TRUEchemie Direct LDL Cholesterol Test Kit (POLYMER – DETERGENT)

for the quantitative determination of low density lipoprotein cholesterol in human serum or plasma

INTENDED USE

The TRUEchemie Direct LDL Cholesterol Test Kit (POLYMER – DETERGENT) is used for the direct quantitative determination of low density lipoprotein cholesterol in human serum or plasma.

INTRODUCTION

Plasma lipoproteins are spherical particles that contain varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, the inner core contains mostly esterified cholesterol and triglycerides. These particles serve to solubilize and transport cholesterol and triglycerides in the bloodstream.

The relative proportions of protein and lipid determine the density of these plasma lipoproteins and provide a basis for their classification. The classes are very low-density lipoproteins (VLDL), low density lipoproteins (LDL), and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have varied effects. The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD), while HDL cholesterol has often been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated risk for CAD.

Over the years a variety of methods have been employed for the determination or estimation of LDL cholesterol. The Friedewald equation, in a variety of forms, has been most frequently used for the estimation of LDL cholesterol. However, its usefulness is limited and its accuracy has been questioned. Determination of LDL cholesterol by beta-quantification is recognized as the reference method, but the procedure is so cumbersome relatively few laboratories use this method. A recent method using immunoseparation has become popular. However, this method still requires sample pre-treatment prior to cholesterol determination, making it unsuitable for full automation of the procedure. The method presented here offers direct determination of LDL cholesterol with a two-part, liquid stable reagent that is easily adapted to most automated chemistry analyzers.

PRINCIPLE

The Direct LDL Cholesterol Reagent has two-part, liquid stable method for directly measuring LDL-C levels in serum or plasma. The method depends on the properties of a unique detergent which eliminates the need for any off-line pre-treatment or centrifugation steps. This detergent (Reagent 1) solubilizes only the non-LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for colour formation. The enzyme reaction with LDL-C in the presence of the coupler produces colour that is proportional to the amount of LDL cholesterol present in the sample.

PACK SIZE

Kit Size	**1 x 40 ml**
Cat. No.	**ADX331**
Kit Contents	
1) DLDL Cholesterol Reagent (R1)	1 x 30 ml
2) DLDL Cholesterol Reagent (R2)	1 x 10 ml
3) DLDL Cholesterol Calibrator	1 x 0.5 ml

REAGENTS COMPOSITION

1) DLDL Cholesterol Reagent (R1)

Buffer	: 100 mmol/L (pH 7.0)
Cholesterol esterase from Pseudomonas	: 800 U/L
Cholesterol oxidase form *Nocardia* sp.	: 500 U/L
Peroxidase from Horseradish	: 800 U/L
4-aminoantipyrine	: 1 mmol/L
Preservative.	

2) DLDL Cholesterol Reagent (R2)

Buffer	: 100 mmol/L (pH 7.0)
N,N-bis (4-sulfhobutyl)- m-Toluidine-disodium (DSBmT) Preservative	: 1.2%
3) DLDL Cholesterol Calibrator	: DLDL 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 Direct LDL Cholesterol Calibrator is required for calibration. Calibrators to be reconstituted with D.W with quantity as given on the calibrator bottle. Calibrate with each bottle change or lot change or if control results are found to be out of range.

CALIBRATOR PREPARATION

Reconstitute with 0.5 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

1. Serum, EDTA-treated or heparinized plasma are the recommended specimens.
2. Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours).
3. Plasma: Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).
4. If not analyzed promptly, specimens may be stored at 2 - 8 °C for up to 5 days. If specimens must be stored for more than 5 days, they may be frozen at -80°C.

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 600 nm.

TEST PROCEDURE

Wavelength	: 600 nm
Temperature	: 37°C

Prewarm the reagents to reaction temperature

	Blank (µL)	**Standard (µL)**	**Sample (µL)**
DLDL Cholesterol Reagent (R1)	750	750	750
DLDL Cholesterol Calibrator	-	10	-
Sample	-	-	10
Mix and incubate for 5 mins			
DLDL Cholesterol Reagent (R2)	250	250	250

Mix well and incubate for another 5 min. at 37ºC.

After incubation, zero spectrophotometer with distilled water blank. Read and record the absorbance of incubated calibrator and sample.

Calculation:

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

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

LDL Cholesterol	**Classifications**
<129 mg/dl (3.35 mmol/L)	Optimal
130-159 mg/dl (3.36-4.11 mmol/L)	Borderline High Risk
160-189 mg/dl (4.14-4.89 mmol/L)	High Risk
≥190 mg/dl (4.91 mmol/L)	Very High Risk

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

Sensitivity: 5 mg/dL

Linearity: Up to 500 mg/dL under the described assay conditions. If the concentration is greater than linearity (500 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=10)	**Mean (mg/dL)**	**SD (mg/dL)**	**CV (%)**
Control Level - 1	134.8	0.5	0.4
Control Level - 2	58.9	0.4	0.7

Inter-assay precision run to run (n=12)	**Mean (mg/dL)**	**SD (mg/dL)**	**CV (%)**
Control Level - 1	136.8	0.9	0.7
Control Level - 2	57.7	0.8	1.4

The reagent was tested for 12 days, using two different Direct LDL 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 LDL 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 0.999 and the regression equation is y=0.994x+0.550. The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

1. Hemoglobin at levels up to 400 mg/dl does not interfere.
2. Bilirubin at levels up to 20 mg/dL does not interfere.
3. Triglycerides up to 1500 mg/dL will not interfere.

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEMS PARAMETERS (Semi-Automated Analyser)

Mode	: Endpoint
Calibrator conc.	: As given in the calibrator vial
Wave length	: 600 nm
Units	: mg/dL
Flow cell temp.	: 37°C
Blank	: Reagent Blank
Reagent 1 volume	: 750 µl
Reagent 2 volume	: 250 µl
Sample volume	: 10µl
Incubation	: 5+5 mins
Normal Range	: <129
Sensitivity	: 5
Linearity	: 500
Reaction slope	: Increasing

REFERENCES

1.Gotto, A.M., Lipoprotein Metabolism and the etiology of Hyperlipidemia, Hospitalpractice, 23:Suppl. 1,4 (1988).

2. Crouse, J.R., et al., Studies of Low Density Lipoprotein Molecular Weight in Human Beings with Coronary Artery Disease, J. Lipid Res., 26:566 (1985).

3. Badimon, J.J., Badimon L., Fuester V., Regression of Athroscierotic Lesions by Highdensity lipoprotein Plasma fraction in the Cholesterol-Fed Rabbit, Journal of Clinical Investigation, 85:1234-41 (1990).

4. Castelli, W.P., et al., Cholesterol and other Lipids in coronary heart disease, Circulation, 55-767 (1977).

5. Barr, D.P., Russ, E.M, Elder, H.A., Protein-Lipid Relationships in Human Plasma, Am. J. Med. 11:480 (1951).

6. Gordon, T., et al, High Density Lipoprotein as a Protective Factor Against Coronary Heart Disease, Am. J. Med., 62:707 (1977).

7. William, P., Robinson, D., Baily A., High Density Lipoprotein and Coronary Risk Factor, Lancet, 1:72 (1979).

8. Kannel, W.B., Castelli W.P., Gordon, T., Cholesterol in the Prediction of Artheriosclerotic Disease; New Perspectives Based on the Framingham Study, Am. Intern. Med., 90:85 (1979).

9. National Institutes on Health Publication no. 93-3095, September (1993).

10.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	Batch code	Use-by date
In vitro diagnostic medical device	Do not re-use	Keep dry
Temperature limit 2-8 °C	Date of manufacture	European Conformity
Manufacturer	Caution	Authorized Representative
Catalogue number	Non-sterile	

Do not use if package is damaged

Keep away from sunlight