet. 1:72 (1979)
2. Gordon,T., Castelli, W.P., Hjortland, M.C. *et al*. Am. J.Med. 62, 707-714 (1977)

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