TRUEchemie Direct HDL Cholesterol Test Kit (Polymer – Detergent)

for the quantitative determination of High-Density Lipoprotein cholesterol (HDL-C) in human serum

INTENDED USE

The TRUEchemie Direct HDL Cholesterol Test Kit (Polymer – Detergent) is used for the "in vitro" quantitative determination of high-density lipoprotein cholesterol (HDL-C) in human serum .

INTRODUCTION

High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in liver as complexes of apolipoprotein and phospholipid and are capable of picking up cholesterol and caring it from arteries to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL-cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized.

Accurate measurment of HDL-C is of vital importance when assessing patient's risk for CHD.

PRINCIPLE

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethyleneglycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER).
The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction.

$$\text{HDL + LDL + VLDL + CM} \xrightarrow[\text{PEGME}]{\text{PVS}} \text{HDL + (LDL+VLDL+CM)}$$
PVS/PEGME

$$\text{HDL} \xrightarrow{\text{CHOD, CHER}} \text{Fatty Acid + } H_2O_2$$

$$2H_2O_2 + \text{4-AA + TODB} \xrightarrow{\text{Peroxidase}} \text{Quinone + } 5H_2O$$

PACK SIZE

Kit size	**1 x 40 ml**
Cat no.	**ADX323**
1) DHDL Cholesterol Reagent (R1)	1 x 30 ml
2) DHDL Cholesterol Reagent (R2)	1 x 10 ml
3) DHDL Cholesterol Calibrator	1 x 1.0 ml

REAGENTS COMPOSITION

1) DHDL Cholesterol Reagent (R1)
Buffer : >5 mmol/L
MgCl2 : >2 mmol/L
TOOS : <2 mmol/L
2) DHDL Cholesterol Reagent (R2)
CHE : >2 U/L
COD : <5KU/L
POD : <10KU/L
3) DHDL Cholesterol Calibrator : DHDL Cholesterol concentration is as stated in vial

REAGENT PREPARATION

Ready to use reagents.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The disposal of the residues has to be done as per local legal regulations.

CALIBRATION

The procedures are calibrated with the calibrator solution which is included with each series of tests. Its absorbance is used to calculate results.

CALIBRATOR PREPARATION

Reconstitute with 1 ml of distilled water. Let it stand for 30 minutes at room temperature. Dissolve the content of the vial swirling gently to avoid the formation of foam.

REAGENT STORAGE & STABILITY

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2-8°C. Do not use reagents over the expiration date.
Calibrator:
Reconstituted calibrator is stable only for 7 days at 2-8°C

SPECIMEN COLLECTION AND STORAGE

Fresh Serum (use samples free from hemolysis).

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes
2. Test tubes/rack
3. Timer
4. 37°C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 578-620 nm

TEST PROCEDURE

Wavelength :578 nm (578 - 620 nm)
Temperature :37°C

Prewarm the reagents to reaction temperature

	Blank (µl)	Calibrator (µl)	Sample (µl)
DHDL Cholesterol Reagent (R1)	450	450	450
Calibrator	-	5	-
Sample	-	-	5
Mix well and incubate for 5 mins at 37ºC.			
DHDL Cholesterol Reagent (R2)	150	150	150

Mix well and incubate for 5 min. at 37ºC. Measure the absorbance of calibrator & sample against reagent blank at 578 nm.

Calculation:

$$\text{Conc. of DHDL in the sample (mg/dL)} = \frac{\text{Sample O.D}}{\text{Calibrator O.D}} \times \text{Conc of calibrator (mg/dL)}$$

QUALITY CONTROL

Quality Controls are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUE

Male : 35 - 80 mg/dL
Female : 42 - 88 mg/dL
It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

Sensitivity: 2.32 mg/dL
Linearity: Up to 150 mg/dL under the described assay conditions. 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. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION:

Intra-assay precision within run (n=20)	**Mean (mg/dL)**	**SD (mg/dL)**	**CV (%)**
Sample - 1	112	3.18	2.84
Sample - 2	28	0.85	3.09

Inter-assay precision run to run (n=20)	**Mean (mg/dL)**	**SD (mg/dL)**	**CV (%)**
Sample - 1	31	0.74	2.36

The reagent was tested for 20 days, using two different Direct HDL concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie Direct HDL cholesterol reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.99 and the regression equation is y=1.072x+0.705. The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Following Substances do not interfere:

Hemoglobin : up to 10 g/L
Bilirubin : up to 40 mg/dL
Triglycerides : up to 2000 mg/dL

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEMS PARAMETERS

Mode : Endpoint
Calibrator conc : As stated on vials
Wave length : 578 nm (578 - 620 nm)
Units : mg/dL
Flow cell temp. : 37°C
Blank : Reagent blank
Reagent 1 volume : 450 µl
Reagent 2 volume : 150 µl
Sample volume : 5 µl
Incubation : 5 + 5 mins
Normal Range : Male : 35 - 80, Female : 42 - 88
Sensitivity : 2.32
Linearity : 150
Reaction Slope : Increasing

REFERENCES

1. Dominiczak M, McNamara J. The system of Cardiovascular prevention.103-125; Nauk M, Wiebe D. Warncik G. Measurement of High-Density-Lipoprotein Cholesterol.221-224. In: Handbook of Lipoprotein Testing (eds. Rifai, Warnick and Dominiczak) 2nd edition.
2. Barr,D.P.,Russ E.M.,Eder,H.A.,Protein-lipid relationships in human plasma, Am.J.Med., 11;480(1951)
3. Gordon,T.et al. High density lipoproteins as a protective factor against coronary heart disease, Am. J, Med.,62;707(1977).
4. Castelli, W.P. et al., HDL Cholesterol and other lipids in coronary heart disease, Circulation, 55;767(1977).
5. National institutes of Health publication No. 93-3095, September (1993).
6. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use	REF Catalogue number	Caution
IVD *In vitro* diagnostic medical device	LOT Batch code	Non-sterile
2°C 8°C Temperature limit 2-8°C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep dry
Do not use if package is damaged	Keep away from sunlight	

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