MEDICA

IVD CE

REF 10224-4 4 x 29 mL / 10 mL

LDL CHOLESTEROL (LDL)
Wedges each contain usable volumes of 29 mL of R1 reagent and 10 mL of R2 reagent.

INTENDED USE
The EasyRA LDL cholesterol reagent is intended for the quantitative measurement of low density lipoprotein cholesterol in human serum or plasma, using the MEDICA EasyRA® Chemistry Analyzer in clinical laboratories. Measurement of LDL is used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis and various liver and renal diseases.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION
Lipoproteins solubilize and transport cholesterol and other lipids in the blood. There are various classes of lipoproteins that exhibit different effects on the heart and the cardiovascular system.[1] The measurement of LDL cholesterol helps in early diagnosis and treatment of lipid and lipoprotein metabolism disorders. The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD).[2-8] The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that in all adults 20 years of age and older, a fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride) be performed every five years to screen for coronary heart disease risk.[4] Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated increased risk for CAD.[9]

PRINCIPLE OF THE PROCEDURE
This direct assay method of measuring LDL cholesterol involves the removal of other non-LDL lipoproteins via selective solubilization and reaction by Reagent 1 into a non-colored product. In the second step, the selective detergent in Reagent 2 solubilizes the LDL cholesterol specifically which then reacts with a chromogen to develop a color which can be read optically at 550 nm. The intensity of the color has a maximum absorbance at 550 nm and is proportional to the concentration of LDL cholesterol in the sample.

REAGENTS

LDL SOLUBILIZING DETERGENT REAGENT (R1):

Buffer	
Detergent 1	<1.0%
Cholesterol esterase	<1500 U/L
Cholesterol oxidase	<1500 U/L
Peroxidase (Horseradish)	<1300 ppg U/L
Ascorbic oxidase	<3000 U/L
Preservative	

LDL CHROMOGEN DETERGENT REAGENT (R2):

Buffer	
Detergent 2	<1.0%
N, N-bis(4-sulfobutyl)-m-toluidine, disodium (DSBmT)	<1 mM
Preservative	

PRECAUTIONS
1. Good laboratory safety practices should be followed when handling any laboratory reagent. (*CLSI,* GP17-A2).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY
The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. The reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values. DO NOT FREEZE.

SPECIMEN COLLECTION AND STORAGE / STABILITY
Fresh serum or plasma drawn from the patient after a 12-14 hour fast is the required specimen. Plasma samples must be collected using lithium heparin as anticoagulant. Remove the serum or plasma as soon as possible after collection (within 3 hrs). If the assays are not completed within 14 hours, serum can be stored up to 5 days at 2 – 8°C. If specimens need to be stored longer than 5 days before testing, they may be frozen at < -80 ºC. Samples may be frozen only one time. Refer to NCCLS H18-A for further instructions on specimen collection, handling and storage.

PROCEDURE

Materials Provided
Medica LDL Reagent Wedge, REF 10224

Additional materials required
Medica LDL Calibrator, REF 10655
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (60 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration
Medica's LDL Calibrator, REF 10655, is recommended for the calibration of the assay. The calibration interval (30 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs. The value of the LDL Cholesterol Calibrator was assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL).

Quality Control
It is recommended that at least two levels of human serum based controls (normal and abnormal) be run with the assay daily, whenever patient testing is performed and with each reagent lot change. Medica recommends the use of additional lipid control material with LDL levels around 160 mg/dL. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA Analyzer calculates the LDL concentration from the ratio of the corrected unknown sample's absorbance to the corrected absorbance of the calibrator multiplied by the calibrator value.

$$\text{LDL(mg/dL)} = \frac{[(A_{U_{550}} - A_{U_{700}}) - (A_{RBlk_{550}} - A_{RBlk_{700}})] - [(A_{U_{550}} - A_{U_{700}})_{SBlk} - (A_{RBlk_{550}} - A_{RBlk_{700}})_{SBlk}] \times dF}{[(A_{C_{550}} - A_{C_{700}}) - (A_{RBlk_{550}} - A_{RBlk_{700}})] - [(A_{C_{550}} - A_{C_{700}})_{SBlk} - (A_{RBlk_{550}} - A_{RBlk_{700}})_{SBlk}] \times dF} \times \text{CalValue}$$

Where A_U and A_C are the absorbance values of the unknown and the calibrator, respectively; A_{RBlk} is absorbance of the reagent blank; SBlk is sample blank; and "Cal Value" is the concentration of LDL in the calibrator (mg/dL or mmol/L). Since the volume of the reaction is changed with the delayed addition of the R2 reagent, there is a dilution correction factor (dF) included in the calculation.

Expected Values[6]
It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

The reference range for LDL in serum is as follows according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines

LDL Cholesterol – Primary Target of Therapy		
<100	mg/dL	Optimal
100-129	mg/dL	Near optimal/above optimal
130-159	mg/dL	Borderline high
160-189	mg/dL	High
>190	mg/dL	Very high

Procedural Limitations
Avoid using heavily hemolyzed and/or icteric serum or plasma samples.

The EasyRA Chemistry Analyzer flags any result above 540 mg/dL as Linearity High "LH". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the LDL test to 1080 mg/dL.

PERFORMANCE CHARACTERISTICS[7]

NOTE: Medica's LDL test has not been certified or tested by the Cholesterol Reference Method Laboratory Network (CRLMN).

Reportable Range
The reportable range is 6 to 540 mg/dL. Extended range is 6 to 1080 mg/dL when half of the sample is used (1:1 dilution)

Inaccuracy/ Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for LDL (y) on the EasyRA Analyzer to the performance of LDL reagent (x) on the Roche COBAS MIRA* Analyzer. The data shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the Roche COBAS MIRA Analyzer.

Number of samples	61	Range of Samples	7 to 540 mg/dL
Slope	0.9904	y Intercept	-0.3123
Correlation Coefficient	0.9976	Regression Equation	Y = 0.9904*X -0.3123

**Cobas Mira is a registered trademark of Roche Diagnostics, INC., Indianapolis, IN.*

The following table lists the data obtained in a comparison of matched serum (x) and Li-heparinized plasma (y) samples using the Medica reagent for LDL on the EasyRA Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	70	Range of Samples	1.62 to 14.71 mg/dL
Slope	0.9854	y Intercept	-0.0643
Correlation	0.9891	Regression Equation	Y = 0.9854*X – 0.0643

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of four levels of QC material were tested twice a day for 20 days. Both total and within-run imprecision were calculated from these data.

Within run imprecision:

QC Level mg/dL	Within Run SD mg/dL	Within Run CV %
193	2.11	1.1
133	1.59	1.2
83	0.99	1.2
49	0.70	1.4

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
193	3.36	1.8
133	3.03	2.3
83	1.93	2.3
49	131	2.7

Linearity (CLSI, EP6-A)
Linear from 6 to 540 mg/dL. , based on the linear regression Y = 1.00*X -4.03.

The linear range can be extended above 540mg/dL. The Medica EasyRA Chemistry Analyzer flags any result above 540 mg/dL as Linearity High "LH". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will effectively extend the reportable range of the LDL test to 1080 mg/dL..

Limit of Quantitation (LOQ): 4.8 mg/dL (CLSI, EP17-A)

Interfering Substances (NCCLS EP7-A)
Less than 10% interference was classified as “no significant interference.”
No significant interference was found in levels of up to 600 mg/dL of hemoglobin.
There is significant interference above 5.5 mg/dL of bilirubin. Do not use icteric samples.
No significant interference was found in levels up to 500 mg/dL of triglycerides (using Intralipid*).
No significant interference was found in levels up to 50 mg/dL of ascorbic acid.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[10,11]

REFERENCES

1. Gotto AM, Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital Practice, 23: Suppl.1,4 (1988).
2. Crouse JR et al., Studies of low density lipoprotein molecular weight in human beings with coronary artery disease, J. Lipid Res., 26; 566 (1985).
3. Badimon JJ, Badimon L., Fuester V., Regression of Atherosclerotic Lesions by High-Density Lipoprotein Plasma Fraction in Cholesterol-Fed Rabbit, Journal of Clinical Investigation, 85:1234 – 41 (1990).
4. Castelli WP et al. Cholesterol and other lipids in coronary heart disease, Circulation, 55; 767 (1977).
5. Barr DP, Russ EM, Eder HA, Protein-lipid relationships in human plasma, Am. J. Med., 11;480 (1951).
6. Gordon T. et al., High density lipoprotein and coronary heart disease, Am. J. Med.,62;707 (1977).
7. William P., Robinson D., Baily A., High density liproprotein and coronary risk factors, Lancet, 1;72 (1979).
8. Kannel WB, Castelli WP, Gordon T., Cholesterol in the prediction of artherosclerotic disease; New perspectives based on the Framingham study, Am. Intern. Med., 90;85 (1979).
9. National Institute of Health publication No. 93-3095, September 1993.
10. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
11. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EasyRA Assay Parameters (LDL)

Primary Wavelength (nm)	550
Secondary Wavelength (nm)	700
Reaction Type	Endpoint (2)
Reaction Direction	Increase
Reagent Blank	Yes (with each calibration)
Sample Blank	Yes
Reaction Time	10.4 min
Calibration interval (maximum)	30 days
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	2.5
Diluent 1 volume (µl)	15
Diluent 2 volume (µl)	20
Reagent volume R1 (µl)	250
Reagent volume R2 (µl)	83
Decimal Places (default)	0
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend measuring range)
Reportable Range	6 to 540 mg/dL

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